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Evaluation of Thyroid Hormones Changes and CD4+ T-cell Count during Menstrual Cycle in Pulmonary TB Infected Women in Nnewi, Nigeria

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Authors' contributions

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

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ABSTRACT

Background: Tuberculosis remains a disease of major public health importance worldwide including Nigeria. Endocrine abnormalities have been reported among Tuberculosis patients with the thyroid inclusive.

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Aims of Study: The present study was designed to evaluate the thyroid hormones changes and CD4+ T-cell count during menstrual cycle in women infected with Pulmonary Tuberculosis in Nnamdi Azikiwe University Teaching Hospital, Nnewi, south east Nigeria.

Materials and Methods: The study comprised 90 premenopausal females aged 15-45 years randomly recruited at Direct Observed Therapy (DOT) Clinic at NAUTH, Nnewi from 2013-2014. 30 participants were Symptomatic TB infected females who were not yet on drugs at the time of sample collection while 30 were Symptomatic TB infected females on Anti tuberculosis therapy. The remaining 30 were apparently healthy hospital staff with regular menstrual cycle. After due consent, a detailed medical history was obtained and routine investigations of pulmonary tuberculosis and confirmation using Ziehl Neelsen and sputum culture techniques for AFB and chest x-ray were done. Blood samples were collected at follicular and luteal phases of their menstrual cycle for analysis of Thyroid hormones (T3, T4 and TSH) using Enzyme Linked Immunosorbent Assay (ELISA) method. CD4 T-cell counts was determined using cyflow SL Green Cytometer.

Results: Result showed significantly lower T3 and CD4 T-cells with normal TSH values in Symptomatic TB compared with control females at both phases of menstrual cycle (*P=.05* respectively). TSH value was significantly lowered in Symptomatic TB than Symptomatic TB on ATT while T3 and CD4 T-cell count were significantly higher in Symptomatic TB on ATT compared to Symptomatic TB at both phases of menstrual cycle (*P=.05*).

Conclusion: Euthyroid sick syndrome was observed among Symptomatic TB females which showed some level of improvements with administration of ATT. A new national strategic plan for TB control is advocated to include routine thyroid function check with special attention paid to the reproductive function.

Keywords: Thyroid hormones; CD4+ T-cell count; menstrual cycle; pulmonary tuberculosis.

1. INTRODUCTION

Despite the advent of highly effective drugs, morbidity and mortality due to *Mycobacterium tuberculosis* are still on the increase in Africa, Nigeria inclusive. This continuous increase can be partly attributable to wide spread human immunodeficiency virus (HIV) epidemic [1,2]. Report estimated about 9 million new cases of TB worldwide and Nigeria comes third behind only India and China in terms of tuberculosis cases [3,4]. About 590,000 new cases of tuberculosis have also been reported in Nigeria with only 91,354 cases placed on treatment [3,4]. This was attributed to the negative effects of the interactions between TB and HIV with increase in Multi-Drug Resistant tuberculosis (MDR-TB) [3].

Tuberculosis is a chronic infectious disease transmitted through air which puts everyone at risk of being infected [3]. Tuberculosis can affect the body systems including the reproductive system in females, which can lead to altered levels of thyroid hormones in the affected individuals [5]. Cytokines such as IL-6 and TNFα can acutely decrease TSH and T3 and increase rT3. HIV infection increases the incidence of extra pulmonary TB (EPTB) especially genital tuberculosis which impacts negatively on menstrual and reproductive function [6,7]. Genital tuberculosis is said to occur in 10% cases of pulmonary tuberculosis [8]. Tuberculosis, whether pulmonary or extra pulmonary has been known to affect the menstrual cycle. Hassan and Darwish [9] reported a prevalence rate of 66% menstrual abnormalities in women with pulmonary tuberculosis. Most of these women (76%) reverted to normal menstruation following anti TB treatment. Of all the menstrual abnormalities reported, secondary amenorrhoea constituted 26.5%, while hypomenorrhoea was 20%. Similar reports have been documented [10,11]. When menstrual abnormalities associated with tuberculosis fail to disappear after anti-TB treatment the possibility of having genital TB becomes very high. Female genital TB is usually an asymptomatic or latent infection and often undiagnosed unless specifically searched for in the course of investigating female infertility and other reproductive problems [12].

In Nigeria, the rate of infertility has been found to vary from 4% to 70% [13,14]. The present study thus seeks to evaluate the changes in thyroid function activity and CD4 T-cell count during menstrual cycle in TB infected females.

2. MATERIALS AND METHODS

2.1 Subjects

A total of 90 premenopausal females aged between 15 and 45(30±15) years were recruited for the study. The participants comprised 30 apparently healthy females recruited amongst the hospital staff which served as Control group while the remaining participants were randomly recruited at Direct Observed Therapy clinic of Nnamdi Azikiwe University Teaching Hospital Nnewi which served as Test subjects. They were all screened for HIV and TB and were classified using WHO and CDC criteria for TB staging as Symptomatic TB infected females (n=30) and Symptomatic TB infected females on ATT (n=30) 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 *M. tuberculosis* were done using concentrated sputum for microscopy and Ziehl Neelsen staining techniques for AFB. Chest x-ray examination results 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 results of Laparoscopic and *M. tuberculosis* Polymerase Chain Reaction Studies.

2.2 Blood Sample Collection

Five 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 10 am by venipuncture and 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℃ until assayed for hormones (T3, T4 and TSH). The remaining 2 mls of blood was dispensed into EDTA bottles and was used immediately for malaria parasite screening HIV screening, and confirmation and CD4+ T-Cell 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.

2.4 Exclusion and Inclusion Criteria

HIV/Malaria infected patients were excluded from the study. Also excluded were extra pulmonary tuberculosis patients. The female participants used were those with pulmonary tuberculosis and with no fertility problems prior to the time of contraction of tuberculosis.

2.5 Drug Administration for TB Infection

The drug regimen given to the participants 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. Continuation therapy includes rifampicin and isoniazid (RH) given orally once daily for 4 months. A category 2 regimen is 8months therapy for relapse and those that failed to respond to drug regimen above. Streptomycin was added for the first 2 months of a 3 months therapy followed by a continuation phase of 5months with rifampicin, isoniazid and ethambutol (RHE).

2.6 Methods

Determination of CD4+ T-Cells Count was done using Cyflow SL Green cytometer Determination of Total Triiodothyronine, Total Thyroxine and Thyrotropin (tT3, tT4 and 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. [15,16]. 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.

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 Mean (±SD) Thyroid Hormones (T3 (ng/ml), T4 (µg/dl), TSH (µlU/ml) at Follicular and Luteal Phases of Menstrual Cycle

Result showed that in Symptomatic TB females, Symptomatic TB females on ATT and Control female subjects, the mean serum T3 (0.68±0.24, 0.93±0.16, 1.01±0.48), T4 (8.58±1.77, 6.51±1.48, 7.86±1.68), TSH (0.90±0.43, 1.25±0.52, 1.32±0.49) at follicular phase were not significantly different when compared with corresponding values at luteal phase T3 (0.67±0.25, 0.92±0.23, 1.03±0.36), T4 (7.71±1.48, 7.42±1.33, 7.11±2.03), TSH (0.93±0.35, 1.48±0.51, 1.40±0.53) of the menstrual cycle (*P>.05* respectively).

The mean T3 at follicular and luteal phases of menstrual cycle dropped significantly in Symptomatic TB females (0.73±0.32, 0.69±0.25) compared with the corresponding values in Control female subjects (1.01±0.48, 1.03±0.36) (*P=.05* respectively).

Furthermore, the mean T3 concentration (ng/ml) at follicular and luteal phases of menstrual cycle was significantly higher in Symptomatic TB females on ATT (0.93±0.76, 0.92±0.23) compared with the corresponding values in Symptomatic TB females (0.68±0.24, 0.62±0.16) (*P=.05* respectively)

The mean T4 concentration at follicular phase of menstrual cycle dropped significantly in Symptomatic TB females on ATT (6.51±1.48) Compared with the corresponding value in Control female subjects (7.86±1.68) (*P=.05*).

The post hoc analysis showed significant drop in the mean T4 concentration (µg/dl) at follicular phase of menstrual cycle in Symptomatic TB females on ATT (6.51±1.48) compared with the corresponding value in Symptomatic TB females (8.58±1.77) (*P=.05*).

The post hoc analysis showed significantly higher mean TSH value (µIU/ml) at follicular and luteal phases of menstrual cycle in Symptomatic TB females on ATT (1.25±0.52, 1.48±0.51) compared with Symptomatic TB females (0.90±0.43, 0.93±0.35) (*P=.05*) (See Table 1).

3.2 CD4+ T-Cells Counts at Follicular and Luteal Phases of Menstrual Cycle

The mean (±SD) CD4+ T-cell count (/µl) in Symptomatic TB females, Symptomatic TB females on ATT and Control female subjects was not significantly different between follicular (217±93, 387±114, 689±172) and luteal (212±97, 367±136, 660±157) phases of menstrual cycle in (*P>.05* respectively).

The mean CD4+ T-cell count dropped significantly at follicular and luteal phases of menstrual cycle in Symptomatic TB (217±93, 212±97) and Symptomatic TB females on ATT (387±114, 367±136) when compared with values in Control group (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 TB females on ATT (387±114, 367±136) compared with follicular value in Symptomatic TB females (217±93, 212±97) (*P=.05* respectively) (See Table 2).

4. DISCUSSION

The significant difference in the level of thyroid parameters (T3, T4 and TSH) observed in Symptomatic TB females and Symptomatic TB females on ATT at follicular and luteal phases of menstrual cycle suggests that the treatment had some impact on thyroid function. This implies a reduction in the incidence of thyroid abnormality in affected subjects. This will have a corresponding effect on menstrual and reproductive function. The thyroid hormone plays a vital role in all physiological activities in humans including menstrual cycle 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 [17,18]. 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. Severe hypothyroidism

Table 1. Mean (+SD) serum levels of Thyroid Hormones in symptomatic TB, Symptomatic TB on ATT and Control female subjects at follicular and luteal phases of menstrual cycle

Parameters	T3 (ng/ml)		P-value	T4 (ug/dl)		P-value	TSH (uIU/ml)		P-value
Groups	**Follicular**	**Luteal**		**Follicular**	**Luteal**		**Follicular**	**Luteal**	
Symptomatic TB (A) (n=30)	0.68±0.24	0.67±0.25	0.847	8.58±1.77	7.71±1.48	0.053	0.90±0.43	0.93±0.35	0.832
Symptomatic TB on ATT (B) (n=30)	0.93±0.16	0.92±0.23	0.875	6.51±1.48	7.42±1.33	0.048	1.25±0.52	1.48±0.51	0.089
Control (C) (n=30)	1.01±0.08	1.03±0.16	0.764	7.86±1.68	7.71±2.03	0.768	1.32±0.49	1.40±0.53	0.794
F-value	9.441	16.151		9.447	0.265		3.067	5.894	
P-value	0.000	0.000		0.000	0.921		0.065	0.010	
AvsB	0.000	0.000		0.000	0.574		0.021	0.006	
AvsC	0.000	0.000		0.122	0.984		0.005	0.008	
BvsC	0.156	0.168		0.004	0.531		0.712	0.707	

is commonly associated with ovulatory dysfunction due to numerous interactions of thyroid hormones with the female reproductive system. Reports have shown that both hyperprolactinaemia due to increased TRH production, and altered GnRH pulsatile secretion could lead to delay in LH response and inadequate corpus luteum [19-21]. Thyroid responsivity by the ovaries could be explained by the presence of thyroid hormone receptors in human oocytes [22]. It has been established that thyroid hormones also synergize with the FSH-mediated LH/hCG receptor to exert direct stimulatory effects on granulosa cell function (progesterone production) [23].

However, the significantly lower levels of T3 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. 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 [24]. 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 [25].

The increased incidence of some degree of thyroid dysfunction observed in 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 [26]. This might lead to immunosuppression and might make extrapulmonary tuberculosis more likely in the infected female subjects thereby leading to gonadal and thyroid TB. Previous report shows that changes in CD4 T-cell counts correlated significantly with progesterone and estrogen concentration [27]. 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 this disease will be increased if appropriate treatment is not administered in time.

The present study reported significantly reduced level of CD4 T-cell count in Symptomatic TB females, and Symptomatic TB females on ATT compared to Control females at both follicular and luteal phases of the menstrual cycle. This signifies a reduction in cellular immunity which is the hallmark of TB and HIV infections [28,29]. Cellular immunity involving CD4+T-cells plays a major role in tuberculosis infection [30,31] and loss of CD4+ T-cells was associated with increased susceptibility to TB [32]. 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) [33].

However, The significantly high levels of CD4+ T- cell count in Symptomatic TB females on ATT 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 also improve the hormonal levels of such subjects. It has been shown that Cell-mediated immunity released effective control of M. tuberculosis [34]. In addition, CD8+ T cells, CD4+ T cells and CD1-restricted irregular T cells have also been reported to be particularly

Table 2. (±SD) blood concentrations of CD4+ T-Cells counts in symptomatic TB, symptomatic TB female subjects on ATT and control female subjects at follicular and luteal phases of menstrual cycle

Parameters	CD4+ T-Cell Count (/µl)		P-value
Groups	Follicular	Luteal	
Symptomatic TB (A) (n=30)	217±93	212±97	0.772
Symptomatic TB on ATT (B) (n=30)	387±114	367±136	0.999
Control (C) (n=30)	689±172	660±157	0.425
F-value	23.561	24.653	
P-value	0.000	0.000	
AvsB	0.000	0.000	
AvsC	0.000	0.000	
BvsC	0.000	0.000	

important in the prevention of latent TB reactivation [35,36]. There is therefore the tendency for any pre-existing hormonal imbalance to normalize thereby correcting any existing menstrual and reproductive abnormality.

5. CONCLUSION

In conclusion, TB infections exert significant changes on thyroid function which resulted to euthyroid sick syndrome and reduction in cell mediated immunity in affected women thereby increasing the severity of infections. This may produce menstrual abnormalities which may affect the reproductive potentials of these women. A new national strategic plan for TB control is advocated to include a counseling module for reproduction and routine screening for thyroid function.

CONSENT

All author(s) hereby declare that all written informed consent was obtained from all the patients who participated in this study.

ETHICAL APPROVAL

All author(s) 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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