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Possible Impact of Thyroid Dysfunction on Menstrual Cycle in HIV/TB Co-infected Females in NAUTH, Nnewi, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author RUN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NUS and NMI managed the analyses of the study. Authors COC, EAJ and AIL managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Thyroid dysfunction is among the commonest endocrinopathies in HIV as well as Tuberculosis infection and can pose unique consequences on women's reproductive health.
Aim of Study: The present study aimed to evaluate the impact of thyroid dysfunction on menstrual

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cycle of HIV/TB Co-infected females in Nnamdi Azikiwe University Teaching Hospital Nnewi, Nigeria.

Materials and Methods: A total of 210 reproductive aged women (15-45 years) were randomly recruited for the study and grouped into: (i) Symptomatic HIV females (n=30); (ii) Symptomatic HIV females on ART (n=30); (iii) Symptomatic TB females (n=30); (iv) Symptomatic TB females on ATT (n=30); (v) Symptomatic HIV/TB females (n=30), (vi) Symptomatic HIV/TB females on therapy (n=30); and (vii) Control females (n=30). After due consent, blood samples were collected at the follicular (Fp) and luteal phases (Lp) of their menstrual cycle for determination of thyroid indices (FT3 (ng/ml), FT4 (µg/dl, TSH (µIU/ml) using enzyme-linked immunosorbent assay (ELISA) method, CD4+ T-cells (/µl) using Cyflow SL Green Cytometer.

Results: There was significantly lower FT3 but significantly higher TSH values in Symptomatic HIV and Symptomatic HIV/TB females and significantly lower FT3 with normal TSH values in Symptomatic TB compared respectively with Control females at both phases of menstrual cycle (*P=.05*). CD4 T-cells counts was significantly lower in all test groups compared to Control females at both phases of menstrual cycle (*P=.05*) and significantly higher in Symptomatic HIV on ART, TB on ATT and HIV/TB females on treatment compared to their counterparts not on therapy (*P=.05* respectively). The thyroid indices showed hypothyroidism in Symptomatic HIV females and Symptomatic HIV/TB females while euthyroid sick syndrome was observed in Symptomatic TB females with significant drop in CD4 T-cells.

Conclusions: The study showed significant derangement in thyroid indices and CD4 T-cells in all the study subjects. Early diagnosis and treatment of thyroid dysfunction is beneficial.

Keywords: HIV; TB; Co-infection; menstrual cycle; thyroid dysfunction; CD4 T-cells.

1. 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 [1,2]. Reports have shown considerable evidence that the rate at which HIV infection progresses in women is different from that in men [3,2,4]. In Nigeria, about 3.1 million people are living with HIV/AIDS while 58% (1.72 million) are females mostly within reproductive age range (15-45 years) [5, 2]. In Nigeria HIV is a leading cause of morbidity and mortality among women of reproductive age [5,6]. The prevalence of HIV among young women aged 15-24 years was reported as three times higher than among men of the same age because HIV generally is easily transmitted from men to women [5].

Large numbers of women of childbearing age become infected with HIV and the infection is transmitted to their babies during pregnancy. Though more than 80% of HIV transmission in Nigeria is through heterosexual intercourse, HIV prevalence is highest among commercial sex workers [5].

HIV infection increases the incidence of extra pulmonary TB (EPTB) especially genital tuberculosis which impacts negatively on menstrual and reproductive function [7,8]. In Nigeria, rate of infertility was 4% to 70% [9,10], while higher rates of infertility in HIV and or TB infected female subjects have been reported in other African countries [11,12]. Therefore, HIV/TB co-morbidity calls for serious concerns since it may have synergistic or antagonistic effects on the reproductive system. HIV and TB infections separately and independently have been known to impact negatively on women's reproductive health [13]. The negative reproductive impact ranges from menstrual disorders and failure of reproductive function.

Thyroid function in HIV-1 and TB individuals has been a special area of concern due to reports of thyroid function abnormalities in these patients. Reports have shown that HIV-1 infection by itself is associated with thyroid abnormalities and that some antiretroviral (ARV) medications may also be related. Thyroid dysfunction may occur as the result of immune system reconstitution [14,15] and are associated with other viral infections in HIV-1 patients, tuberculosis and hepatitis C virus included [16,17].

The thyroid hormone enhances mitochondrial oxidation, and thus, augments metabolic rate. This effect on metabolic rate is probably responsible for the association between the thyroid hormone and respiratory drive [18]. The

terms “Non-thyroidal Illness Syndrome (NTIS)” and “Euthyroid Sick Syndrome (ESS)” have been used to describe alterations in thyroid function tests in critical illness, such as starvation, sepsis, surgery, myocardial infarction, and also in chronic, systemic diseases including chronic heart failure, chronic liver or hematologic diseases, cancer, diabetes, connective tissue diseases and chronic obstructive pulmonary diseases [19].

The advent of antiretroviral therapy (ART) and anti-tuberculosis therapy (ATT) has revolutionized HIV and TB management thereby raising the hope for effective management of both infections. Since the reproductive function is not immuned to HIV infection, the present study sets to investigate the thyroid status of females presenting with HIV/TB co- morbidities during menstrual cycle in Nnewi, Anambra State, Nigeria.

2. MATERIALS AND METHODS

2.1 Subjects

A total of 210 premenopausal females aged between 15 and 45(30±15) years were recruited for the study. The participants consist of 30 apparently healthy females who were recruited amongst the hospital staff using a cluster random sampling method which served as Control group while the remaining participants were also randomly recruited at Heart to Heart center, HIV clinic and Direct Observed Therapy clinic of Nnamdi Azikiwe University Teaching Hospital Nnewi which served as Test subjects. All the participants were screened for HIV and TB and were classified into the following groups using WHO and CDC criteria for HIV and TB staging (i) Symptomatic HIV infected participants (n=30); (ii) Symptomatic HIV infected participants on ART (n=30); (iii) Symptomatic TB infected participants (n=30); (iv) Symptomatic TB infected participants on ATT (n=30); (v) Symptomatic HIV/TB infected participants (n=30); Symptomatic HIV/TB Co-infected participants on Therapy (n=30) who have been on therapy for not less than six months.

A well-structured questionnaire was administered to each participant to ascertain the history of their menstrual cycle, reproductive history and other biodata. Routine investigations for *Mycobacterium tuberculosis* were done using concentrated sputum for microscopy and Zielh Neelson staining techniques for AFB. Chest x-ray examination results and and results of Laparoscopic and *M. tuberculosis* Polymerase Chain Reaction Studies of participants who have been placed on ATT before the commencement of the study were obtained from their respective EPI data files for confirmation of pulmonary tuberculosis and other data.

2.2 Blood Sample Collection

Six ml of blood sample was collected from each participant at follicular (7-13th day) and at luteal (21-23rd day) phases of menstrual cycle. The blood sample was collected between 8 to 10am by venepuncture. Four ml was dispensed into dry plain bottles and allowed to clot, retracted and centrifuged. The serum was separated from the clot immediately and transferred into the well labeled container and stored frozen at -20^{0}c until assayed for hormones (FT3, FT4, TSH). The remaining two mls of blood was dispensed into EDTA bottles and was used immediately for malaria parasite screening HIV screening and confirmation, CD4+ T-Cell count and absolute lymphocyte count.

2.3 Ethical Clearance and Informed Consent

The Ethics Committee of Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra state, Nigeria approved the study design. The participants were informed about the study design and only those who gave their consent were recruited for the study. The informed consent form was written and approved along with the ethical clearance obtained from the ethics committee board of NAUTH Nnewi. The consent form was issued with the questionnaire during recruitment which only those who agreed and volunteered to participate signed before their blood samples were collected. All the participants recruited were assured that information obtained from them would be treated with uttermost confidentiality and they had the full right not to participate in or withdraw from the study at any point they desired to. The ethics committee approval included that of individuals from 15 years to 45 years and the written informed consent for participant between 15 and 18 years was obtained from their parents/guardians.

2.4 Exclusion and Inclusion Criteria

Participants with HIV Stage-1 (Asymptomatic HIV), HIV Stage –3 and 4 were excluded from the study. Only those adjudged as symptomatic-

HIV (stage-2) were included in the study. Participants with malaria parasite infection as at the time of study were also excluded. Participants with extra pulmonary tuberculosis were excluded and subjects with known fertility problems before contracting HIV infections were also excluded. Women on any contraceptives were also excluded. Hence the female participants used were those with no prior fertility problems until the existence of HIV.

2.5 Drug Administration for HIV/TB Infection

HIV infected patients with TB co-infection received antiretroviral therapy concurrently with anti- tuberculosis therapy. The drug regimen comprised of (1) two months intensive therapy and 4 months continuation therapy and the combination of drugs were as follows (RHZE):

> Rifampicin (R) 150 mg + isoniazid (H) 75 mg + pyrazinamide (Z) 400 mg + ethambutol (E) 275 mg orally once daily for 2 months at dosage according to the patient's body weight.
>
> For continuation therapy, rifampicin and isoniazid (RH) were given orally once daily for 4 months.

2.6 Methods

Antibodies to HIV-1 and HIV-2 in Human Plasma were detected using Abbott Determine 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.

Examination of Sputum for AFB by Ziehl Neelson method and Culture Methods for the determination of Mycobacterium Tuberculosis using Lowenstein Jensen medium technique as described by chessbrough [20].

Determination of Free Triiodothyronine, Free Thyroxine and Thyrotropin (FT3, FT4, TSH) were determined using a commercially available "second generation" enzyme-linked immunosorbent assay (ELISA) kits (Glory Science Co., Ltd, USA) as described by Chopra et al. [21]. Intra-assay and inter-assay coefficients of variation were <6% and <10%, respectively, which had a range of measurement from 0.8-2.0 ng/ml for tT3 and 4.5-12.5 µg/dl for T4. The measurement range for TSH was from 0.5-4.7 µIU/ml.

CD4+ t-cell count was done by the Becton Dickinson FACS flow cytometer.

2.7 Statistical Analysis

The version 16 of SPSS package was used in statistical analysis. The variables were expressed as mean (±SD). The Student independent 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. The Spearman's correlation coefficient was used to assess the level of association between two variables. The level of significance was considered at *P=.05*.

3. RESULTS

3.1 Thyroid Hormones (FT3) at Follicular and Luteal Phases of Menstrual Cycle

The mean (±SD) serum FT3 level (ng/ml) in Symptomatic HIV, Symptomatic HIV females on ART, Symptomatic TB females, Symptomatic TB females on ATT, Symptomatic HIV/TB females and Symptomatic HIV/TB females on treatment was not significantly different between follicular (0.73±0.37, 1.11±0.30, 0.68±0.24, 0.93±0.16, 0.59±0.15, 0.93±0.33) and luteal (0.65±0.29, 0.95±0.31, 0.67±0.25, 0.92±0.23, 0.62±0.16, 1.02±0.34) phases of menstrual cycle (*P>.05* respectively). In Control female subjects, there was no significant difference in the mean serum FT3 concentration (ng/ml) between follicular (1.01±0.48) and luteal (1.03±0.36) phases of menstrual cycle (*P>.05*).

When the mean FT3 concentration (ng/ml) at follicular and luteal phases of menstrual cycle were compared between the Control group and Test groups, the mean FT3 dropped significantly in Symptomatic HIV females (0.73±0.37, 0.65±0.29), Symptomatic TB females (0.73±0.32, 0.69±0.25), and Symptomatic HIV/TB females (0.59±0.15, 0.62±0.16) compared with the follicular and luteal values in Control female subjects (1.01±0.48, 1.03±0.36) (*P=.05* respectively).

The post hoc analysis showed significant drop in the mean FT3 value (ng/ml) at follicular and luteal phases of menstrual cycle in Symptomatic HIV females (0.73±0.37, 0.65±0.29) and Symptomatic HIV/TB females (0.59±0.15, 0.62±0.16) compared with follicular and luteal values in the Symptomatic HIV females on ART

(1.11±0.30, 0.95±0.31) and Symptomatic HIV/TB on treatment (0.93±0.33, 1.02±0.34) (*P=.05* respectively). The post hoc analysis showed significantly higher mean FT3 value (ng/ml) at follicular and luteal phases of menstrual cycle in Symptomatic HIV/TB females on treatment (0.93±0.33, 1.02±0.34) compared with follicular value in Symptomatic HIV females (0.73±0.37, 0.65±0.29) and Symptomatic HIV/TB females (0.59±0.15, 0.62±0.16) (*P=.05* respectively). Furthermore, the mean FT3 concentration (ng/ml) at follicular and luteal phases of menstrual cycle was significantly higher in Symptomatic TB females on ATT (0.93±0.16, 0.92±0.23) and Symptomatic HIV/TB females on treatment (0.93±0.33, 1.02±0.34) compared with follicular and luteal values in Symptomatic TB females (0.68±0.24, 0.62±0.16) and Symptomatic HIV/TB females (0.59±0.15) (*P=.05* respectively) (See Fig. 1).

3.2 Thyroid Hormone (FT4) at Follicular and Luteal Phases of Menstrual Cycle

The mean (±SD) serum FT4 concentration (µg/dl) in Symptomatic HIV females, Symptomatic HIV females on ART, Symptomatic TB females, Symptomatic TB females on ATT and Symptomatic HIV/TB females on treatment was not significantly different between follicular (7.83±2.51, 7.79±1.66, 8.58±1.77, 6.51±1.48, 7.49±1.45) and luteal (8.71±2.20, 7.61±2.31, 7.71±1.48, 7.42±1.33, 7.40±1.38) phases of menstrual cycle (*P>.05* respectively). But The mean (±SD) serum FT4 concentration (µg/dl) in Symptomatic HIV/TB female subjects was significantly lower at follicular phase (5.81±1.60) compared with luteal phase (8.35±3.03) of menstrual cycle (*P=.05*). In Control female subjects, there was no significant difference in the mean FT4 concentration (µg/dl) between follicular (7.86±1.68) and luteal (7.11±2.03) phases of menstrual cycle (*P>.05*).

When the mean FT4 concentration (µg/dl) at follicular phase of menstrual cycle were compared between the Control group and Test groups, the mean FT4 dropped significantly in Symptomatic TB females on ATT (6.51±1.48) and Symptomatic HIV/TB females (5.81±1.60) Compared with follicular value in the Control female subjects (7.86±1.68) (*P=.05* respectively).

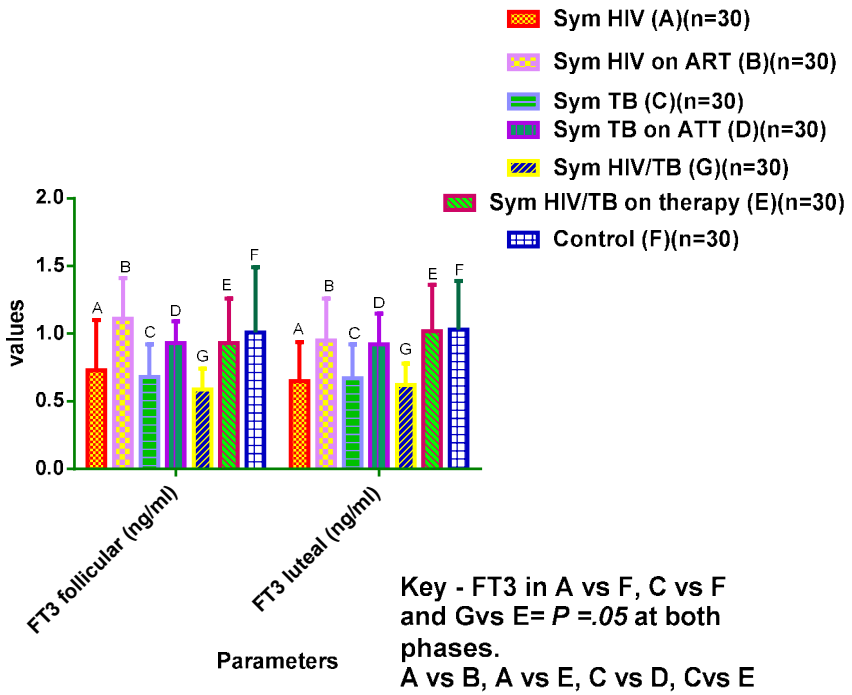


Fig 1: Comparison of mean (±SD) serum levels of FT3 in Test groups and Control group at Follicular and luteal phases of menstrual cycle

The post hoc analysis showed significant drop in the mean FT4 concentration (µg/dl) at follicular phase of menstrual cycle in Symptomatic TB females on ATT (6.51±1.48) and in Symptomatic HIV/TB females on treatment (7.49±1.45) compared with follicular value in Symptomatic TB females (8.58±1.77) (*P=.05* respectively) (See Fig. 2).

3.3 Thyroid Hormone (TSH) at Follicular and Luteal Phases of Menstrual Cycle

The mean (±SD) serum TSH level (µIU/ml) in Symptomatic HIV females, Symptomatic TB females, Symptomatic TB females on ATT, Symptomatic HIV/TB female and Symptomatic HIV/TB females on treatment was not significantly different between follicular (4.17±3.65, 0.90±0.43, 0.85±0.52, 3.71±3.12, 3.58±3.01) and luteal (4.56±1.17, 0.93±0.35, 1.48±0.51, 4.81±3.96, 1.74±0.18) phases of menstrual cycle (*P>.05* respectively). On the contrary, the mean serum TSH value (µIU/ml) was significantly higher at follicular phase (3.26±1.88) compared with luteal phase (1.24±0.39) of menstrual cycle in Symptomatic HIV females on ART (*P=.05*). In Control female subjects, There was no significant difference in the mean TSH value (µIU/ml) between follicular (1.32±0.49) and luteal (1.40±0.53) phases of menstrual cycle (*P>.05*).

When the mean TSH concentration (µIU/ml) at follicular and luteal phases of menstrual cycle were compared between the Control group and Test groups, the mean TSH was significantly higher in Symptomatic HIV females (4.17±3.65, 4.56±1.17) and Symptomatic HIV/TB females (3.71±3.12, 4.81±3.96) compared with follicular value in the Control female subjects (1.32±0.49, 4.81±3.96) (P=.05 respectively) while TSH was significantly higher only at follicular phase in Symptomatic HIV females on ART (3.26±1.88) and Symptomatic HIV/TB females on treatment (3.58±3.01) compared with follicular value in the Control female subjects (1.32±0.49) (*P=.05*).

The post hoc analysis dropped significantly in the mean TSH value (µIU/ml) at luteal phase of menstrual cycle in Symptomatic HIV females on ART (1.24±0.39) and Symptomatic HIV/TB females on treatment (1.74±0.18)

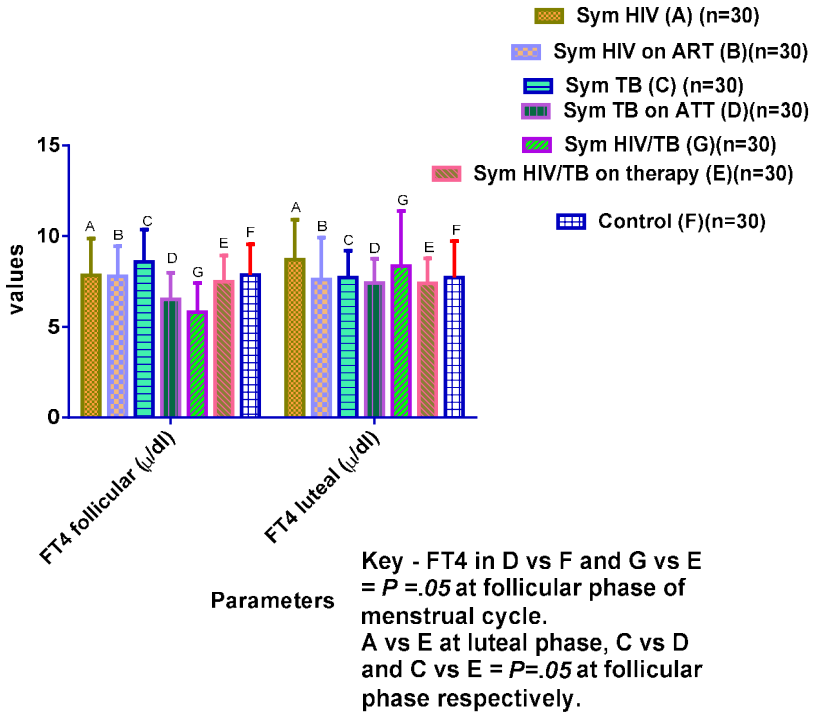
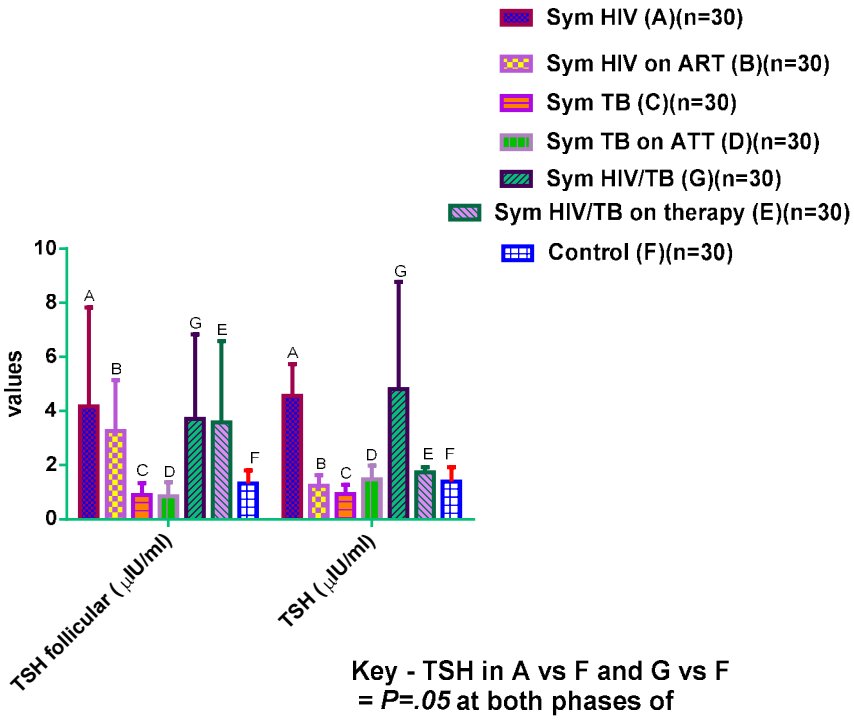


Fig 2: Comparison of mean (±SD) serum levels of FT4 in Test groups and Control group at Follicular and luteal phases of menstrual cycle

compared with Symptomatic HIV females (4.56±1.17) and Symptomatic HIV/TB females (4.81±3.96) (*P=.05* respectively). The post hoc analysis showed significantly higher mean TSH value (µIU/ml) at luteal phase of menstrual cycle in Symptomatic TB females on ATT (1.48±0.51) compared with Symptomatic TB females (0.93±0.35) (*P=.05*). The post hoc analysis also showed significantly higher mean TSH at follicular phase of menstrual cycle in Symptomatic HIV/TB females on treatment and Symptomatic HIV/TB females (3.58±3.01, 3.71±3.12) compared with Symptomatic TB female subjects (0.90±0.43) (*P=.05* respectively). There was significantly higher mean TSH at luteal phase of menstrual cycle in Symptomatic HIV/TB females (4.81±3.96) compared with Symptomatic TB female subjects (0.93±0.35) (*P=.05*) (See Fig. 3).

3.4 CD4+ T-Cells Counts at Follicular and Luteal PHASES of Menstrual Cycle

The mean (±SD) CD4+ T-cell count (/µl) in Symptomatic HIV females, Symptomatic HIV females on ART, Symptomatic TB females, Symptomatic TB females on ATT, Symptomatic HIV/TB female was not significantly different between follicular (211±88, 403±100, 217±93, 387±114, 107±77, 299±112) and luteal (231±96, 389±105, 212±97, 367±136, 122±69, 266±124,) phases of menstrual cycle (*P>.05* respectively). The mean CD4+ T-cell count (/µl) was not significantly different between follicular (689±172) and luteal (660±157) phases of menstrual cycle in Control female subjects (*P>.05*).



Key - TSH in A vs F and G vs F = *P=.05* at both phases of menstrual cycle. B vs F and E vs F = *P=.05* at follicular phase.
A vs B, A vs E and C vs D and G vs E = *P=.05* at luteal phase respectively.
C vs E and D vs E = *P=.05* at follicular phase.
TSH in B = *P=.05* between follicular and luteal phases of menstrual cycle.

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

When the mean CD4+ T-cell count (/µl) at follicular and luteal phases of menstrual cycle were compared between the Control group and Test groups, the mean CD4+ T-cell count dropped significantly in Symptomatic HIV (211±88, 231±96), Symptomatic HIV females on ART (403±100, 389±105), Symptomatic TB (217±93, 389±105), Symptomatic TB females on ATT (387±114, 389±105), Symptomatic HIV/TB females (107±77, 122±69) and Symptomatic HIV/TB females on treatment (299±112, 266±124) compared with follicular and luteal values in the Control female subjects (689±172, 660±157) (*P=.05* respectively).

The post hoc analysis showed significantly higher mean CD4+ T-cell count (/µl) at follicular and luteal phases of menstrual cycle in Symptomatic HIV females on ART (403±100, 389±105) and Symptomatic HIV/TB females on treatment (299±112) compared with follicular and luteal values in the Symptomatic HIV female subjects (211±88, 231±96) and Symptomatic HIV/TB females (107±77, 122±69) (*P=.05* respectively).

The post hoc analysis showed significantly higher mean CD4+ T-cell count (/µl) at follicular and luteal phases of menstrual cycle in Symptomatic TB females on ATT (387±114, 367±136) and Symptomatic HIV/TB females on treatment (299±112, 299±112) compared with follicular value in Symptomatic TB females (217±93, 212±97) and Symptomatic HIV/TB females (107±77, 122±69) (*P=.05* respectively). The post hoc analysis showed significant drop in the mean CD4+ T-cell count (/µl) at follicular and luteal phases of menstrual cycle in Symptomatic HIV/TB females on treatment (299±112, 266±124) compared with follicular value in Symptomatic TB females on ATT (387±114, 367±136) (*P=.05* respectively) (See Fig. 4).

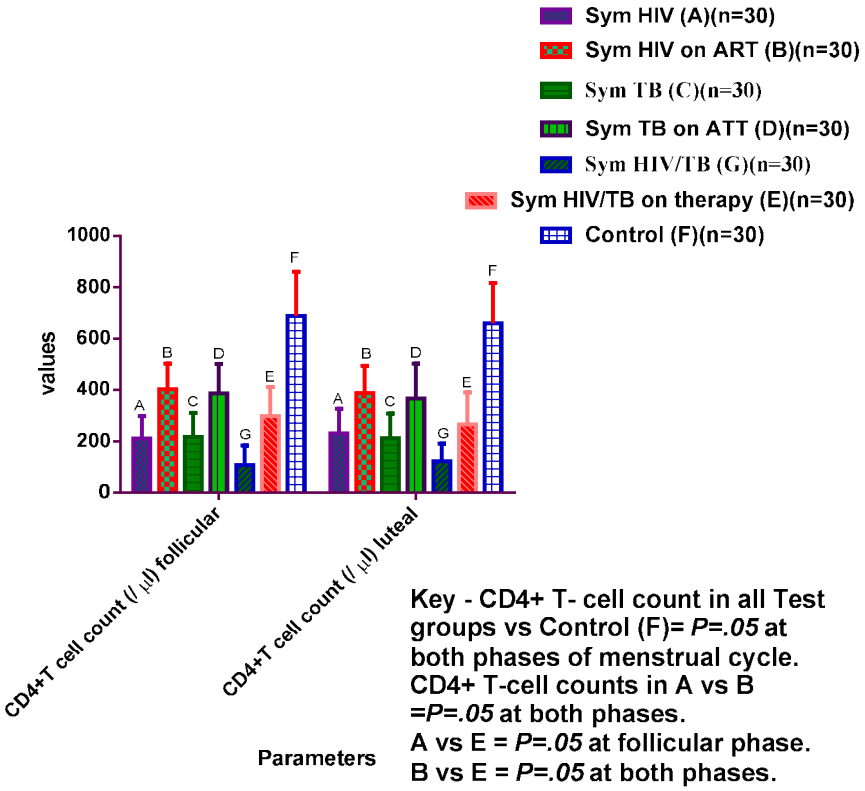


Fig 4: Comparison of mean (±SD) concentrations of of CD4+ T-cell counts in Test groups and Control group at Follicular and luteal phases of menstrual cycle

4. DISCUSSION

The insignificant difference in the level of thyroid parameters (FT3, FT4 and TSH) observed in Symptomatic HIV, Symptomatic HIV females on ART, Symptomatic TB females, Symptomatic TB females on ATT, Symptomatic HIV/TB females and Symptomatic HIV/TB female subjects on treatment between follicular and luteal phases of menstrual cycle suggests that the impact of drugs on thyroid function was not enough to return the subjects back to the predisease states However, the significantly lower level of FT3 with higher level of TSH observed in Symptomatic HIV females and Symptomatic HIV/TB female subjects may suggest hypothyroidism possibly as result of HIV infection. Previous reports in developed countries and in general population has been documented [22,23,24,25]. The thyroid hormone plays a vital role in all physiological activities in humans including menstrual functions in females. Increased thyroid function (hyperthyroidism) may lead to premature menstruation or precocious puberty, menorrhagia or hypermenorrhea whereas reduced thyroid function (hypothyroidism) may lead to delayed menstruation or oligomenorrhoea and pregnancy loss [25,26]. This has been attributed to the connection between thyroid hormone levels and the menstrual cycle which is mainly mediated by thyrotropin-releasing hormone (TRH), which has a direct effect on the ovary. Additionally, abnormal thyroid function can alter levels of sex hormone-binding globulin, prolactin, and gonadotropin-releasing hormone, contributing to menstrual dysfunction. For example, increased levels of TRH may raise prolactin levels, contributing to the amenorrhea associated with hypothyroidism [27].

The significantly lower level of FT3, FT4 with higher levels of TSH in Symptomatic HIV/TB suggests significant hypothyroidism. HIV and TB are two chronic infections that seem to potentiate each other. The endocrine effects of the two diseases became more pronounced in a situation of co-morbidity hence the hypogonadism and hypothyroidism reported in the two illnesses above became more severe suggesting that HIV/TB co-infection had greater impact on endocrine function than either of the infections.

The insignificant variation in the level of thyroid hormones in Symptomatic HIV/TB female subjects on drugs suggests a level of stability which ART and ATT conferred on those patients. However, prolonged use these drugs (ART and ATT) have been associated with endocrine dysfunction [28,29,30,31] as discussed previously.

The insignificant difference in the levels of FT3 and TSH observed in Symptomatic HIV female subjects on ART indicates a significant impact of treatment on these patients and possible reduction on the incidence of hypothyroidism or euthyroid syndrome. However, prolonged use of ART has been associated with thyroid abnormalities [32,33] and this was attributed mostly to the use of protease inhibitors. However, the present study was carried out in women within their reproductive age at different phases of menstrual cycle and did not observe this drug effects. It is important to mention that the limitation of this study includes that when stratifying in so many groups, the size of individuals in each group is very small, as well as the number of controls. Some studies have also reported that HIV patients on HAART experienced a kind of immune reconstitution [34,35] which may be associated with Hashimoto's thyroiditis [36]. Hashimotos disease is another known cause of hypothyroidism.

On the other hand, the significantly lower levels of FT3 with normal TSH observed in Symptomatic TB female subjects at both follicular and luteal phases of menstrual cycle shows a state of euthyroid sick syndrome which is often associated with chronic systemic illnesses including tuberculosis. This has been reported in previous findings [37,38]. It has been reported that during prolonged infections, the blood levels of selenium, T3, T4 and TSH may decrease and the conversion of T4 to T3 slows down thus inducing a hypothyroid state [39]. It has also been reported that pro-inflammatory cytokines especially IL-6 produced during TB infection suppressed T3 activity thereby inducing hypothyroidism in affected individuals [40]. The insignificant difference in the level of FT3, FT4 and TSH between Symptomatic TB female subjects on ATT and Control female subjects showed that treatment had some positive impact on thyroid function of these subjects. This implies a reduction in the incidence of thyroid abnormality in affected subjects. This will have a corresponding effect on menstrual and reproductive function. However, it has been reported that some of the drugs used in the treatment of multidrug-resistant tuberculosis (MDR-TB) could induce hypothyroidism in some patients [30,41,31].

The thyroid abnormalities observed in Symptomatic HIV and TB female subjects individually became more manifest in a state of co-morbidity (HIV/TB). This implies a worsening state of hypothyroidism. HIV and TB are two chronic infections that seem to potentiate each other. The endocrine effects of the two diseases became more pronounced in a situation of co-morbidity hence the increased incidence of hypogonadism and hypothyroidism as reported above. HIV/TB co-morbidity will certainly have greater impact on endocrine function than either of the infections alone. The increased incidence of hypothyroidism observed in HIV and TB infected females in the present study may be due to low CD4+T-cell counts as a result of increased incidence of opportunistic infections. In a recent study, incidence of opportunistic infections has been documented as an independent risk factor for decreased thyroid function [42]. The immunosuppression associated with HIV infection might make extra pulmonary tuberculosis more likely in co-infected female subjects thereby leading to gonadal and thyroid TB. This has direct effects on these endocrine organs leading to reduction in function. The implication of this is that the incidence of menstrual and reproductive abnormalities associated with either of the diseases will be increased if appropriate treatment is not administered in time.

The significantly reduced level of CD4 T-cell count in Symptomatic HIV females, Symptomatic HIV females on ART, Symptomatic TB females, Symptomatic TB females on ATT, Symptomatic HIV/TB females and Symptomatic HIV/TB female on treatment compared to Control females at both follicular and luteal phases of the menstrual cycle signify a reduction in cellular immunity which is the hallmark of HIV and TB infections. This has been previously reported [43,44,45]. Cellular immunity involving CD4+T-cells plays a major role in tuberculosis infection [46,47] and loss of CD4+ T-cells was associated with increased susceptibility to TB [48]. However, it has been postulated that CD4 T-cells could promote rather than control tuberculosis in the absence of PD-1 (protein derivative mediated inhibition) [49].

The significantly high levels of CD4+ T- cell count in Symptomatic HIV females on ART, Symptomatic TB females on ATT and Symptomatic HIV/TB female subjects on treatment compared to their counterparts without treatment indicates improvement in immune functions showing the benefits of the treatment and some levels of restoration in cellular immunity in these patients. This may be probably due to significantly reduced viral load in the affected subjects. Hormonal levels in such subjects tend to improve. The tendency is for any pre-existing hormonal imbalance to normalize thereby correcting any existing menstrual and reproductive abnormality. However, menstrual disorders have been reported in subjects after prolonged treatment with HAART [50,51]. Such menstrual irregularities were linked to the use of protease inhibitors.

5. CONCLUSION

From the foregoing, HIV and TB infections exert significant thyroid and cellular immune changes in affected women and may potentiate each other thereby increasing the severity of these double infections in the affected women. This may produce menstrual abnormalities which may affect the reproductive potentials of these women. Further studies are required to explain some of these changes with a view to tackling effectively the challenges raised by the co-morbidity.

CONSENT

All authors hereby declare that all written informed consent was obtained from all the patients who participated in this study.

ETHICAL APPROVAL

All authors hereby declare that all experiment and procedure have been examined and approved by the appropriate board of ethics committee of Nnamdi Azikiwe University Teaching Hospital Nnewi, South East Nigeria, and research have therefore been performed in accordance with the standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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