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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

associated with rifampicin sensitive TB patients than rifampicin resistant group (*OR* = 0.213; 95% *CI*: 0.048–0.951; $P < 0.05$), the level of anti-*Toxoplasma* antibodies wasn't significantly higher among rifampicin sensitive TB patients ($P = 0.082$). Moreover, we found that rifampicin resistance is significantly lower among toxoplasmosis seropositive patients as compared to toxoplasmosis seronegative patients (13.8% *vs* 42.9%, $P = 0.03$) (Table 1).

Table 1

T. gondii seropositivity and anti-*Toxoplasma* IgG titre among control group and TB patients.

Indexes	Control group	TB patients				
		Total	Rifampicin sensitive	Rifampicin resistant	Toxoplasma positive*	Toxoplasma negative
Number	30	43	33	10	29	14
T. gondii seropositivity[&]	13 (43.3)	29 (67.4)[a]	25 (75.7)[c]	4 (40.0)	29 (100.0)	0
Anti-*Toxoplasma* IgG[△]	47.81 (2.0–218.2)	168.72 (4.5–868.9)[b]	223.01 (4.5–868.9)	30.90 (14.7–247.3)	237.34 (55.0–868.9)	16.30 (4.5–30.9)

[&]: *T. gondii* seropositivity data are expressed as [*n* (%)]; [△]: Anti-*Toxoplasma* IgG (IU/mL) Median (range); *: Cut off value for anti-*Toxoplasma* IgG = 32 IU/mL. [a]$P < 0.05$ compared with control group; [b]$P < 0.01$ compared with control group; [c]$P < 0.05$ compared with rifampicin-resistant group.

Serum level of MDA was found to be significantly higher among TB patients (22.91 ± 11.59) nmol/mL, compared to the control group (4.40 ± 1.18) nmol/mL; $P < 0.001$. There was a positive correlation between *Toxoplasma* IgG titre and MDA levels in TB patients ($r = 0.75$, $P = 0.001$), so associated toxoplasmosis could be a considerable factor affecting the severity of TB.

The mean of serum MDA level was significantly higher among rifampicin resistant more than rifampicin sensitive TB-patients, (25.4 ± 11.67) & (14.70 ± 6.68), respectively, $P < 0.01$. Also it was significantly higher in TB positive/toxoplasmosis-positive patients than TB positive/toxoplasmosis-negative patients (26.50 ± 10.43) & (15.47 ± 10.55), nmol/mL respectively, $P < 0.01$.

4. Discussion

Co-infection of tuberculosis with other infections as parasitic infections represents a serious and challenging health problem. Both *M. tuberculosis* and *T. gondii* are intracellular opportunistic microorganisms that are prevalent in developing countries [24].

There is a paucity of studies on TB/toxoplasmosis co-infection and the only available data were from case reports [11–13]. Therefore, we intended to determine the frequency of toxoplasmosis co-infection in Egyptian TB patients.

Our study showed that seroprevalence of anti-*Toxoplasma* IgG antibodies in the control group was 43.3%. This is broadly consistent with previous data in our locality where a prevalence of 59.6% among healthy blood donors was recorded [25].

Among TB patients, the prevalence of parasitic disease varies widely in different areas and different survey sites [24]. We found that the seroprevalence of *T. gondii* infection among TB patients was 67.4% and this was significantly higher than the control group. This was in accordance with Yassen [26], who also showed that patients with active TB have significantly a higher seroprevalence of toxoplasmosis infection compared to control group. Conversely, Ledru *et al.* [27] reported no significant difference for anti-*Toxoplasma* antibodies among the controls and TB patients. Many factors possibly affect co-infection of TB and parasitic diseases such as socio-demographics, decreased immunity as in renal transplant recipients, patients on haemodialysis, HIV positive patients and immigration [28–30].

The key cytokines in cell mediated immunity against intracellular parasites like *T. gondii* and *M. tuberculosis* are TH1 cytokines; (IFN-γ, TNF-α, IL-12 and IL-18). They promote isotype switching from IgM to IgG1 to facilitate phagocytic cell mediated immune responses [31]. Also, IFN-γ and TNF-α are the central controlling factors of tachyzoite growth [32].

During active TB infection, there is a decrease in production of TH1 cytokines and overproduction of TH2 cytokines [33,34]. Consequently, this shift of the TH1 response towards TH2 responses with subsequent suppression of cell mediated immunity against toxoplasmosis which could reactivate the old lesions or increases the susceptibility to new infection.

T. gondii infection was significantly higher among rifampicin sensitive TB patients than rifampicin resistant group ($P < 0.05$), however, the level of anti-*Toxoplasma* IgG antibodies wasn't significantly higher among rifampicin resistant TB patients. Small number of patients in this study couldn't be relied on our observation for the low prevalence of *Toxoplasma* infection among rifampicin resistant TB patients.

Combined infections with more than one organism are one of the factors that affect the outcome and the severity of the infections. So, we evaluated the influence of toxoplasmosis co-infection on the severity of pulmonary TB by measuring serum malondialdehyde (MDA) concentration as a marker of oxidative stress and a parameter linked to disease severity. Our study clarified that MDA was significantly higher among TB patients compared to the control group. Furthermore, serum level of MDA was significantly higher among TB/*Toxoplasma* co-infected patients as compared to *Toxoplasma* negative TB patients. This was similar to several studies that have shown that *M. tuberculosis* and *T. gondii* co-infection was associated with increased oxidative stress characterized by increased levels of circulating plasma lipid peroxides [17–19,35].

Oxidative stress in pulmonary TB could be attributed to many factors such as tissue inflammation, poor intake of micronutrients in addition to the release of free radicals from macrophages or anti-TB drugs treatment side effects [36–38].

Our data indicated that MDA levels were significantly higher among rifampicin resistant than rifampicin sensitive TB-patients, This finding is in support of Alli *et al.* [38] who reported that patients with rifampicin resistant TB had increased levels of MDA than both rifampicin sensitive TB-patients group and negative TB-group. Our finding could be supported by a previous study concluded the use of oxidative

stress index as a marker of response to antituberculous therapy (ATT). Also, they found that supplementing ATT with antioxidant has improved outcome of TB [39]. High concentration of MDA in rifampicin resistant group is an indication of oxidative stress which may be due to severe inflammatory process often associated with such infection [17,38].

There is a considerable association between *M. tuberculosis* and *T. gondii* infections in our locality. A reciprocal impact of each infection on the other exists. TB could be a contributing factor for reactivation of a latent toxoplasmosis or increasing the susceptibility to a new infection, and severity of pulmonary tuberculosis could be increased by *Toxoplasma* co-infection. Further researches are needed to elucidate the effects of both combined infections on their outcomes. Given the high rate of *Toxoplasma* infection among TB patients, we recommend their screening for toxoplasmosis.

Conflict of interest statement

The authors declare no conflict of interest.

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