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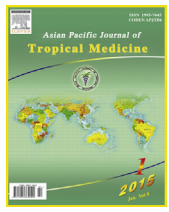

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Tuberculosis/toxoplasmosis co-infection in Egyptian patients: A reciprocal impact

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ABSTRACT

Objective: To assess the concurrent toxoplasmosis infection in Egyptian TB patients and the impact of each infection on the other in terms of increased severity of TB or reactivation of latent *Toxoplasma* infection.

Methods: Three hundred suspected pulmonary TB cases were initially screened for TB using direct Ziehl Neelsen staining and Lowenstein Jensen culture of their sputa. Rifampicin resistance was detected by Xpert MTB/RIF assay. Control group of 30 age and sex-matched healthy individuals negative for TB was included for comparison. All subjects were further assessed for serum levels of anti-*Toxoplasma* IgG antibodies and malondialdehyde (MDA).

Results: Forty three confirmed TB-infected patients including 10 (23.3%) rifampicin-resistant patients were detected. Associated toxoplasmosis was found to be significantly higher among TB patients (*OR* = 2.709; 95% *CI*: 1.034–7.099; $P < 0.05$) and among rifampicin sensitive than rifampicin resistant TB patients (*OR*=0.213; 95% *CI*: 0.048–0.951; $P < 0.05$). Serum levels of anti-*Toxoplasma* IgG antibodies and MDA were significantly higher among TB patients than the control group. Furthermore, serum level of MDA was significantly higher among TB/*Toxoplasma* co-infected patients as compared to toxoplasmosis free-TB patients. Strong positive correlation was detected between serum levels of anti-*Toxoplasma* IgG and MDA in TB patients ($r = 0.75$, $P = 0.001$).

Conclusions: Among pulmonary TB Egyptian patients, there is a considerable prevalence of toxoplasmosis. Severity of pulmonary tuberculosis could be increased by *Toxoplasma* co-infection.

1. Introduction

One third of the world population has been estimated to be infected with *Toxoplasma gondii* (*T. gondii*) parasite [1]. Mostly it does not cause serious illness in healthy adults, but causes severe diseases in immunocompromised patients [2].

In immunocompetent individuals, effective immune response produces a balance for both parasite and host survival but does not eradicate the infection. Weakness of the host immune function may allow reactivation of actively replicating tachyzoites, sometimes and results in extensive organ damage [3].

Tuberculosis (TB) remains a major cause of morbidity and mortality worldwide in spite of great effort for eradication. Globally, there were an estimated 9.27 million incidences of TB and new infections occur at a rate of one per second [4,5]. About 33% of the world population infected with *Mycobacterium tuberculosis* (*M. tuberculosis*) reside in developing countries including Egypt. An incidence rate of 15/100000 population was recorded in Egypt in 2014 [5]. Furthermore, this situation is exaggerated by HIV pandemic with almost 13 million people currently co-infected with HIV and TB [5,6]. The increasing number of multi-drug resistant, extensively drug-resistant and extremely resistant strains of *M. tuberculosis* made the control of the tuberculosis spread more difficult [6].

Both TB and parasitic diseases are infectious diseases causing serious harm to humans with an overlap in endemic regions, which may lead to frequent co-infection in these areas. Cases of co-infection of TB with intracellular parasites were

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reported as with malaria [7,8], visceral leishmaniasis [9,10], *T. gondii* [11,12]. Hwang *et al.* [13] reported a cerebral toxoplasmosis case with disseminated tuberculosis in an immunocompetent patient. In the cases of co-infection, modification of the immune response was suggested [14,15].

The response of the immune system against infections includes the generation of reactive oxygen species (free radicals) which are toxic to human tissues and cells. Malondialdehyde (MDA), a lipid peroxidation product, is considered an indicator for oxidative stress; a process that results in damage of proteins and DNA within cells [16].

Oxidative stress was recorded, and implicated in the pathogenesis of both TB [17,18], and toxoplasmosis [19]. However, no studies on oxidative stress have been conducted on TB/*T. gondii* co-infected patients. Therefore, we investigated the association between toxoplasmosis and TB in our locality. Also, we explored the potential impact of *Toxoplasma* co-infection on the severity of pulmonary TB via measuring serum level of MDA as a marker of oxidative stress in TB/*T. gondii* co-infected patients in comparison to toxoplasmosis free-TB patients and healthy controls.

2. Materials and method

2.1. Participants

This study was a cross-sectional study in the period from January 2015 to May 2016. It was carried out on 300 patients attending the outpatient clinic of Mansoura Chest Hospital for suspicion of having tuberculosis. 'A suspected TB case' was defined as adult complaining of clinical symptoms as chronic cough for more than three weeks, presence of blood streaked sputum or haemoptysis, radiological findings suggesting TB infection not yet confirmed by positive *M. tuberculosis* bacilli on smear examination and/or culture examination. Exclusion criteria included patients less than 18 years old, pregnant women, patients with chronic obstructive pulmonary diseases and cancers. Thirty age and sex-matched healthy individuals negative for TB participated as a control group for this study.

From all patients and controls, three consecutive early morning sputum samples were collected for TB diagnosis. After confirmation of TB, five ml venous blood was withdrawn from each TB patient in plain tubes. After 15 min allowing for blood clotting, tubes were centrifuged at 1500 ×g for 5 min and aliquots of sera were stored at −20 °C for the assessment of anti-*Toxoplasma* IgG antibodies and MDA levels.

2.2. Detection of pulmonary tuberculosis and rifampicin resistance

Diagnosis of TB was done by direct Ziehl Neelsen staining of sputum samples for detection of acid fast bacilli and Lowenstein Jensen culture for isolation of *M. tuberculosis* [20]. Detection of rifampicin resistance (as a surrogate marker of multi drug resistance) was performed for TB positive samples by Xpert MTB/RIF assay (an automated molecular test for simultaneous detection of both *M. tuberculosis* and rifampicin resistance). It is performed on the MTB/RIF test platform (GeneXpert, InC; Sunnyvale, CA, USA). Processing of the sample and PCR were integrated in a disposable plastic cartridge containing all reagents needed for lysis of bacteria, extraction of nucleic acid, amplification, and detection of amplicon.

Briefly, sputum samples were treated with sample reagent (Sodium Hydroxide and Isopropanol) and after repeated shaking and incubation, they were transferred into a multichambered cartridge. The MTB/RIF cartridge is then loaded into the GeneXpert device where heminested real-time PCR is done to identify rifampicin resistance inducing mutations in the RNA polymerase beta (rpoB) gene in the *M. tuberculosis* genome using 3 specific primers and 5 unique molecular probes to ensure a high degree of specificity [21,22].

2.3. Detection of anti-Toxoplasma IgG antibodies

Anti-*Toxoplasma* IgG was measured using a commercially available ELISA kit (*Toxoplasma* IgG enzyme immunoassay test, Biocheck, Inc.) following manufacturer's instructions.

2.4. Determination of the malondialdehyde (MDA) level in serum

MDA, as an indicator of the oxidative stress in the tissues, its levels were measured in the sera of TB patients and the controls using thiobarbituric acid reaction method [23].

2.5. Data analysis

The SPSS software package version 17.0 was used for statistical analysis. Qualitative values were expressed as absolute frequencies and percentages, and quantitative values as median and range or mean ± SD. Categorical variables were compared using the Pearson's Chi-square test (x^2) and quantitative variables using Mann Whitney U test or student's *t*-test. Differences were considered significant at P value <0.05.

2.6. Ethical aspects

This study was approved from our Local Ethical Committee of Faculty of Medicine – Mansoura University (MFM-Institutional Research Board) with a code number R/16.05.14.

3. Results

Out of 300 TB suspects enrolled in this study; only 43 patients whose direct ZN sputum smears were positive for acid fast bacilli and/or their sputa were positive for *M. tuberculosis* by Lowenstein Jensen culture were diagnosed as pulmonary TB patients. Of these, 10/43 (23.3%) were resistant to rifampicin using Xpert MTB/RIF assay. The mean age in TB patients group was (40.9 ± 8.3) years and in the control group was (37.1 ± 8.1) years. Among TB patients group, male gender (86%) was more prevalent than females (14%), $P < 0.001$. Smokers were significantly higher among TB group, *OR* = 5.721; 95% *CI*: (1.994–16.418); $P = 0.001$ than in controls. No statistical difference was detected regarding the distribution of hypertension or diabetes between TB patients and control group.

Associated *T. gondii* infection was found to be significantly more frequent among TB patients than the control group, *OR* = 2.709; 95% *CI*: (1.034–7.099); $P < 0.05$. Moreover, serum level of anti-*Toxoplasma* IgG antibodies was significantly higher among TB patients [median (range): 168.72 (4.50–868.99)] than the control group [median (range): 47.817 (2.00–218.25)]; $P < 0.01$ (Table 1). Although *T. gondii* infection was significantly

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