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CYTOKINES CHANGES ASSOCIATED WITH MENSTRUAL CYCLE IN HIV INFECTED FEMALES AT NAUTH, NNEWI, SOUTH-EAST NIGERIA

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Abstract

Background: HIV infection is characterized by hormonal and immunological changes which may grossly affect the reproductive cycle in affected women. **Aim:** To evaluate Cytokine changes in HIV infected women during menstrual cycle. **Materials and methods**: A total of 90 women aged between 15 and 45 years were randomly recruited for the study. 30 of the women were normal healthy seronegative for HIV and served as control. Blood samples were collected under sterile conditions during the follicular and luteal phases of menstrual cycle after due informed consent had been obtained and the samples were analyzed for Cytokines (IL-8, IL-6,

IL-4, and TNFα) using Enzyme Linked Immunosorbent Assay (ELISA) method. **Results**: The Cytokines (IL-8, IL-6, IL-4 and TNFα) were significantly higher at both phases of menstrual cycle in HIV infected women when compared with the Control ($P<0.05$). **Interpretation and Conclusion:** The study showed significant cytokine changes with some degree of inflammatory reactions in HIV infected women. The implication of these changes within reproductive life of the women is discussed.

Keywords: HIV, Women, Cytokine changes, South East, Nigeria

INTRODUCTION

The Human Immune deficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) pandemic has become a major public health problem worldwide especially in sub-Saharan Africa where more than 80 percent of all people living with HIV/AIDS reside (UNAIDS 2004; 2012). Reports have shown considerable evidence that the rate at which HIV infection progresses in women is different from that in men (Farzadegan *et al*., 1998, UNAIDS 2012; USAID, 2012). In Nigeria, about 3.1 million people are living with HIV/AIDS while 58% (1.72 million) are females mostly within reproductive age (UNAIDS 2011; UNAIDS 2012). In Nigeria HIV is a leading cause of morbidity and mortality among women of reproductive age (UNAIDS 2011; NACA 2012).

HIV infection is known to depress the immune system because of the tropic attraction of the virus to the immune cells. Once the immune cells are overwhelmed, the infected host becomes immunocompromised and is then prone to lots of opportunistic infections.

HIV has been known to impact negatively on women's reproductive health (Ikechebelu *et al*., 2002; Fallahian and Ilkhani, 2006). The negative reproductive impact ranges from menstrual disorders and failure of reproductive function.

Cytokines are low molecular weight extracellular signaling proteins secreted by immune inflammatory cell populations (Desair, 2007). They have been closely associated with ovarian function in females and are believed to be produced locally in the ovulatory follicle where they assist granulose cell growth and it inhibits their differentiation. They are also believed to stimulate the secretion of ovulation associated substances such as prostaglandins which aid in the ovulatory process (Brannstrom *et al*., 1995). Cytokines are believed to play a role in menstruation and implantation since they contribute to the defense of the endometrial mucosal epithelium.

Since HIV infection affects all body system including the reproductive system, cytokine changes occur and this may have some undesirable effects on the female reproductive potential.

The present study was therefore designed to evaluate the cytokine changes which are associated with the menstrual cycle in HIV infected females of reproductive age group in Nnewi, South- East, Nigeria.

MATERIALS AND METHODS

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

After obtaining informed consent of the participants, a well detailed questionnaire was administered to each participant to ascertain their medical and reproductive history. Eight milliliters of venous blood was collected aseptically between 9.00am and 12noon from each participant at follicular ($7\text{-}13^{th}$ day) and luteal ($21\text{-}23^{rd}$ day) phases of menstrual cycle. All the participants were double screened for malaria parasite and HIV infection using rapid antigen diagnostic techniques for *malaria falciparum* and immunoassay and immunochromatographic methods for HIV screening respectively. The serum content was separated immediately after clot retraction, labeled and stored at $-20^{o}C$ for determination of IL-8, IL-6, IL-4 and TNFα using ELISA (Enzyme Linked Immunosorbent Assay) method.

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

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

Methods

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

Determination of TNF-α, Interleukin-4, 6 and 8 were done using Enzyme Linked Immunosorbent Assay (ELISA) kits (Glory Science Laboratory USA)

Statistical analysis

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

RESULTS

Levels of Cytokines (IL-8) at Follicular and Luteal Phases of Menstrual cycle

The mean (±SD) plasma IL-8 concentration (pg/ml) dropped significantly at luteal phase (742.5±197.7, 377.1±174.2) compared with follicular phase (876.4±387.2, 550.9±183.6) of menstrual cycle in HIV seropositive females and HIV seropositive females on ART ($P<0.05$ respectively). When the mean IL-8 value (pg/ml) at follicular and luteal phases of menstrual were compared between the Control group and Test groups, the mean IL-8 was significantly higher in HIV seropositive females (876.4±387.2, 742.5±197.7) and HIV seropositive females on ART (550.9±183.6, 377.1±174.2) compared with follicular and luteal values in the Control female subjects (280.1±47.7, 276.9±56.3) ($P<0.05$ respectively).

The post hoc analysis showed significant drop in the mean IL-8 value (pg/ml) at follicular and luteal phases of menstrual cycle in HIV seropositive females on ART (550.9±183.6, 377.1±174.2) compared with follicular and luteal values in the HIV seropositive females (876.4±387.2, 742.5±197.7) ($P<0.05$ respectively) (See fig 1).

Levels of Cytokines (IL-6) at Follicular and Luteal Phases of Menstrual cycle

The mean (±SD) plasma IL-6 concentration (pg/ml) dropped significantly at follicular phase (474.1±153.2) of menstrual cycle compared with the luteal phase (584.3±271.3) in HIV seropositive female subjects ($P<0.05$). On the other hand, there was no significant difference in the mean IL-6 value (pg/ml) between follicular (224.9±54.6) and luteal (296.6±143.7) phases of menstrual cycle in HIV seropositive females on ART ($P>0.05$). Similarly, there was no significant difference in the mean IL-6 value between follicular (217.6±64.9) and luteal (204.6±36.7) phases of menstrual cycle in Control female subjects ($P>0.05$).

When the mean IL-6 concentration (pg/ml) at follicular and luteal phases of menstrual cycle were compared between the Control group and

Test groups, the mean IL-6 was higher in HIV seropositive females (474.1±153.2, 584.3±271.3) compared with follicular and luteal values in the Control female subjects (217.6±64.9, 204.6±36.7) (P<0.05respectively).

The post hoc analysis showed significant drop in the mean IL-6 value (pg/ml) at follicular and luteal phases of menstrual cycle in HIV seropositive females on ART (224.9±54.6, 296.6±143.7) compared with follicular and luteal values in the HIV seropositive females (474.1±153.2, 584.3±271.3) (P<0.05 respectively) (See fig 2)

Levels of Cytokines (IL-4) at Follicular and Luteal Phases of Menstrual cycle

The mean (±SD) plasma IL-4 concentration (pg/ml) in HIV seropositive females significantly dropped at luteal (354.9±207.7) compared with follicular (497.6±216.1) phase of menstrual cycle (P<0.05). But the mean IL-4 value (pg/ml) dropped significantly at follicular (527.2±231.3) compared with luteal phases (660.2±254.2) in HIV seropositive females on ART (P<0.05). The mean IL-4 value (pg/ml) dropped significantly at follicular phase (210.7±71.2) compared with luteal phase (334.8±76.5) in Control female subjects (P<0.05).

When the mean IL-4 concentration (pg/ml) at follicular and luteal phases were compared between the Control group and Test groups, the mean IL-4 was significantly higher in HIV seropositive females (354.9±207.7, 497.6±216.1) and HIV seropositive females on ART (527.2±231.3, 660.2±254.2) compared with follicular and luteal values in the Control female subjects (210.70±71.2, 334.8±76.5) (P<0.05 in each case).

The post hoc analysis showed significantly higher mean IL-4 value (pg/ml) at follicular and luteal phases of menstrual cycle in HIV seropositive females on ART (527.2±231.3, 660.2±254.2) compared with follicular value in HIV seropositive females (354.9±207.7, 497.6±216.1) (P<0.05 respectively) (See fig 3).

Levels of Cytokines (TNFα) at Follicular and Luteal Phases of Menstrual cycle

The mean (±SD) plasma TNFα concentration (pg/ml) dropped significantly at luteal phase (788.2±191.7) compared with follicular phase (949.6±335.7) of menstrual cycle in HIV seropositive female subjects (P<0.05). There was no significant difference in the mean TNFα value (pg/ml) between follicular (483.6±160.0) and luteal (519.2±177.8) phases of menstrual cycle in HIV seropositive females on ART (P>0.05). The mean TNFα value (pg/ml) dropped significantly at follicular phase (211.8±57.6) compared to luteal phase (333.0±72.2) of menstrual cycle in Control female subjects (P<0.05).

When the mean TNFα concentration (pg/ml) at follicular and luteal phases of menstrual cycle were compared between the Control group and Test groups, the mean TNFα was significantly higher in HIV seropositive females (949.6±335.7, 788.2±191.7) and HIV seropositive females on ART (483.8±160.0, 519.2±177.8) compared with follicular and luteal values in the Control female subjects (211.8±57.6, 333.0±72.2) ($P<0.05$ in each case).

The post hoc analysis showed significant drop in the mean TNFα concentration (pg/ml) at follicular and luteal phases of menstrual cycle in HIV seropositive females on ART (483.8±160.0, 519.2±177.8) compared with follicular and luteal values in HIV seropositive female subjects (949.6±335.7, 788.2±191.7) ($P<0.05$ respectively) (See Fig 4)

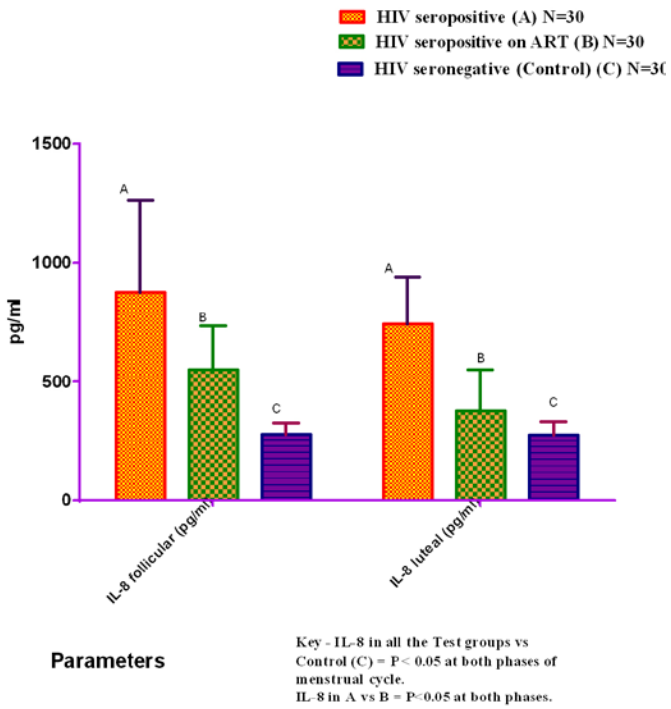

Fig 1: Comparison of mean (±SD) plasma levels of IL-8 in Test groups and Control group at Follicular and luteal phases of menstrual cycle

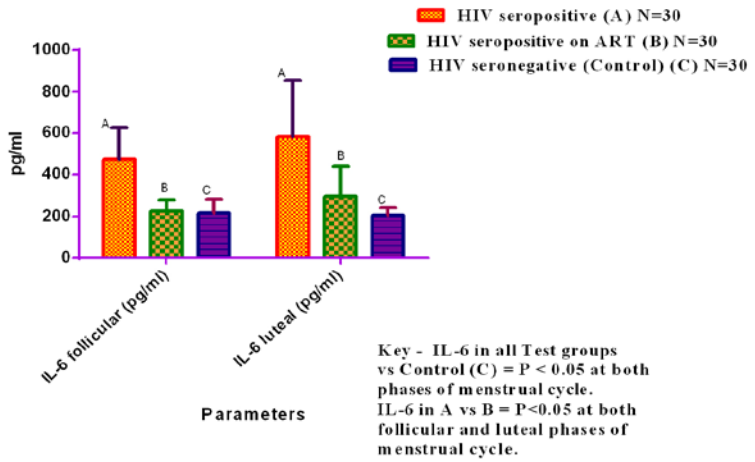

Fig 2: Comparison of mean (±SD) plasma levels of IL-6 in Test groups and Control group at Follicular and luteal phases of menstrual cycle

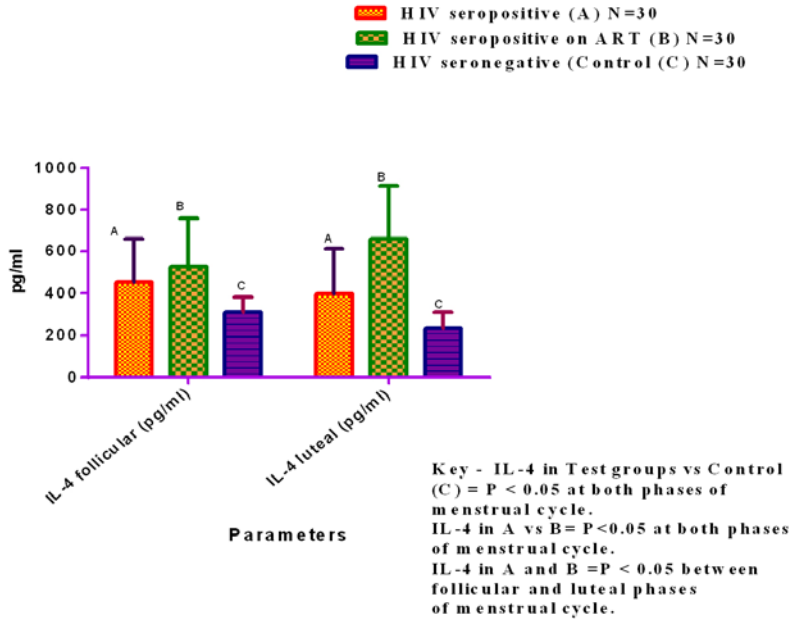

Fig 3: Comparison of mean (±SD) plasma levels of IL4 in Test groups and Control group at Follicular and luteal phases of menstrual cycle

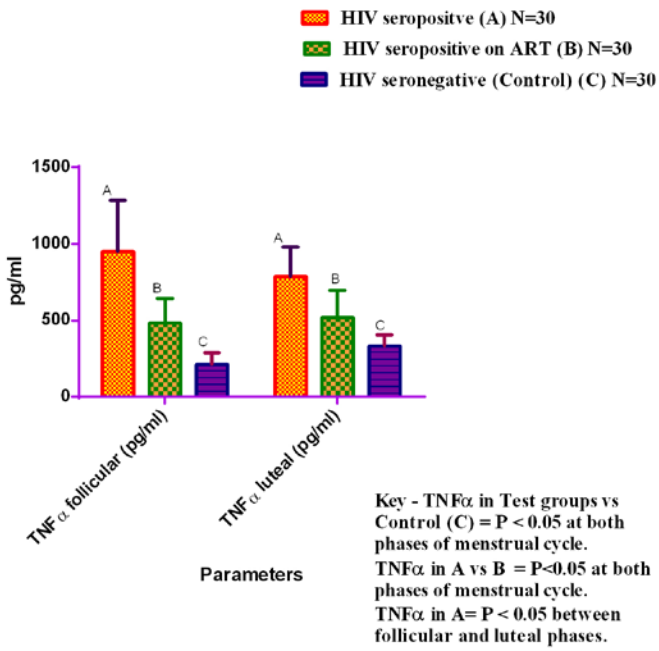

Fig 4: Comparison of mean (±SD) plasma levels of TNFα in Test groups and Control group at Follicular and luteal phases of menstrual cycle

DISCUSSION

The study showed significantly higher levels of IL-8 and TNFα in HIV seropositive females and HIV seropositive females on ART at follicular phase compared to luteal phase of menstrual cycle. Cytokines including TNFα and IL-8 are secreted by activated monocytes. It is worthy to note that monocytes populations are reduced in diseased states such as HIV infection indicating reduced cellular immunity which is one of the problems in these subjects. The increased secretion of these cytokines therefore may be due to hyperstimulation of the surviving monocytic cells. It has been reported that endotoxin stimulated monocytes of women in the luteal phase produced more TNFα when compared to follicular phase (Brannstrom *et al*., 1999; Bouman *et al*., 2001). IL-8 was also found to be significantly elevated at the follicular phase compared with the luteal phase (Al- Harthi, 2001) which implied increased inflammatory response and enhanced cellular immunity in this phase and this may have been induced by reduced progesterone and estrogen production. This report is consistent with the observation in the present study.

The significantly higher level of TNFα in HIV seropositive female subjects at the follicular phase when compared with the luteal phase is inconsistent with findings in healthy women. It has been documented that in apparently healthy women, TNFα is generally higher at the luteal phase during which the population of activated monocytes, which secret TNFα, are reported to be higher (Marthur *et al*., 1979; Northern *et al*., 1994; Brannstrom *et al*., 1999). This is probably due to the fact that the luteal phase is the time when fertilization takes place and the fertilized ovum requires protection from infective agents hence the increased pro- inflammatory activity (Norman, 2001).

The significantly higher level of IL-6 at the luteal phase compared with the follicular phase in the present study is comparable with the previous reports done in developed countries (Konecna *et al*., 2000). IL-6 is said to stimulate B-lymphocytes (thereby enhancing humoral immunity) and T-lymphocytes differentiation and activate macrophages and NK- cells (thereby enhancing cellular immunity). It is also said to possess antinflammatory properties (Marijke *et al*., 2011). Since IL-6 production is said to be decreased by estrogen, the low estrogen production associated with hypogonadism in HIV subjects result in increased IL-6 production since sex hormones perform immunoregulatory functions.

Reports of IL-6 levels during the menstrual cycle phases have been controversial. Whereas Angstwurm *et al*., (1997) reported higher levels of IL-6 during the follicular phase of menstrual cycle, Abrahamsen *et al*., (2003) reported no difference while Schwarz *et al*., (2000) reported decreased IL-6 in the follicular phase during the menstrual cycle. It has been reported that during the luteal phase of menstrual cycle, the immune response is shifted toward a Th2-type response and this was found to correspond to increased levels of progesterone and 17β-E_2 (Marijke *et al*., 2000). This account for the anti-inflammatory immune response associated with IL-6 production at this phase and reduced cellular immunity.

The significantly high levels of IL-8, IL-6, IL-4 and TNFα in HIV seropositive females and HIV seropositive females on ART compared to Control females at both follicular and luteal phases of menstrual cycle also showed higher degree of inflammation in diseased subjects when compared to healthy people. Excessive production of some cytokines for instance TNFα has been associated with problems such as fever or even tumor formation hence certain neoplastic conditions such as Karposi sarcomas are said to be common in chronic HIV patients (Locksley *et al*., 2001). The increased IL-4 may be due to antiiflammatory effects of estrogen thereby promoting Th2 immune response. Moreover, HIV is a chronic inflammatory disease and is said to induce a Th-1type immune responses which are characterized by the production of pro-inflammatory cytokines some of

which have been associated with harmful effects such as fever and tumors formation (Chowdbury *et al*., 2010). The implications of elevated cytokines on endocrine and metabolic functions have been previously reported (Hashimoto *et al*., 1994; Gartner, 2009) and this has far reaching effects on menstrual and reproductive functions of the affected women (O' Brien *et al*., 2007).

However, the significantly reduced levels of IL-8, IL-6, IL-4 and TNFα in HIV seropositive females on ART compared to their counterparts without treatment showed significant reduction of inflammation and perhaps some level of improvement in the reproductive function in these subjects due to a reduction in viral load (AL-Harthi *et al*., 2001). This signifies beneficial effects of treatment which results in significant restoration of the cellular immunity and reduction of inflammation in these patients. This has been previously reported (Sachdeva *et al*., 2010).The fluctuations in the levels of cytokines have been reported to be related to hypogonadal function as has been discussed previously (Marijke *et al*., 2000).

The significantly increased levels of the anti inflammatory cytokine (IL-4) in HIV seropositive females on ART especially at the luteal phase were consistent with the value observed in Control females. These signify a reduction or modulation of inflammatory activities and humoral immunity which is a Th2 type of response. IL-4 is also secreted by activated immune cells such as monocytes. It has been reported that variations in progesterone and estrogen (sex hormones) induced by HIV infections drive the immune response to either a Th1 type or Th2 type response depending on the concentration of these hormones. This leads to the exaggeration of either pro-inflammatory cytokines such as TNFα or anti-inflammatory cytokines such as IL-4 (Marijke *et al*., 2000). It has also been reported that cytokines are elevated in systemic diseases and this can directly inhibit the ovary in females (Marijke *et al*., 2000).

The present study therefore, concludes that there were cytokine changes with some degrees of inflammatory reactions in HIV infected females.

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