European Journal of Scientific Research
ISSN 1450-216X / 1450-202X Vol. 156 No 4 June, 2020, pp.371 - 382
http://www. europeanjournalofscientificresearch.com

Impact of Progesterone Base Hormonal Contraceptive on some Inflammatory Markers in HIV Seropositive Females attending Fertility Clinic in NAUTH, Nnewi, Nigeria

Victoria Ogechi Agu
Department of Medical Laboratory Science, College of Health Sciences
Nnamdi Azikiwe University, Nnewi Campus, P.M.B 5001
Nnewi, Anambra State, Nigeria

Nkiruka Rose Ukibe
Corresponding Author: Department of Medical Laboratory Science
College of Health Sciences, Nnamdi Azikiwe University
Nnewi Campus, P.M.B 5001, Nnewi, Anambra State, Nigeria
E-mail1: nr.ukibe@unizik.edu.ng
Tel: +2348062915510

Charles Chinedum Onyenekwe
Department of Medical Laboratory Science, College of Health Sciences
Nnamdi Azikiwe University, Nnewi Campus, P.M.B 5001
Nnewi, Anambra State, Nigeria

Charles German Ikimi
Department of Biochemistry, Nnamdi Azikiwe University Awka
P.M.B 5025, Awka, Anambra State, Nigeria

Ikechukwu Uzoma Chukwuagwu
Department of Medical Laboratory Science, College of Health Sciences
Nnamdi Azikiwe University, Nnewi Campus, P.M.B 5001,
Nnewi, Anambra State, Nigeria

Ofia Anya Kalu
Department of Internal Medicine, College of Health Sciences
Nnamdi Azikiwe University
Nnewi Campus, P.M.B 5001, Nnewi, Nigeria

Joseph Chimezie Awalu
Department of Medical Laboratory Science, College of Health Sciences
Nnamdi Azikiwe University, Nnewi Campus, P.M.B 5001
Nnewi, Anambra State, Nigeria

Abstract

This is a case-controlled retrospective study designed to assess the impact of hormonal contraceptive (progesterone base) on levels of some immunoglobulin and cytokines in HIV infected females in Nnewi, Nigeria. One hundred and eighteen (118)

consented premenopausal females on regular menstrual cycle including; 29 HIV infected females on contraceptive (A); 29 HIV infected females not on contraceptive (B); 30 HIV seronegative females on contraceptives (C) and 30 HIV seronegative females not on contraceptive (D) were conveniently recruited with aid of questionnaire. Immunoglobulin (IgG and IgM) and cytokines (TNF-α and IL-2) were assayed using ELISA method. Results showed that IgG, IgM and TNF-α were significantly increased in at follicular phase while only IgM was increased at luteal phase of menstrual cyle in HIV infected females on/not on contraceptives when compared with HIV seronegative counterparts ($p \leq 0.001$ respectively). Similar observation was made for IL-2 at follicular phase of menstrual cycle in HIV infected females on contraceptive when compared with their seronegative counterparts on contraceptive ($p = 0.002$). The significant elevation in IgG, IgM and TNF-α in HIV infected females on contraceptives indicates active inflammation which was more marked at follicular phase of menstrual cycle thereby, predisposing them to adverse sexually transmitted infections and disease progression as a result of combined effect of the progesterone base contraceptives.

Keywords: HIV, Hormonal contraceptive, Immunoglobin, Cytokines.

1. Introduction

There has been an increasing evidence of spread of new infection of Human immunodeficiency virus (HIV) with an average of 2.5 million per year globally. Heterosexual transmission and epidemic in women have been greatly implicated worldwide (UNAIDS, 2016). Previous studies have documented the negative impact of HIV on women's reproductive health (Ikechebelu et al., 2002; Fallahian and Ilkhani, 2006, Ukibe et al., 2015). This 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 (Ukibe et al., 2015).

Some evidence of variable changes in antibody (immunoglobulin isotypes) and cytokines levels have been recorded by various researchers (Ukibe et al., 2015; Ifeanyichukwu et al., 2016; Nwokolo et al., 2017). In apparently healthy individuals, Immunoglobins appears in the plasma in the course of initial infection and re-infections thereby, conferring humoral immunity to individuals at the risk of infection. Cytokines however, plays immunomodulatory role in controlling the homoeostasis of the immune system. They function in the integrity of the central nervous system and endocrine (gonodal) system (Aruna et al., 2018). In human immunodeficiency virus infection, there are deregulated productions of a number of these cytokines. Some cytokines, such as IL-1, IL-6, TNF-alpha, interferon-gamma, are produced in increased amounts in vivo, whereas the production of IL-2 is decreased. Cytokines generally are categorized into pro-inflammatory cytokines, which stimulates the immune system or the anti-inflammatory cytokines which suppresses the immune system (Ukibe et al., 2015; Dinarello, 2000). Ukibe et al. (2015) reported cytokine changes in HIV infection with some undesirable effects on the female reproductive potential.

Contraceptives (fertility control) on the other hand, are methods or devices used to prevent pregnancy. Contraceptive use by female will reduce the burden imposed by HIV and increasingly, reduce the number of unwanted pregnancies and birth of HIV infected children (UNAIDS, 2014). Some cultures however discourage access to birth control because they consider it to be morally, religiously or politically undesirable (Hanson and Burke, 2010). According to *World Health Organization, (2011)*, of the various types of contraceptives, sterilization by means of vasectomy in males and tubal ligation in females, intrauterine devices (IUDs) and implantable birth control appears to be the most used and effective method. This is followed by a number of hormone-based methods including oral pills, patches, vaginal rings and injections. The less effective methods are physical barriers such as condoms, diaphragms, sponges and fertility awareness methods while spermicides and

withdrawal by the male before ejaculation are considered the least method. Previous research has shown that hormonal contraceptives modulate the immune system in such a way that may affect the immune response to HIV infection (Stringer and Antonsen, 2009). The public health implication of the combined effects of HIV infection and hormonal contraceptive use on some immunoglobin and cytokine levels in NAUTH, Nnewi, Nigeria, therefore, formed the basis for the present study.

2. Materials and Methods

2.1. Study Design

This research is a case-controlled, observational and retrospective study, designed to assess the effects of hormonal contraceptive use on some cytokines (IL-2 and TNF-alpha) and immunoglobulin (IgG and IgM) levels in HIV infected females on hormonal contraceptives attending Fertility Clinic at Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria using purposive sampling technique.

2.2. Study Population

A total of 118 premenopausal female participants (17-49yrs) were recruited and were divided into test groups: HIV infected females on hormonal contraceptives (n=29), HIV infected females not on hormonal contraceptive (n=29) and control groups: HIV seronegative females on hormonal contraceptive (n=30) and HIV seronegative females not on hormonal contraceptive (n=30). All the HIV infected females were on HAART for a minimum period of two years prior to sample collection and they were on any of the following fixed regimen: 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. In these regimen, 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 also been on it for minimum of two years before sampling, the contraceptive used could either be; Depo-provera (medroxyprogestrone acetate) an injectable hormonal contraceptive containing 150mg of progesterone given once in three months, Jadelle an implantable hormonal contraceptive containing 150mg of progesterone (levonorgestol) which once implanted last for a period of 5 years or Implanol an implantable hormonal contraceptives containing 68mg of progesterone (etonogestrol) which once implanted last for a period of 3 years.

2.3. Inclusion Criteria and Exclusion Criteria

HIV stages 1 and II infected females on HAART aged 17-49 years on/not on hormonal contraceptive were included in the study. Age matched HIV seronegative females on/not on hormonal contraceptive were also included as control. HIV stage III and IV infected females were excluded from the study. HIV co-infected females with tuberculosis and hepatitis were excluded. Females with diabetes and hypertension, pregnant women and females below 17 years or above 49 years were all excluded from the study.

2.4. Ethical Consideration

The ethical approval for this work was sought and obtained from the board of ethics committee of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, in accordance with Helsinki declaration by the World Medical Association (WMA) on the ethical principles for medical research

involving human (Levine, 2006). Written informed consent was also obtained from all the participants before sampling.

2.5. Sample Collection

Approximately 5mls 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 (Test) as well as members of staff (Control) of Nnamdi Azikiwe University Teaching Hospital, Nnewi at both follicular (7-13th 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. The samples were then spun for 5 minutes at 1500 revolution per minutes (rpm) using a bench centrifuge after which the serum was separated for the analysis of IL-2, TNF alpha, IgG and IgM levels. The separated samples were preserved at -80^{o}c in the retroviral laboratory of Nnamdi Azikiwe University Teaching Hospital prior to assay.

2.6. Laboratory Analysis

Determination of HIV-1/2 assays: HIV-1/2 assay was done using determine as described by Alere medical company limited, Japan (Piot et al., 1988).

Determination of human IgG and IgM assay were done with ELISA kit by the method of Terpstra et al. (1985) while IL-2 and TNF-α were also determined using ELISA kit as described by (Hedeyati et al., 2001) and Malek, (2010) respectively.

2.7. Anthropometric Data Collection

The blood pressures were 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)

2.8. 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 analysis of variance (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 $p<0.05$.

3. Results

3.1. Levels of IgG (mg/ml), IgM (µg/ml), TNF α (pg/ml) and Interleukin 2 (ng/ml) in HIV Infected Females and Control Females On/Not on Hormonal Contraceptive (Mean ±Std Deviation)

The mean serum IgG and IgM (mg/ml) values showed no significant difference in HIV infected females on hormonal contraceptive between follicular (2.82 ± 1.63) and luteal (1.96 ± 0.87) phase of menstrual cycle ($p > 0.05$ respectively). Likewise, no significant difference was observed in the mean serum IgG values in HIV infected females who are not on hormonal contraceptive between follicular (1.05 ± 0.19) and luteal (1.17 ± 0.24) phase of menstrual cycle ($p > 0.05$ respectively). Similarly, there was no significant difference in the mean serum IgG value in control females on hormonal contraceptive between follicular (0.81 ± 0.32) and luteal (2.30 ± 3.10) phase of menstrual cycle ($p >$

0.05 respectively). However, a significantly lower value was observed in the mean serum IgG in control females not on hormonal contraceptive at follicular (0.48 ± 0.10) compared with luteal (0.74 ± 0.13) phases of the menstrual cycle (p = 0.002 respectively).

When the mean serum IgG and IgM values at follicular phase of menstrual cycle were compared between test and control groups, IgG and IgM values were significantly higher in HIV infected females on contraceptive (2.82 ± 1.63, 62.30 ± 17.32), HIV infected females not on contraceptive (1.05 ± 0.19, 84.54 ± 20.06) and control females on contraceptive (0.81 ± 0.32, 62.30±17.32) compared with control females not on contraceptive (0.48 ± 0.10, 45.65 ± 13.81) (p≤ 0.001 respectively). Similar observation was made same parameters at luteal phase of menstrual cycle in HIV infected females on contraceptive (1.96 ± 0.87, 130.71 ± 40.08), HIV infected females not on contraceptive (1.17 ± 0.24, 90.85 ± 28.08), control females on contraceptive (2.30 ± 3.10, 77.02 ± 19.73) compared with control females not on contraceptives (0.74 ± 0.13, 56.71 ± 15.44) (p = 0.045, p ≤ 0.001 respectively).

There were significant increases in IgG and IgM values at follicular phase of menstrual cycle in HIV infected females on contraceptive (2.82 ± 1.63, 164.87 ± 68.81) compared with HIV infected females not on contraceptive (1.05 ± 0.19, 84.54 ± 20.06) (p ≤ 0.001 respectively). Similarly, a significant increase was observed in IgG and IgM at follicular phase of menstrual cycle in HIV infected females on contraceptive (0.81 ± 0.32, 164.87 ± 68.81) when compared with the control females on hormonal contraceptive (0.48±0.10, 62.30 ± 17.32) (p ≤ 0.001 respectively).

At luteal phase of menstrual cycle, IgG and IgM were significantly increased in HIV infected females on contraceptive (1.96 ± 0.87, 130.71 ± 40.08) and control females on contraceptive (2.30 ± 3.10, 77.02 ± 19.73) compared with control females not on contraceptive (0.74 ± 0.13, 90.85 ± 28.08) (p = 0.031, 0.003, 0.018, respectively) (Table 1).

3.2. Levels of TNF α, and Interleukin 2 in HIV Infected Females and Control Females On/Not on Hormonal Contraceptive

When the mean serum TNF-α at follicular and luteal phases of menstrual cycle was compared between test and control groups, TNF-α was significantly higher in HIV infected females on contraceptive (8.89 ± 4.14, 5.93 ± 2.48), HIV infected females not on contraceptive (3. 07 ± 0.61, 3.82 ± 1.22) and control females on contraceptive (2.32 ± 0.78, 6.76 ± 9.20) compared with the control females not on contraceptive (1.30 ± 0.63, 1.78 ± 0.36) (p ≤ 0.001, 0.032 respectively).

A significant increase in the mean serum TNF-α value was observed at follicular phase of menstrual cycle in HIV infected females on contraceptive (8.89 ± 4.14) compared with HIV infected female subjects not on contraceptive (3. 07 ± 0.61) and control females on/not contraceptive (2.32 ± 0.78, 1.30 ± 0.63) (p ≤ 0.001 respectively).

At luteal phase of menstrual cycle, TNF-α was significantly increased in HIV infected females on contraceptive (5.93 ± 2.48) compared with control females not on contraceptive (1.78 ± 0.36) (p = 0.016 respectively). The same observation was made in control females on contraceptive (6.76 ± 9.20) compared with control females not on contraceptive at luteal phase of menstrual cycle (1.78 ± 0.36) (p = 0.013 respectively).

However, when the mean serum IL-2 value at follicular phase of menstrual cycle was compared between test and control groups, IL-2 was significantly lower in HIV infected females on contraceptive (0.38 ± 0.24), HIV infected females not on contraceptive (0.12 ± 0.05) and control females on contraceptive (0.14 ± 0.09) compared with control females not on contraceptive (0.80 ± 0.08) (p = 0.002 respectively).

However, there was significant increase in IL-2 value at follicular phase of menstrual cycle in HIV infected females on contraceptive (0.38 ± 0.24) compared with the counterpart not on contraceptive (0.12 ± 0.05) and control females on contraceptive (0.14 ± 0.09) (p = 0.002, 0.005 respectively) (Table 2).

Table 1: Levels of IgG and IgM in HIV infected females and control females on/not on hormonal contraceptive (mean ±std deviation)

Group	IgG (mg/ml)		p-value	IgM (µg/ml)		p-value
	Follicular	Luteal		Follicular	Luteal	
HIV infected females on contraceptive (A) (29)	2.82±1.63	1.96±0.87	0.200	164.87±68.81	130.71±40.08	0.232
HIV infected females not on contraceptive (n=29) (B)	1.05±0.19	1.17±0.24	0.252	84.54±20.06	90.85±28.08	0.580
HIV seronegative females on contraceptive (n=30) (C)	0.81±0.32	2.30±3.10	0.228	62.30±17.32	77.02±19.73	0.228
HIV seronegative females not on contraceptive (n=30) (D)	0.48±0.10	0.74±0.13	0.002	45.65±13.81	56.71±15.44	0.238
F-value	8.886	2.980		11.452	10.730	
p-value	0.001	0.045		0.001	0.001	
A vs B	0.001	0.122		0.001	0.003	
A vs C	0.001	0.608		0.001	0.003	
A vs D	0.001	0.031		0.001	0.001	
B vs C	0.652	0.064		0.335	0.384	
B vs D	0.358	0.368		0.001	0.005	
C vs D	0.592	0.018		0.349	0.226	

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

Group	TNFa (pg/ml)		p-value	IL-2 (ng/ml)		p-value
	Follicular	Luteal		Follicular	Luteal	
HIV infected females on contraceptive (A) (29)	8.89±4.14	5.93±2.48	0.963	0.38±0.24	0.23±0.10	0.145
HIV infected females not on contraceptive (n=29) (B)	3.07±0.61	3.82±1.22	0.125	0.12±0.05	0.17±0.07	0.096
HIV seronegative females on contraceptive (n=30) (C)	2.32±0.78	6.76±9.20	0.225	0.14±0.09	0.76±1.31	0.234
HIV seronegative females not on contraceptive (n=30) (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	0.001	0.032		0.002	0.082	
A vs B	0.001	0.171		0.002	0.749	
A vs C	0.001	0.678		0.005	0.052	
A vs D	0.001	0.016		0.002	0.654	
B vs C	0.576	0.109		0.827	0.818	
B vs D	0.266	0.155		0.618	0.859	
C vs D	0.525	0.013		0.499	0.618	

3.3. Correlation of BMI and Blood Pressure with Female Reproductive Hormones, Cytokines and Immunoglobulins in HIV Infected Females and Control Females On /Not on Contraceptive.

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

SBP showed a moderate positive correlated with IgM ($r = 0.455$, $p = 0.022$) and IgG ($r = 0.459$, $p = 0.021$) in HIV infected females on hormonal contraceptive.

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 contraceptive and a strong negative

correlation with IL-2 in HIV infected females who are not on hormonal contraceptive (r = - 0.481, p = 0.015) (Table 3).

Table 3: Correlation of BMI and blood pressure with cytokines and immunoglobulins in HIV infected females and control females on /not on contraceptive

Parameters	HIV infected females on contraceptive (n=29)		HIV infected females not on contraceptive (n=29)		HIV seronegative females on contraceptive (n=30)	
	R	p-value	R	p-value	R	p-value
Contraceptive use vs age	-	-	-		0.661	0.002
IgG vs SBP	0.459	0.021	0.510	0.009	-	-
IgM vs SBP	0.455	0.022	-	-	-	-
IL2 vs SBP	0.422	0.036	-	-	-	-
TNF vs SBP	0.479	0.015	-0.481	0.015	-	-

4. 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. This has also resulted to so many unintended pregnancies which need to be checkmated with contraception to reduce; the spread of HIV infection, birth of HIV infected children and other sexually transmitted infections.

The similar findings in blood pressure values observed between HIV infected females on hormonal contraceptives and control females suggests that HIV infection may not be associated with increase in blood pressure nor predispose to hypertension. This is consistent with report by Akello et al., (2016). However, previous works done on the relationship between blood pressure and HIV infections produced controversial findings (Okeke et al., 2017; American Heart Association, 2018*). However, a significant increase in DBP observed in control females on hormonal contraceptives suggests that the observed increase in blood pressure may be attributed to the hormonal contraceptive. This* observation is similar to the work of (Haroon and Naveed, 2015) who also reported an increase in diastolic blood pressure in hormonal contraceptive users.

Furthermore, *the study showed significantly increased levels of IgG and IgM in HIV infected females on hormonal contraceptives, HIV infected females not on hormonal contraceptives and control females on hormonal contraceptives compared with control females not on hormonal contraceptives at both follicular and luteal phases of the menstrual cycle. This observation is* an indication that HIV infection as well as hormonal contraceptive use stimulates immunoglobulin production as a consequence of the immunological reaction induced by the virus and the exogenous hormone. A research in Uganda and Norway also reported high levels of IgG in HIV positive females with higher values observed for Uganda compare to Norway where the researcher attributed the differences in the two populations to environmental factors (Lugada et al., 2014). This study also agrees with the previous findings by Panagiotis et al. (2007) who also observed that significant proportions of HIV positive patients have elevated level of one or more immunoglobulin, usually IgG isotype. The persistence increase of IgM and IgG at both follicular and luteal phase of menstrual cycle in HIV infected females on hormonal contraceptive are in line with earlier observation that increased production of immunoglobulin is associated with increased opportunistic infection requiring immunoglobulin response possibly to confer a protective immunity to the infected females (Lugada et al., 2014; Ifeanyichukwu et al., 2009). Studies have shown that hormonal contraceptives (especially those containing progesterone when compared to those containing estrogen) modulate the immune response in HIV infection with increased viral load, depletion of CD4+T cells and disease progression (Stringer and Antonsen, 2009; Wang et al., 2004). Baeten et al. (2007) also reported that Progesterone affect naturally occurring vaginal barriers such as Lactobacillus species, increases vaginal secretions thus increasing susceptibility to various sexually transmitted infections and potential to infection with

multiple HIV strains. Therefore, the use of hormonal contraceptive in HIV infection and in HIV-uninfected females may worsen the health condition in females due to increased susceptibility to other sexually transmitted infection if not properly monitored.

Similarly, the increased level of IgG observed in HIV infected females not on hormonal contraceptive and in control females on contraceptives compared with control females not on hormonal contraceptive at luteal phase of menstrual cycle at both follicular and luteal phases of menstrual cycle in this study is in agreement with the previous work done by (Stringer and Antonsen, 2009; Ifeanyichukwu et al., 2009; Ukibe et al., 2015). The authors confirmed that hormonal contraceptives and HIV infection modulate the immune response to microorganism. A similar report of increased level of immunoglobulin G has been previously published (Ezegwui et al., 2013). This observation elucidated the role of hormonal contraceptive in the immune system.

The significantly increased level of immunoglobulins observed in HIV infection and hormonal contraceptive use in this study implies greater effect on the immune system because the presence of IgG in the circulation generally indicate the presence of immunogen that triggers immune response to offending microorganism. While IgM on the other hand is produced first in the course of an infection and it usually disappear only to reappear following subsequent re-infection by the same organism.

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 (Aruna et al., 2018). In this study, significantly increased level of TNF-α in HIV infected females on hormonal contraceptives, HIV infected females not on hormonal on contraceptives, control females on hormonal contraceptive compared with control females not on hormonal contraceptive at both follicular and luteal phases of the menstrual cycle indicates higher degree of inflammation. Excessive TNF-α has been linked with inflammation, trauma, sepsis, cancer, depression, viral replication (Dowlati et al., 2010; Swardfager et al., 2010) and ovarian failure (Kellie et al., 2009; Mamta et al., 2016). This elevated level of TnF-α may account for the high inflammation, depression, cancer (kaposis sarcoma) and hypogonadism observed in HIV infection (Ukibe et al., 2015). Similarly, the increased level of this cytokines observed in hormonal contraceptive users may be implicated in the increased risk of cancers of the breast, cervix and ovary observed with hormonal contraceptive use. 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 (Ukibe et al., 2015). Thus the elevated levels of TNF observed in HIV infection and contraceptive use 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 not on hormonal contraceptive at luteal phase of menstrual cycle is a strong indication 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.

The significant increase in TNF at follicular phase of menstrual cycle in HIV infected females on hormonal contraceptive compared with their counterparts not on hormonal contraceptive and control females on/not on hormonal contraceptive, may suggest increased inflammatory activity and severity of HIV infection in the HIV contraceptive users. This may further predispose these females to increased risk of viral replication, increased viral load and depletion of CD4+ T-cells as well as opportunistic infections. This finding is in tandem with the work of (Enrique et al., 2014; Ukibe et al., 2015)**.**

Hormonal contraceptives and ARDs interactions can work antagonistically to each other thereby, can significantly affect the potency of hormonal contraceptives vis-avis ARDs used in HIV infection. It is documented that hormonal contraceptives especially those containing progesterone selectively, compromises antiviral activity of Tenofavir and Tenofavir-alafenamide (Shen et al., 2017) and this may suggests decrease ARD protection in hormonal contraceptive users. Similarly, Efavirenz

and Nevirapine significantly reduce the effectiveness of progesterone containing contraceptive (Landolt et al., 2013; Thurman et al., 2014) thus resulting to contraceptive failures. The interaction of hormonal contraceptives with ARDs may be responsible for the pronounced increase in inflammatory response observed in HIV infected females on hormonal contraceptives in this study.

This study also observed significant decrease in IL-2 level 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, this further suggest a depressed immunity in HIV infected individuals and hormonal contraceptive users at follicular phase of menstrual cycle. This finding was similar to the previous work done by Stringer and Antonsen, (2009)

Furthermore, in this study, IgG showed positive correlation with SBP in HIV infected females on hormonal contraceptives and HIV infected females who were not on hormonal contraceptive. IgM also showed moderate positive correlation with SBP in HIV infected females on hormonal contraceptive. This shows that increase in blood pressure can be immunologically mediated. The relationship between hypertension and inflammation are well documented (Siddiqui et al., 2014; Christopher et al., 2014). Immunoglobulin causes hypertension through various mechanisms such as induction of apoptosis of suppressor T cells, binding to the arteries, glomerulus and kidney basement membrane thus altering hemodynamic parameters, 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 (Xia et al., 2013).

Hormonal contraceptive use showed strong positive correlation with age in control females on hormonal contraceptive. Age is an important risk factor for cardiovascular diseases in healthy females. At younger age, women has lower risk of CVD due to the protective effect of endogenous ovarian hormones (estrogen) but at menopause the levels of this hormone falls and they become prone to increased risk of cardiovascular diseases (Barrey et al., 2003; Margolis et al., 2007). 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

Significant variations observed in the levels of TNF-α, IL-2, IgG and IgM in HIV infected females on hormonal contraceptive particularly at follicular phase of menstrual cycle suggests active inflammatory reaction which may have been caused by the combined effect of hormonal contraceptive and HIV infection thereby, worsening the ongoing immune depression in the affected individuals. Screening for inflammatory markers should be included in the regular checkup for all HIV subjects irrespective of contraceptive use. Also, anti-inflammatory drugs should be included in the drugs of HIV infected females on hormonal contraceptives to reduce the drug interaction between ARDs and hormonal contraceptives.

Acknowledgements

Authors wish to acknowledge all the HIV female participants who voluntarily gave their informed consent for this study.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interests: All authors have none to declare.

Authorship: NRU conceptualized and designed the study. VOA, CGI, IUC, OAK and JCA acquired, analysed and interpreted the data. VOA, NRU, CCO drafted and revised the article critically for important intellectual content. All authors read and approved the final version of the manuscript.

References

[1] Akello S, Ueda P, Kenyesigye M, Byaruhanga E, Kiyimba A, Amanyire G, Kintu A, Fawzi W.W, Muyindike W.R and Danaei G, 2017. Association between human immunodeficiency virus and blood pressure in adults and roles of body weight as a mediator: Cross sectional study in Uganda. *Journal of Clinical Hypertension* 19 pp. 1181-1191.

[2] American Heart Association News, 2018. What's the connection between high blood pressure and human immunodeficiency virus? Retrieved online 28th July, 2018 http://news.hearth.org>what is the connection between high blood pressure and human immunodeficiency virus.

[3] Aruna B, Harmandeep K.S, Gurpreet K, 2014. Sex hormones and immune dimorphism. *The Science World Journal.* http://dx.doi.org/10.1155/2014/159150. Accessed 8th August, 2018.

[4] Baeten J.M, Lavreys L and Overbaugh J, 2007. The influence of hormonal contraceptive use on human immunodeficiency virus-1 transmission and disease progression. *Clinical Infectious Diseases* 45, pp. 360-369.

[5] Barrey M.C.N, Johnson B.D, Sharaf B.L, Bitner V, Berga S.L, Braunstein G, et al, 2003 Hypoestrogenemia of hypothalamic origin and coronary artery disease in premenopausal women: a report from the national heart, lung and blood institute-sponsored wise study. *Journal of American College of Cardiology* 41, pp. 413-419.

[6] Christopher T.C, Maggie L, Banhook T, Tin S.K, Alexander B, Christopher G.S et al, 2014. Antibodies in the pathogenesis of hypertension. *Biomedical Research International* pp. 504045. http://dx.doi.org/10.1155/2014/504045.

[7] Dinarello C.A, 2000. Proinflammatory cytokines. *Chest* 118, pp. 503–508.

[8] Dowlati Y, Herrmann N, Swardfager W. A, 2010. A meta-analysis of cytokines in major depression. *Biology and Psychiatry* 67, pp. 446–457.

[9] Enrique V.T, Manpreet K.S, Minette L, Judy L, Nishita P, Cesar O et al, 2014 Cytokine Levels in Plasma Samples of Individuals with human immunodeficiency virus Infection. *Austin Journal of Clinical Immunolology* 1, pp. 1003.

[10] Ezegwui H, Ikeako L.C and Obiora-Okafor N.C, 2013. The use of depot medroxyprogesterone acetate (DMPA) injectable contraceptive in Enugu, Nigeria. *Nigerian Journal of Medicine* 21, pp. 266-271

[11] Fallahian M and IIkhani M, 2006. "Menstrual Disorders in Nongenital Tuberculosis". *Infectious Disease in Obstetrics and Gynaecology* 18452, pp. 1–3.

[12] Hanson S.J and Burke A.E, 2010. Fertility control: contraception, sterilization and abortion. Wolters Kluwer Health/Lippincott Williams and Wilkins: pp. 382–395.

[13] Haroon S and Naveed A. 2015. Effect of hormonal contraceptives on serum electrolyte and blood pressure. *Journal of Postgraduate Medical Institute* 28, pp. 409-413.

[14] Hedeyati M, Yazdanparast R, Azizi F, 2001. Determination of human Tumour Necrotic Factor-α by highly sensitive enzyme immunoassays. *Biochemical Biophysiology* 289, pp. 295-298.

[15] Ifeanyichukwu M, Onyenekwe C, Ele P, Ukibe N, Meludu S, Ezeani M, et al, 2009. Evaluation of immunoglobulin classes (IgA, IgG and IgM) levels and complement fixation activity in HIV infected subjects. *International Journal of Biological and Chemical Sciences* 3, pp. 1504-1508.

[16] Ifeanyichukwu M.O, Odozi E.B, Meludu S.C, Okeke C.O, 2016. Effect of human immunodeficiency virus infection on some haematological parameters and immunoglobulin levels in HIV patients in Benin City Southern, Nigeria. *Journal of Human Immunodeficiency Virus and Retrovirus* 2, pp. 2.

[17] Ikechebelu J.I, Ikegwuonu S.C, Joe-Ikechebelu N.N, 2002. Human immunodeficiency virus Infection and Sexual behaviours among infertile women in Southwestern Nigeria. *Journal of Obstetrics and Gynecology* 22, pp. 306-307.

[18] Kellie A.C, Hagen K, Robin S, Monica E.C, Toby L, Anne S, et al. 2009. The tumour-promoting actions involve Tumour necrotic factor receptor-I and Interleukin-17 in ovarian cancer in mice and human. *The Americal Society Clinical Investigation* 119, pp. 3011-3022.

[19] Landolt N.K, Phanuphak N, Ubolyam S, Pinyakorn S, Kriengsinyot R, Ahluwalia J, et al, 2013. Efavirenz, in contrast to nevirapine, is associated with unfavorable progesterone and antiretroviral levels when coadministered with combined oral contraceptives. *Journal of Acquired Immunodeficiency Syndrome* 62, pp. 534-539. doi:10.1097/QAI.0b013e31827e8f98.

[20] Levine R.J. 2006. Some recent development in the international guidelines on the ethic of research involving human subjects. *Annals of New York Academy of sciences* 918, pp. 170-180.

[21] Lugada E.S, Mermin Y.J, Asjo Y.B, Kaharuza Z.F, Downing Y.R, Langeland Y.N, Ormaasen V, Bruun J, Awory A.C and Ulvestad E.Z, 2014. Immunoglobulin Levels Amongst Persons with and without Human Immunodeficiency Virus Type 1 Infection in Uganda and Norway. Blackwell Publishing Ltd. *Scandinavian Journal of Immunology* 59, pp. 203–208

[22] Malek T.R and Castro I. Human IL2 immunoassay quantikine ELISA. *Immunity* 33, pp. 153.

[23] Mamta G, Kataryn T.S, 2016. TNF expression, risk factors & inflammatory exposures in ovarian cancer: evidence for an inflammatory pathway of ovarian carcinogenesis? *Human Pathology* 54, pp. 82-91.

[24] Margolis K.L, Adami H.O, Luo J, Ye W and Weiderpass E, 2007. A respective study of oral contraception use and risk of myocardial infarction in Swedish women. *Fertility and Sterility* 88, pp. 310-316.

[25] Nwokolo H.I, Onyenekwe C.C, Emeremmdu F.O and Ukibe N.R. Some Immunological Profiles in Women Practising DMPA Contraception in Malaria Endemic Area of Nnewi and Environs. *Archive of Clinical and Biomedical Research* ISSN: 2572-5017.

[26] Okeke N.L, Thibaut davy, Joseph J.E and Soma N, 2016. Hypertension among human immunodeficiency virus -infected patients in clinical care, 1996-2013. *Clinical Infectious Diseases* 63, pp. 242-248.

[27] Panagiotis A.K, Bruce J.D, Liron P, Gary L.H and Bruce A.B, 2007. Protein electrophoresis and immunoglobulin analysis in human immunodeficiency virus-infected patients. *American Journal of Clinical Pathology* 128, pp. 596- 603.

[28] Piot P, Plummer F.A, Mhalu F.S, Lamboratory J.L and Chin J.M, 1988. *An International Prospective of Sciences* 239, pp. 573-579.

[29] Shen Z, Rodriguez-Garcia M, Mickey V.P, Jack B, Angela D.M.K and Charles R.W, 2017. Hormonal Contraceptives Differentially Suppress Tenofavir and Tenofavir-alafenamide Inhibition of human immunodeficiency virus Infection and Tenofavir in Blood and Genital Tract CD4+ T cells. *Science Reports* 7, pp. 17697.

[30] Siddiqui A.H, Irani R.A, Blackwell S.C, Ramin S.M, Kellems R.E and Xia Y, 2014. "Angiotensin receptor agonistic autoantiboby is highly prevalent in preeclampsia: correlation with disease severity". *Hypertension* 55, pp. 386-393.

[31] Stringer E and Antonsen E, 2009. Hormonal contraceptive and human immunodeficiency virus disease progression. *Acquired Immunodeficiency Syndrome* 23, pp. 10.

[32] Swardfager W, Lanctôt K, Rothenburg L, Wong A, Cappell J, Herrmann N, et al, 2010. A meta-analysis of cytokines in Alzheimer's disease. *Biology and Psychiatry* 68, pp. 930–941.

[33] Terpstra W.J, Ligthart G and Schoone G.J, 1985. Enzyme linked immunosorbent assay for the detection of specific IgG and IgM in human leptospirosis. *General Microbiology* 131, pp. 377-385.

[34] Thurman A.R, Anderson S and Doncel G.F, 2014. Effects Hormonal Contraceptives on Anti Retroviral Drug Metabolism, Pharmacokinetics and Pharmacodynamics. *American Journal of Reproductive Immunolology* 71, pp. 523-530.

[35] Ukibe N.R, Emelumadu O.F, Ukibe S.N, Onyenekwe C.C, Ahaneku J.E, Aboh N.A, and Nnadozie O.J, 2015. Sex hormones changes associated with menstrual cycle in human immunodeficiency virus infected females at Nnamdi Azikiwe University Teaching Hospital Nnewi, Southeast Nigeria. *European Journal of Sientific Research* 11, pp. 134-141.

[36] United Nations programme on human immunodeficiency virus /acquired immunodeficiency syndrome, 2006. Report on the global Acquired immunodeficiency syndrome epidemic. Report/default.asp. (Retrieved 6th August 2018). http://www.unaids.org/en/HIV_data/2006Global

[37] United Nations programme on Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome, 2014. The GAP Report. http://www.unaids.org/sites/default/files/en/media/unaids/contentassets/documents/unaidspublication/2014/UNAIDS_Gap_report_en.pdf. Accessed July 1, 2017.

[38] Wang C.C, McClelland R.S, Overbaugh J, Reilly M, Panteleeff D.D, Mandaliya K, et al, 2004. The effects of hormonal contraception on genital tract shedding of human immune deficiency virus -1. Acquired Immunodeficiency Syndrome 18, pp. 205-209.

[39] World Health Organization, 2011. Department of Reproductive Health and Research. Family planning: A global handbook for providers: Evidence-based guidance developed through worldwide collaboration (PDF) (Review and Updated Edition). Geneva, Switzerland: WHO and Center for Communication Programs.

[40] Xia Y and Kellems R.E, 2013. Angiostension receptor agonistic autoantibodies and hypertension: Pre-eclampsia and beyond". *Circulation Research* 113, pp. 78-87.