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Original article

Toxoplasmosis and associated risk factors in tuberculosis patients attending the chest hospital in Beni Suef Governorate, Egypt

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ABSTRACT

Background: Toxoplasmosis is a significant health issue among Egyptian patients, exhibiting a high prevalence rate. Several studies have reported tuberculosis (TB)-*Toxoplasma* co-infection that could pose a double burden in healthcare facilities in developing countries. The present study aimed to study *T. gondii* seropositivity and IFN-γ levels among TB patients who had positive toxoplasmosis compared to those without toxoplasmosis and also identify the associated risk factors. **Methods**: Cross-sectional research on patients who attended the outpatient clinic at the chest hospital in Beni Suef Governorate, Egypt, from May 2023 to November 2023. Sputum samples were examined for TB positivity using Ziehl-Neelsen stain, Lowenstein Jensen culture and GeneXpert assay. Blood samples of TB patients screened for anti-*Toxoplasma* IgG/IgM antibodies, measuring interferon-gamma (IFN-γ) levels, and identifying the associated risk factors. **Results:** Out of 124 patients, 80 were TB positive and 44 were TB negative. Seropositivity of *Toxoplasma* infection was 51.25% (41/80) in TB-positive patients and 18.18% (8/44) in TB-negative patients, revealing a significant association between *Toxoplasma* positivity and TB (P<0.001). Statistical analysis indicated a significant association between *Toxoplasma* infection seropositivity in TB patients and the associated chronic diseases, taking immunosuppressive drugs, contacting pet animals, eating processed meat, washing hands and vegetables before eating and cooking meat well. **Conclusion**: The study findings showed *Toxoplasma* infection is prevalent amongst Egyptian TB patients. This gives insight into the importance of earlier identification and managing patients with TB-toxoplasmosis coinfection to avoid any serious complications.

Introduction

Toxoplasma gondii (*T. gondii*) and *Mycobacterium tuberculosis* (*M. tuberculosis*) are pathogenic organisms that are widespread among humans and may result in long-lasting infections and life-threatening diseases, particularly in people with a compromised immune system [1]. *T. gondii* could infect a third of the world's population. It can be transmitted through food, water, or transplacentally [2]. Human toxoplasmosis may

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result in a serious health issue, although the vast majority of cases are mild or asymptomatic, particularly if the host immune system is weakened. This is because it might enable actively reproducing tachyzoites to reactivate, which may severely damage organs [3].

Tuberculosis is one of the world's deadliest infections and may cause serious health problems. It is caused by *M. tuberculosis*, an intracellular bacterium primarily a respiratory pathogen. Pulmonary *tuberculosis* is one of the leading causes of infection-related mortality, particularly in low-income settings and among patients who have weakened immune systems. About 15% of infections occur at extra-pulmonary sites, creating new challenges for the disease's diagnosis and management. It is thought that *M. tuberculosis* can disseminate out of the lungs more frequently rather than a rare occurrence throughout all infections, causing secondary lesions that can either become dormant or active [4]. Even though tuberculosis is a preventable and treatable disease, an estimated 1.6 million individuals die from it annually [5].

Co-infection of TB and toxoplasmosis is a growing problem that has received more attention in developing countries. Mashaly et al. [6] studied the effect of concomitant *Toxoplasma* infection on immunocompetent TB patients and evaluated the implications of each on the other regarding TB severity and latent toxoplasmosis reactivation. In China, Zhao et al. [7] proved *T. gondii* seropositivity among TB patients. According to Ali et al. [8], TB patients in Iraq have an elevated level of anti-*Toxoplasma* IgG antibodies. In India, Vasantham et al. [9] documented a case of pulmonary TB combined with toxoplasmosis. Also, an immunocompetent case of ocular co-infection concerning *T. gondii* and *M. tuberculosis* was reported by Agarwal et al. [10].

The primary cytokines involved in the cell-mediated immune response towards *M. tuberculosis* and *T. gondii* are T-helper1 (Th1) cytokines, including IFN-γ which play a crucial role in the host's defense towards these intracellular microorganisms. IFN-γ is considered a TB biomarker [11] and protects against tachyzoites proliferation during infection with *T. gondii* [12]. In newborns with suspected congenital toxoplasmosis or TB infection, IFN-γ released from mononuclear cells in the peripheral blood could be used to confirm these infections [13]. Throughout an active tuberculosis infection, Th2 cytokines are overproduced while Th1 cytokines are decreased. As a result, this suppresses the effectiveness of cell-mediated immunity against toxoplasmosis, which may result in either a new infection being more susceptible or an old infection reactivating [6].

Even though co-infection with these two pathogens has been researched, they are few and relatively scattered. Hence, the current research studied *T. gondii* seropositivity and IFN-γ levels among TB patients who had positive toxoplasmosis compared to those without toxoplasmosis and also identified the associated risk factors.

Subjects and Methods

Study participants and settings

The subjects of the current cross-sectional research were 124 (87 males and 37 females) patients who attended the outpatient clinic at the chest hospital in Beni Suef Governorate, Egypt, from May 2023 to November 2023. Patients who meet the following criteria are eligible for the study: (a) those who have a fever, night sweats, coughing up sputum for more than three weeks, weight loss, lack of appetite, chest pain, hemoptysis, and/or radiological evidence of tuberculosis; (b) those who are at least 21 years old; and (c) those who voluntarily consented to participate. However, patients who had already been diagnosed or received treatment, those with AIDS, chronic obstructive lung disorders, and cancer were excluded.

According to Mashaly et al. [6], *T. gondii* seropositivity was 29/43 (67.4%) in TB patients. Our study sample size was estimated to involve a 67.4% prevalence rate of *T. gondii* among TB patients, a confidence level of 95%, and a level of precision of 10.27%.

A questionnaire was applied to all participants fulfilling the following data: age, sex, occupation, residence, having any chronic diseases and immunosuppressive diseases, taking any immunosuppressive drugs, history of blood transfusion, owing pet animals in the house, dietary habits (especially with regard to eating processed meat, cooking meat well), and personal hygiene (washing hands and vegetables before eating).

Specimens collection

Three consecutive morning sputum samples were collected from each participant under aseptic conditions. Diagnosis of TB was done by examining sputum samples by staining with Ziehl-Neelsen stain, culturing on Lowenstein Jensen

culture, and GeneXpert assay. In addition, 5 ml of venous blood samples were collected. Each sample was subjected to serum extraction after centrifugation at 1500×g for 5 min. Serum samples were kept at -20 °C until the screening of anti-*Toxoplasma* IgG/IgM antibodies and IFN-γ.

Diagnosis of pulmonary tuberculosis

Direct detection of *M. tuberculosis*

Sputum samples were subjected to concentration/decontamination by N-acetyl-L-cysteine-sodium hydroxide, followed by Ziehl-Neelsen (ZN) staining. The microscopic screening was done for *M. tuberculosis*, which was acid-fast bacilli, measuring 0.2-0.5μm wide and 2-4μm long and stained bright red against a blue background [14].

Culturing on Lowenstein–Jensen medium

Within 2 hours, sputum samples were inoculated on Lowenstein Jensen culture following decontamination and neutralization, and the media was incubated in a CO^2 atmosphere at 37 ºC for a maximum of 8 weeks. The presence of *Mycobacteria* was seen as irregular, dry, and off-white growth after 2-3 weeks. *Mycobacteria* were identified by their specific morphology after staining with Ziehl-Neelsen stain as thin pink rods in a contrasting blue background using x100 objective of the light microscope. After 8 weeks of incubation, cultures that had no growth are considered negative.

GeneXpert assay

This is an automated molecular assay to detect *M. tuberculosis* and rifampicin resistance. It was carried out using the MTB/RIF test platform (GeneXpert, Inc., Sunnyvale, CA, USA). All reagents required for the lysis of bacteria, nucleic acid extraction, amplification, and amplicon detection, were added into a disposable plastic cartridge for sample processing and PCR. Sputum samples were processed using sample reagents (sodium hydroxide and isopropanol), and they were placed into a multi-chambered cartridge after repeated shaking and incubation. After loading the MTB/RIF cartridge, heminested Real-Time PCR was conducted using the GeneXpert device. For a high level of specificity, 5 different molecular probes and 3 specific primers were used [15].

Detection of anti-Toxoplasma IgG/IgM antibodies

According to the manufacturer's instructions, the OnSite™ Toxo IgG/IgM Combo Rapid Test (CTK Biotech, Inc., San Diego, California, USA), a lateral flow immunochromatography test, was used to detect and differentiate anti-*Toxoplasma* IgG and IgM antibodies in sera samples. A mixture of 10 μl of the patient's serum and 70 μl of the kit's diluent was applied onto the sample pad. During this procedure, a recombinant *T. gondii* antigen that has been conjugated with colloidal gold binds to *T. gondii* antibodies present in the patient's serum. The resulting colored complex is captured in the test window coated by mouse anti-human antibodies. After ten minutes, the result was visually read. To ensure that the test strip is functioning, a positive control line must always be present at the strip's top. The test is considered to be negative if only this line appears. A positive response for *Toxoplasma* infection is indicated by the appearance of a second and/or third line.

Determination of IFN-γ serum levels

According to the manufacturer's instructions, serum levels of IFN-γ were estimated by enzyme-linked immunosorbent assay (RayBio® Human IFN-γ ELISA Kit, USA), which is a quantitative sandwich enzyme immune-assay using one polyclonal antibody and another monoclonal antibody specific for IFN-γ. At 450 nm, optical density (OD) values were measured, and the concentrations of serum IFN-γ were calculated from the standard curves. The sensitivity cutoff was 20 pg/ml.

Statistical analysis

SPSS version 25 (Armonk, NY, IBM Corp) was used to analyze the collected data. Chi-square ($\chi 2$) was employed to analyze the prevalence of anti-*Toxoplasma* antibodies among suspected TB patients and the associated risk factors with *Toxoplasma* seropositivity in TB patients. Cohen's Kappa coefficient (κ) was employed to assess inter-rater reliability among TB diagnostic tests. The serum level of IFN-γ was expressed as mean and standard deviations (mean ± SD) and the student t-test was employed for comparing the means between groups. $P<0.05$ was statistically significant and $P< 0.001$ was highly statistically significant.

Results

This study involved 124 patients attending outpatient clinic, TB positivity was detected by examining sputum samples using Ziehl-Neelsen, Lowenstein Jensen culture, and Gene expert assay. Out of 124, 80 (64.52%) were TB positive and 44

(35.48%) were TB negative. In comparison to Gene expert assay results, Ziehl-Neelsen´s results were statistically significant (P<0.001) with substantial agreement as kappa coefficient = 0.752. While Lowenstein Jensen culture results were statistically significant (P<0.001) with an almost perfect agreement as kappa coefficient = 0.947 (Table 1).

Overall, 39.52% (49/124) of participants had *Toxoplasma* antibodies. Seropositivity of *Toxoplasma* infection based on IgG/IgM was 51.25% (41/80) in TB-positive patients and 18.18% (8/44) in TB-negative patients, revealing a significant association between *Toxoplasma* positivity and TB (P<0.001) (Table 2).

Our results recorded that TB patients with *Toxoplasma* infection had higher IFN-γ concentration levels compared to those without *Toxoplasma* infection (P<0.0001). Also, non-TB individuals with *Toxoplasma* infection have higher IFN-γ concentration levels compared to those without infection (P<0.0001) (Table 3).

Statistical analysis indicated a significant association between *Toxoplasma* infection seropositivity in TB patients and the associated chronic diseases, taking immunosuppressive drugs, owing pet animals in the house, eating processed meat, eating improperly cooked meat, consumption of unwashed or inappropriately washed vegetables, and washing hands before eating. The participant's gender and blood transfusion history were not significantly correlated with the participants' seropositivity for *Toxoplasma* infection among TB patients (Table 4).

Table 1. Validation of Ziehl-Neelsen stain and Lowenstein Jensen culture results for diagnosis of TB in comparison to Gene expert assay.

Applied tests	**Gene expert assay**					**Sensitivity**	**Specificity**	**PPV**	**NPV**	**κ (P)**	**Level of agreement**
	Positive		**Negative**		**Total**						
	No. 80	**%** 64.52%	**No.** 44	**%** 35.48%	**No.** 124						
Ziehl-Neelsen											
Positive	66	82.5	1	2.3	67 (54.03%)	82.5	97.7	98.5	75.4	0.752 (<0.001**)	Substantial agreement
Negative	14	17.5	43	97.7	57 (45.97%)						
LJ culture											
Positive	78	97.7	1	2.3	79 (63.71%)	97.5	97.7	98.7	95.6	0.947 (<0.001**)	Almost perfect agreement
Negative	2	2.5	43	97.7	45 (36.29%)						

κ: Kappa test
PPV: positive predictive value
NPV: negative predictive value
** Highly statistically significant P< 0.001

Table 2. Anti-*Toxoplasma* IgG/IgM positivity among tuberculosis patients.

Studied Patients	**No.**	**Anti-*Toxoplasma* IgG/IgM**		**Chi–square (χ^2)**	***P*-value**
		Positive No. (%)	**Negative No. (%)**		
TB +ve	80	41 (51.25%)	39 (48.75%)	12.9876	0.0003**
TB -ve	44	8 (18.18%)	36 (81.82%)		
Total	124	49 (39.52%)	75 (60.48%)		

** Highly statistically significant P< 0.001

Table 3. Interferon-gamma (IFN- γ**)** levels among TB patients

Studied Patients		**No.**	**IFN- γ level Mean±SD (IU/ml)**	**T-test**	***P*-value**
TB patients	Toxo positive	41	41.98±4.36	8.5247	< 0.0001**
	Toxo negative	39	32.2±5.83		
Negative TB	Toxo positive	8	30±2.8	15.9270	< 0.0001**
	Toxo negative	36	13.6±2.6		

**Highly statistically significant *P*<0.001

Table 4. Associated risk factors with *T. gondii* seropositivity among TB patients.

Risk factors	**TB patients (80)**				**χ2**	**P-value**
	Toxo positive (41)		**Toxo negative (39)**			
	No.	**%**	**No.**	**%**		
Gender						
Male	33	80.49	29	74.36	0.4306	0.5117
Female	8	19.51%	10	25.64%		
Immuno-suppressive drugs						
Yes	16	39.02%	3	7.6 %	9.174	0.024*
No	25	60.9. %	36	92.4%		
Chronic disease						
Diabetic	20	48.78%	9	23.08%	6.9414	0.031095*
Hypertensive	4	9.76%	10	25.64%		
No	17	41.46%	20	51.28%		
Washing vegetables						
Yes	15	36.58 %	37	94.87%	27.34	< 0.00001**
No	26	63.42. %	2	5.13%		
Cooking meat well						
Yes	28	68.29%	10	25.64%	12.9205	0.00325*
No	13	31.71 %	29	74.36%		
Washing hands before eating						
Yes	11	26.82 %	33	84.61%	24.6826	0.00001**
No	30	73.18%	6	15.39%		
Eating processed meat						
Yes	22	53.66 %	5	12.82%	13.1378.	0.000289 **
No	19	46.34%	34	87.18%		
Blood transfusion						
Yes	5	12.2%	9	23.08%	0.9723	0.200417
No	36	87.8%	30	76.92%		
Contact with Pet animals						
Yes	28	68.29%	17	43.59%	4.9563	0.025996*
No	13	31.71%	22	56.41%		

* Statistically significant *P*< 0.05
** Highly statistically significant *P*< 0.001

Discussion

Several studies reported an association between toxoplasmosis and chronic diseases, including rheumatoid arthritis [16], chronic liver diseases [17], chronic renal failure [18], and diabetes mellitus [19]. *T. gondii* infection stimulates a potent and sustained Th1 response via the releasing of proinflammatory cytokines such as IFN-γ, tumor necrosis-α and interleukin-12. Effects of these cytokines in combination with other immune mechanisms safeguard the host from consequent pathogenic effects caused by rapid multiplication of *Toxoplasma* tachyzoites. In chronic diseases, the depletion of these cells can cause latent toxoplasmosis to reactivate, which may lead to serious complications. As TB's effects on human health have increased lately with *M. tuberculosis* drug-resistant strains emerging [20], the current study estimated *Toxoplasma* seropositivity and related risk factors in TB patients.

Regarding the diagnostic methods used in this study to detect *M. tuberculosis*, GeneXpert was superior to Ziehl-Neelsen staining and culturing into Lowenstein Jensen medium, in which 80 cases (64.52%) tested *M. tuberculosis* positive by GeneXpert compared to 79 positive cases by culture (63.71%) and 67 cases (54.03%) by Ziehl-Neelsen staining. The difference in detection rates between the three diagnostic methods may be attributed to the variable sensitivity of different diagnostic methods and technical proficiency among the persons conducting diagnostic procedures. In this study, GeneXpert was used as the gold standard due to its high level of sensitivity and specificity. This method has an increased screening capacity besides requiring minimal bacillary concentrations in the samples for the examination, and the problem of cross-contamination is eliminated due to the self-contained cartridges [21].

Compared to GeneXpert, Ziehl-Neelsen staining method's sensitivity and specificity were 82.5% and 97.7%, respectively. This finding agrees with the results of Al Olimey et al. [22], who recorded similar sensitivity and specificity for the Ziehl-Neelsen staining method. However, it contradicts the results of Salam et al. [23] which indicated lower sensitivity and specificity. Lowenstein Jensen medium's sensitivity was 97.5%, which is higher than the sensitivity recorded in an earlier study by Kumari et al. [24]. However, our findings are similar to Al Olimey et al. [22] who reported 100% sensitivity for the Lowenstein Jensen medium. These varying results could be the consequence of a variety of causes, like sample processing.

Overall, 39.52% (49/124) of the study participants had *Toxoplasma* antibodies. Toxoplasmosis is regarded as a serious health issue amongst the Egyptian population, having high seroprevalence levels ranging from 30% among patients with chronic liver diseases [17] to 54% among those with rheumatoid arthritis [16]. In the current study, 51.25% of TB-positive patients also had *T. gondii* infection. According to various research on this topic, there's a substantial association between infection with *T. gondii* and *M. tuberculosis*, and each infection may influence the progression and severity of the other one. Pulmonary tuberculosis severity may be exacerbated by concurrent toxoplasmosis, as well as TB may enhance vulnerability to new infections and could contribute to the reactivation of latent toxoplasmosis [1]. The recorded *T. gondii* seropositivity among TB patients in the present study was less than the reported results of Mashaly et al. [6] (67.4%), Ali et al. [8] (86.2%), Parsaei et al. [25] (71.1%) and higher than that found by Li et al. [26] (17.07%) and Zhao et al. [7] (13.2%). The variations in *T. gondii* infection results between this study and prior research studies could be attributed to varying housing circumstances, economic and social status, climatic circumstances, management systems for cats, eating behaviors of research subjects, and diagnostic strategies, as well as the level of host immunity.

While our findings, as well as those of several other studies, suggested a significant association between TB and toxoplasmosis, it is important to highlight that not all studies have identified this association. Ledru et al. [27] observed no significant relationship between *Toxoplasma* serology and TB-infected patients in Burkina Faso. Similarly, a recent study by Jafari-Shakib et al. [28] in Northern Iran found no significant co-infection between toxoplasmosis and active tuberculosis.

The cytokine IFN-γ has a variety of roles within various components of the immune system. It is essential for the development, maintenance, and regulation of both innate and adaptive immune cells. By activating macrophages, IFN-γ enhances their ability to perform pinocytosis and receptor-mediated phagocytosis and effectively eliminate mycobacteria and other microbes [29]. Our results recorded high IFN-γ levels in TB patients having *Toxoplasma* infection in comparison to those without *Toxoplasma* infection. The obtained result indicated that the higher release of IFN-γ may be due to the immune system activation by *Toxoplasma* infection, which might lead to a higher immunological response to TB. It is essential in avoiding *T. gondii* transmission [30] and has a significant role in TB's pathogenesis [31]. The results obtained are in line with earlier research that revealed high IFN-γ levels in *Toxoplasma* patients [32] and TB patients [33]. It may be necessary to investigate the underlying mechanisms that could explain the high IFN-γ levels in TB patients with *Toxoplasma* infection.

Toxoplasmosis/Tuberculosis co-infection in the present study is significantly associated with some risk factors, including the presence of chronic diseases, taking immunosuppressive drugs, owing pet animals in the house, eating processed meat,

eating improperly cooked meat, consuming improperly washed or unwashed vegetables, and washing hands before eating. The findings of an earlier study done by Mashaly et al. [6] are in agreement with these findings. Existing chronic diseases or taking immunosuppressive drugs probably increase the complications in TB patients. In those patients, *T. gondii* is regarded as an opportunistic and potentially fatal parasite due to immune breakdown, which might change the course of the disease and induce more severe and frequent complications [34]. In addition, consuming improperly washed vegetables is a significant contributor to *T. gondii* oocysts dissemination [2]. Regarding this, the probability of acquiring *Toxoplasma* infection was considerably decreased by salt and detergent washing of vegetables [35].

Human toxoplasmosis is commonly linked to eating raw or improperly cooked meat that contains infected *T. gondii* tissue cysts. It was proven that *Toxoplasma* cysts retain their infectiousness for 30 days at a temperature of 4 °C [36]. This study showed that raw meat consumption considerably increases the probability of *Toxoplasma* infection in TB patients. Results from previous research support our study's conclusion [37]. Moreover, there was a significant association between having pet animals, especially cats, and *T. gondii* seropositivity in this research. This conclusion seems to be compatible with findings from another research [38]. This could be caused by frequent contact with cat waste and accidental *T. gondii* oocysts intake. Additionally, the oocysts could survive in sandy soil for a long time, posing a risk of environmental contamination [39].

Despite the fact that several studies have documented TB and toxoplasmosis coinfection, several aspects remain unclear such as whether toxoplasmosis poses a risk for developing tuberculosis or vice versa, how TB-toxoplasmosis manifests clinically, and the impact of the coinfection disease and how the infections will progress. The study's limitations include the geographic and sample size constraints, which limit the generalizability of the findings. The use of the OnSite™ Toxo IgG/IgM Combo Rapid Test as the sole diagnostic tool may result in potential false positives or negatives without additional testing. Furthermore, the cross-sectional design does not allow for determining causality or examining the long-term impact of coinfection.

Conclusion

This study found a significant association between Toxoplasmosis and *Mycobacterium tuberculosis* infections, with 51.25% of TB patients being positive for *T. gondii*. Co-infection may worsen TB severity and reactivate latent toxoplasmosis, as indicated by increasing IFN-γ levels. Key risk factors include chronic diseases, immunosuppressive drugs, pet contact, and dietary habits. This gives insight into the importance of earlier identification and managing patients with TB-toxoplasmosis coinfection to avoid any serious complications.

Disclosure of potential conflicts of interest

The authors declared that they have no conflicts of interest.

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Ethical approval

The study protocol was approved by the institutional review board (IRB) at the Faculty of Medicine, Suez University, Suez, Egypt (Approval Number 111222, Dec 2022). The study was conducted according to the Helsinki Declaration's guidelines in 2013. All participants in the study received enough information about the study's purpose, and informed written consent was obtained from each one after making a voluntary decision to participate while maintaining the confidentiality of their data.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contribution

N.M. El-Sayed and M.E. Ramadan proposed the research idea and the study design, S. A-S. Mahmoud and D. A. Ezzeldin collected the study samples. All authors are equally contributed in laboratory performance of the work, interpreted the results, wrote the manuscript and approved the final version of the manuscript.

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