RELATIONSHIP BETWEEN TUBERCULOSIS AND FEMALE HORMONE LEVELS IN POST-MENOPAUSAL WOMEN

Gulru Erbay[1], Gunes Senol[2], Ceyda Anar[1], Ali Riza Meral[1] and Ozlem Tuzel[1]

[1]Department of Chest Diseases, [2]Department of Infectious Diseases and Clinical Microbiology, Dr Suat Seren Chest Diseases and Surgery Training and Research Hospital, Izmir, Turkey

Abstract. Tuberculosis (TB) is an intracellular infection controlled by T-lymphocyte. After menopause, T-lymphocyte cells counts increase suggesting a possible link between T-lymphocyte cell counts and estrogen and progesterone levels. In this study we compared post-menopausal women with and without TB to determine any differences in estrogen and progesterone levels between the two groups. The study group consisted of 38 post-menapausal women hospitalized for TB at Dr Suat Seren Chest Diseases and Surgery Training and Research Hospital for Tuberculosis, in Izmir, Turkey. The control group consisted of 30 post-menopausal women without TB. Both groups were selected from patients without immunosuppressive diseases or malignancies in whom PPD testing was not contraindicated. The mean progesterone level in the control group was 0.3896 ± 0.2014 ng/ml and in the study group was 0.3607 ± 0.3006 ng/ml (p=0.638). The mean estrogen level in the control group was 18.9873 ± 6.5332 pg/ml and in the study group was 26.2768 ± 14.3418 pg/ml; the difference was significant (p=0.007). The body mass index in the study group was significantly (p=0.034) lower than in the study group. In post-menopausal women, the mean estrogen level was significantly higher in participants with TB than those without TB.

Keywords: estrogens, menopause, progesterone, tuberculosis

INTRODUCTION

Tuberculosis (TB) is the world's most common and sometimes fatal, infectious disease in adults (WHO, 2010). Approximately one-third of the world's population is estimated to be infected with *Mycobacterium tuberculosis* (Kilicaslan, 2002). TB is an intracellular infection controlled by the cellular immune response (Daniel, 1980). The host's ability to control TB depends on an effective cellular immune response (Daniel, 1980). T-lymphocytes play an important role in control of TB (Fauci *et al*, 1997).

CD4 lymphocytes with major histocompatibility complex (MHC) class 2 play a central role in TB immunity (Fauci *et al*, 1997). Th1 cells in the CD4 class increase immune activity by producing promoting cytokines, such as interleukin-2, interleukin-12 and interpheron-gamma (Mosmann *et al*, 2005). Stimulation of macrophages by interpheron-gamma results

Correspondence: Gunes Senol, 1703 St. No: 42. D.3 35600 Karsiyaka-Izmir, Turkey.
Tel: 90232 4333333
E-mail: drshenol@yahoo.com

in an increase in the expression of MHC class 2 surface molecules (Townsend *et al*, 1989), resulting in an increased ability to present antigen. CD4 T helper cells have a unique response to this antigen presentation method (Townsend *et al*, 1989). Th2 cells secrete a different set of cytokines, primarily interleukin-4, interleukin-10, and interleukin-13, which promote humoral immunity and depress cell mediated immunity (Elenkov, 2002). These Th2 cytokines inhibit macrophage activation, T cell proliferation, and the production of proinflammatory cytokines (Fauci *et al*, 1997).

Women spend approximately one-third their life post-menopause (Sternberg, 2001). Post-menopausal women (PMW) may experience physiological and psychological symptoms from diminished hormone production. Waning estradiol (E2) in the body is a major factor associates with these symptoms. Changes occur in the immune system and other organ systems during menopause. (Bates, 1990; Godsland *et al*, 1993). Estrogens and progesterone have an effect on the immune system. They can inhibit pro-inflammatory cytokine expression in immune cells expressing the respective receptor (McKay and Cidlowski, 1999). Estrogen receptors are expressed on a variety of immunocompetent cells, CD4+ and CD8+ T cells and macrophages (Salem, 2004). Estrogens have modulator effects on Th1 and Th2 cells (Salem, 2004). Salem (2004) reported estrogen inhibits Th1 proinflammatory cytokine (IL-2, IFN-gamma, and TNF-alfa) production and stimulates Th2 anti-inflammatory cytokine production. Thus, estrogen may suppress Th-dependent diseases while potentiating Th2-dependent diseases. One study suggests that estrogen may enhance the accumulation of Th1 T cells in response to antigens (Maret *et al*, 2003).

During the post-menopausal period, estrogen is synthesized by extraglandular tissue and androgens by aromatization (Wich and Carnes, 1995). Aromatase is expressed in adipose tissue and skin after menopause; these tissues are an important source for estrogen production during the post-menopausal period (Kilicturgay, 2003). Therefore, body mass index (BMI) may be an important parameter for estimating endogenous estrogen production (Wich and Carnes, 1995).

In this study we investigated the relationship between estrogen and progesterone levels in patients with and without TB.

MATERIALS AND METHODS

Setting

Thirty-eight consecutive PMW hospitalized at the Izmir Dr Suat Seren Chest Diseases and Surgery Training and Research Hospital during 2004 and 2008 with tuberculosis were included in the study. Exclusion criteria were: having a malignancy, having immune suppression, taking hormone replacement therapy, corticosteroids, beta agonists or antagonist, or taking other immunosuppressive drugs.

Control subjects were 30 consecutive PMW hospitalized at the same institution. Exclusion criteria were: having TB, having a malignancy, being immunosuppressed, and taking hormone replacement therapy, corticosteroids, beta agonists or antagonist or immunosuppressive drugs. None of the patients, control or study groups refused participation in the study.

Definition of menopausal state

Being post-menopausal was defined as not having a menstrual period for 12 months. A BMI was calculated for each subject as weight in kilograms divided by

height in meters squared (kg/m^2). A morning venous blood sample was collected from each patient for estradiol and progesterone levels, which were determined using an electrochemiluminescence technique in an autoanalyzer (Cobas e601, Roche Diagnostics, Istanbul, Turkey).

Diagnosis of TB

TB was diagnosed using any of the following criteria (WHO, 2007): isolation of *M. tuberculosis* from a culture of the sputum or other body samples, a biopsy specimen showing caseation in a granuloma with or without acid-fast bacilli, a biopsy specimen showing non-caseation in a granuloma in cases responding to treatment, a patient with a positive PPD test having signs and symptoms consistent with active TB, such as having an abnormal chest radiograph or clinical evidence of active TB disease (*eg*, fever, night sweats, cough, weight loss, hemoptysis).

Microbiological diagnosis

Microscopic examination of sputum by Kinyoun staining and culture for *M. tuberculosis* in both Lowenstein-Jensen medium and liquid medium (BACTEC 960 system; Becton Dickinson, Sparks, MD) were used to identify cases of TB.

Treatment of TB

All patients with TB received treatment following WHO criteria (WHO, 2007). Treatment was extended or modified, based on response to treatment and occurrence of drug resistance.

Patients were transferred to respective regional TB treatment offices after hospitalization to continue treatment following national TB treatment policies. Treatment outcomes were not recorded as part of this study.

A PPD test (BBMCIPD, Sofia, Bulgaria; 5 TU/0.1 ml) was performed intradermally (0.1 ml, or 5 TU) on all subjects. The test was read at 72 hours and induration >10 mm was considered positive.

The data were evaluated using SPSS, version 10.0 (SPSS, Chicago, IL). We compared group averages and parameters using a *t*-test and Pearson's correlation. A p-value < 0.05 was considered statistically significant. This study was approved by the Dr Suat Seren Chest Diseases and Surgery Research and Training Hospital Ethics Committee. All participants gave written informed consent prior to participation.

RESULTS

The mean age of the TB subjects was 61 (range: 48-83) years and of the controls was 61 (range, 49-76) years (p=0.920) (Table 1). Of the 38 TB cases studied, 32 had pulmonary TB and 6 had extrapulmonary TB (TB lymphadenitis in 5 patients and TB pleuritis in one patient). The mean ages of menarche, menopause, the social status, education level, economic level and residence location (urban *vs* rural) were not significantly different between subjects with and without TB (p>0.05) (Table 1).

The mean progesterone level among TB cases was 0.4 (± 0.2) ng/ml and among controls was 0.4 (± 0.3) ng/ml (p=0.638) (Table 1).

The mean estrogen level among TB subjects was 26.3 (± 14.3) pg/ml and among controls was 19 (± 6.5) pg/ml. This difference was significant (p=0.007). The mean BMI among TB cases (25 ± 4) was significantly higher than controls (23 ± 4) (p=0.034) (Table 1).

There were no significant differences in mean hormone levels between pulmonary TB cases and extrapulmonary TB cases studied (Table 2).

Table 1
Progesterone, estrogen and BMI levels among study and control subjects.

	Study group (n = 38)	Control group (n = 30)	p-value
Mean age (years)	61 ± 11	61 ± 10	0.920
Menarche age (years)	14 ± 1	14 ± 1	0.940
Menopause age (years)	46 ± 5.7	46 ± 4.9	0.816
Mean progesterone level (ng/ml)	0.4 ± 0.2	0.4 ± 0.3	0.638
Mean estrogen level (pg/ml)	26.3 ± 14.3	19 ± 6.5	0.007
Mean BMI (kg/m^2)	25 ± 4	23 ± 4	0.034

BMI, body mass index.

Table 2
Mean progesterone and estrogen levels in pulmonary and extrapulmonary TB patients.

	Pulmonary TB (n = 32)	Extrapulmonary (n = 6)	p-value
Progesterone (ng/ml)	0.4 ± 0.2	0.3 ± 0.2	0.341
Estrogen (pg/ml)	26 ± 12.1	28.1 ± 24.6	0.739

DISCUSSION

One-third of the world's population is estimated to be infected with *M. tuberculosis* (WHO, 2010). About half of these are women (Kilicaslan, 2002). About 700,000 women die annually from TB and three million contract the disease (WHO, 2013).

The incidence of TB is greater among adolescent men than women (Atre *et al*, 2004). Some studies have speculated this is due to differences in exposure, infection risk and progression from inactive infection to active disease (Holmes *et al*, 1998; Sony *et al*, 2003; WHO, 2013). Progression to active TB disease occurs in about 10% of inactive infections (Andrews *et al*, 2012). Studies conducted in countries with a high TB prevalence show the disease progression rate is higher in reproductive females than the males of the same age period (Holmes *et al*, 1998; Zeana and El-Sadr, 2004). Some studies have found women with TB have a higher case fatality rate when the subject is in her early reproductive years (Holmes *et al*, 1998; WHO, 2013). The Ministry of Health China reported the mortality rate for TB among reproductive age women is higher than among males the same age in China (Ministry of Health, 2001).

By the third year post-menopause, ovaries stop producing estrogen and progesterone (Wich and Carnes, 1995), and the adipose tissue becomes a more important source of estrogen (Kiliçturgay, 2003). Studies have found serum estrogen concentrations are directly associated with BMI in PMW (Lamar *et al*, 2003; Karim *et al*, 2009). Women with a BMI > 25 kg/m^2 were found to have significantly higher estrogen levels than women with a BMI < 25 kg/m^2 (Karim *et al*, 2009). In our study PMW with TB had signifi-

cantly higher BMI than those without TB. This may explain why the estrogen levels were significantly higher in TB patients than patients without TB in our study.

There is very limited numbers of literature regarding sex hormones and TB disease in humans. Neyrolles and Quntana-Murci (2009) reported both innate immune cells (monocytes, macrophages, and dendritic cells) and T cells expressed specific receptors for steroid hormones. They suggested that variation in the X chromosome may be involved in the genetic predisposition to TB and sex-specific genetic effects lead to differences in the prevalence of TB between men and women. Nhamoyebonde and Leslie (2014) indicated that low levels of estrogens promoted Th1 differentiation and production of cytokines such as TNF-α, while high levels promoted Th2 polarization, with a consequent effect on cytokines. That might explain our results.

Our study has some limitations. The major limitation is low number of subjects, the other is the absence of immunological marker measurements.

In conclusion, in our study, estrogen levels in PMW with TB were significantly higher than those without TB, but progesterone levels were not different.

REFERENCES

Andrews JR, Noubary F, Walensky RP, Cerda R, Losina E, Horsburgh CR. Risk of progression to active tuberculosis following reinfection with *Mycobacterium tuberculosis*. *Clin Infect Dis* 2012; 54: 784-91.

Atre SR, Kudale AM, Morankar SM. Cultural concepts of tuberculosis and gender among the general population without tuberculosis in rural Maharashtra, India. *Trop Med Int Health* 2004; 9: 1228-38.

Bates GW. Menopause. In: Riulin ME, Marrison JC, Bates GW, eds. Manual of clinical problems in obstetrics and gynecology. Boston: Little Brown and Co, 1990: 390-3.

Daniel TM. The immunology of tuberculosis. *Clin Chest Med* 1980; 1: 189-201.

Elenkov I. Systemic stress-induced Th2 shift and its clinical implications. *Int Rev Neurobiol* 2002; 52: 163-86.

Fauci AS, Braunwald E, Issebacher K. Harrison's principles of internal medicine. 14th ed. New York, NY: McGraw-Hill, 1997: 2097-114.

Godsland IF, Walton C, Stevenson JC, *et al*. Impact of menopause on metabolism. Symposium on metabolism in the female life cycle. Ares-Serono Symposia Publications, 1993: 171-89.

Holmes C B, Hausler H, Nunn P. A review of sex differences in the epidemiology of tuberculosis. *Int J Tuberc Lung Dis* 1998; 2: 96-104.

Karim R, Mack WJ, Hodis HN, *et al*. Influence of age and obesity on serum estradiol, estrone, and sex hormone binding globulin concentrations following oral estrogen administration in postmenopausal women. *J Clin Endocrinol Metab* 2009; 94: 4136-43.

Kilicaslan Z. Pulmonary tuberculosis and atypical mycobacteria infections. In: Arseven O, ed. Pulmonary diseases. Istanbul: Nobel Medical Publishing, 2002: 283-301.

Kiliçturgay K. Interactions between immune and neuroendocrin systems. Istanbul: Nobel and Gunes Publishing, 2003: 331-7.

Lamar CA, Dorgan JF, Longcope C, *et al*. Serum sex hormones and breast cancer risk factors in postmenopausal women. *Cancer Epidemiol Biomarkers Prev* 2003; 12: 380-3.

Maret A, Coudert JD, Garidou L, *et al*. Estradiol enhances primary antigen-specific CD4 T cell responses and Th1 development in vivo: essential role of estrogen receptor alpha expression in hematopoietic cells. *Eur J Immunol* 2003; 33: 512-21.

McKay LI, Cidlowski JA Molecular control of immune/inflammatory responses: inter-

actions between nuclear factor-kappa B and steroid receptor-signaling pathways. *Endocr Rev* 1999; 20: 435-59.

Ministry of Health, the People's Republic of China. Report on nationwide random survey for the epidemiology of tuberculosis in 2000. Beijing: Ministry of Health, 2001: 1-67.

Mosmann T, Cherwinski H, Bond MW, *et al*. Two types of murine helper T- cell clone. I. Definition according to profiles of lymphokine activites and secreted proteins. *J Immunol* 2005; 175: 5-14.

Neyrolles O, Quntana-Murci L. Sexual inequality in tuberculosis. *PLOS Med* 2009; 6: e1000199.

Nhamoyebonde S, Leslie A. Biological differences between the sexes and susceptibility to tuberculosis. *J Infect Dis* 2014; 209: S100-6.

Salem ML. Estrogen, a double-edged sword: modulation of TH1 and TH2 mediated inflammations by differential regulation of TH1/TH' cytokine production. *Curr Drug Targets Inflamm Allergy* 2004; 3: 97-104.

Sony A. El Beyers DN, Enarson DA, Chan Yeung M. Tuberculosis in women. In: Buist S, Mapp CE, eds. Respiratory diseases in women. *ERS Monograph* 2003: 152-66.

Sternberg EM. Neuroendocrine regulation of autoimmune/inflammatory diseases. *J Endocrinol* 2001; 169: 429-35.

Townsend A, Ohlen C, Bastin J, *et al*. Association of class 1 major histocompatibility heavy and light chains induced by viral peptides. *Nature* 1989; 340: 443-8.

World Health Organization (WHO). WHO report 2007. *WHO/HTM/TB/2007.376.*

World Health Organization (WHO). WHO tuberculosis fact sheet. Geneva: WHO, 2010. [Cited 2015 Oct 6]. Available from: http://www.who.int/mediacentre/factsheets/fs104/en

World Health Organization (WHO). Tuberculosis and gender. Geneva: WHO, 2013. [Cited 2015 Oct 6]. Available from: http://www.who.int/tb/challenges/gender/page_1/en/index.html

Wich BK, Carnes M. Menopause and the aging female reproductive system. *Endocrinol Metab Clin North Am* 1995; 24: 273.

Zeana C, El-Sadr W. The effect of gender on epidemiology, clinical presentation and management of tuberculosis. In: Legato MJ, ed. Principles of gender specific medicine. San Diego: Elsevier Academic Press, 2004: 938-46.