

PREVALENCE OF TOXOPLASMOSIS AS COINFECTION IN IRAQI PATIENTS INFECTED WITH TUBERCULOSIS

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ABSTRACT : The aim of the paper is to study the prevalence of *Toxoplasma* infection in he TB patients in comparison with individuals apparently healthy. A total of one hundred and twenty-eight selected TB patients have collected from the Specialized Center for Chest and Respiratory Disease in Baghdad who have the TB symptoms. They divided into three groups; newly diagnosed TB (NDTB) (58), old diagnosed patients(ODTB) (36) and multidrug resistance patients (MDRTB) (34) compare with forty-six apparently healthy individuals as a control group. The detection depend on Anti-Toxoplasma IgG in their serums by using Toxoplasma IgG enzyme-linked immunosorbent assay kit. The results of the anti-Toxoplasma IgG showed significant differences among the groups and control; mean±SD 15.756±3.1, 14.28±3.04, 14.14±3.27, 10.35±3.14 respectively, and there were a highly significant differences between subgroups of patients and the control group in the percentage of infection with toxoplasma P=0.000. The present study concludes that the prevalence of anti-toxoplasma IgG among TB patients was highly significant than the healthy control group, and MDRTB subgroup has a high prevalence of *Toxoplasma* infection than other subgroups due to a weak immunological status, and increase antibacterial tolerance and the deteriorate prognosis of a disease.

Key words : Toxoplasma, IgG, TB patients

INTRODUCTION

Tuberculosis and Toxoplasmosis are an infectious disease; Tuberculosis is a disease caused by bacteria called *Mycobacterium tuberculosis* which attack the lungs, and may damage other parts of the body which affects nearly one-third of the world's population (Mathema B *et al*, 2015) and Toxoplasmosis is a common zoonosis disease caused by a single-celled parasite called *Toxoplasma gondii* which affects 30% of the population worldwide (Dubey J P and Jones J L, 2018).

The relationship between the different infectious diseases considers as one of the common problems in developing countries which is known as co-infection (Xin-Xu Li1 and Xiao-Nong Zhou, 2013 and Zhao Y J *et al*, 2017).

The role of coinfections may make the patients more immuno-competent or susceptible to another pathogen; increased level of clinical suspicion for co-infections with tuberculosis with many diseases as Cryptococcal (Singh U B *et al*, 2017 and Fang W *et al*, 2017), HIV (Sardar P *et al*, 2009 and Bell LC K, Noursadeghi M, 2017), Toxoplasma (Kang Y H *et al*, 2016 and Zhao Y J *et al*,

2017) and other diseases thereby indicating that tuberculosis could be the reason for immunosuppression and induce occurrence of the opportunistic infections.

Toxoplasmosis and Tuberculosis are a global problem Which afflicts adequate probability of the people in the world and these two diseases may present in a latent state (Pontarelli A1 *et al*, 2018; Mueller-Hermelink M1 *et al*, 2018 and Egorov A I *et al*, 2018) these diseases can be transformed from the latent state into an effective state depending on many factors, and the most important factor is the health of the patient. Many studies investigate the causal agent of diseases in isolation state while we are in contact with the environment microbes so in a whole time we are vulnerable to coinfections of different pathogens causes the aim of this study was to investigate the prevalence of *Toxoplasma* in TB patients.

MATERIALS AND METHODS

Study samples

Patients group were recruited at the Specialized Center for Chest and Respiratory Disease in Baghdad between July 2016 to February 2017, One hundred and twenty-eight male and female patients were collected and

divided into three subgroups according to the type and duration of the antibiotic treatment; first group included (58) patients with newly diagnosed TB who exposed to the tuberculosis for the first time (NDTB). The second group included (36) patients with old diagnosed TB who received first-line TB therapy and the treatment was with isoniazid, rifampicin, and pyrazinamide for two months, followed by isoniazid and rifampicin for four months (ODTB). The third group was (34) patients with multidrug resistance who define as bacillary TB resistance to at least both Rifampacin & Isoniazid (MDRTB).

The control group (C) consist (46) apparently healthy male and female individuals, who selected from the people that attend to the same center to get the healthy card which was necessary to get a job that deals with human health like restaurant worker, who investigated for TB and other chronic disease and found negative

Sputum and blood collection

Sputum and blood specimens were collected from patients have clinical symptoms such as chronic cough for more than a month, have a bloody sputum, and X-ray positive for TB. Five ml of real sputum, not saliva and were obtained from the patients groups and the control group in the early hours of the morning and included in sterile sputum collection containers for TB and three smears replicates of the sputum were directly made and stained by Ziehl-Neelsen stain. All sputum specimens were cultured in Lowenstein Jensen culture

Two ml of the blood were collected and serum obtained and stored at -20°C until used for toxoplasma IgG antibodies investigations.

Detection of anti-Toxoplasma IgG antibodies

Anti-Toxoplasma IgG was measured by using Toxoplasma IgG enzyme-linked immunosorbent assay kit, Bioactiva Diagnostica, Germany, the manufacturer's

instructions were adopted.

RESULTS AND DISCUSSION

The patients group was divided, according to the type of the medication, in to three subgroups, a new diagnosed patients, under treatment; old diagnosed patients, and multi-drug resistance patients, there was no a significant difference between these patient subgroups and control group in the prevlance regarding to gender as seen in table 1, the percentage of gender was 67.2 male and 32.8 female in patients subgroups while in the control group were 73.9 and 26.1 respectively, $X^2 = 3.915$, $P = 0.271$, and there were no a significant differences between all groups in the mean of age $P = 0.435$ (Table 1).

The statistical binomial test utilizes for the three parameters of fever, chest X-ray, and weight loss as described below for detecting the significantly between positive and negative of each subgroup, the higher percentage of fever was found in newly diagnosed TB patient subgroup 43 (74.1%) and a highly significant ($p \leq 0.01$) as shown in the table (2), in the other hand the under-treatment subgroup was significant ($p \leq 0.05$) and not significant in multi-drug resistance subgroup.

Beside that Table (2) indicated that chest X-ray was a highly significant difference between positive and negative in three subgroups of patients at probability ($p \leq 0.01$). Otherwise, weight loss parameter in the two subgroups, multi-drug resistance subgroup and newly diagnosed TB subgroup patient has a highly significant difference ($p \leq 0.01$), furthermore old diagnosed TB patient subgroup was a significant difference ($p \leq 0.05$).

In addition, the chi-square test showed non-significant differences between three subgroups of patients for three parameters.

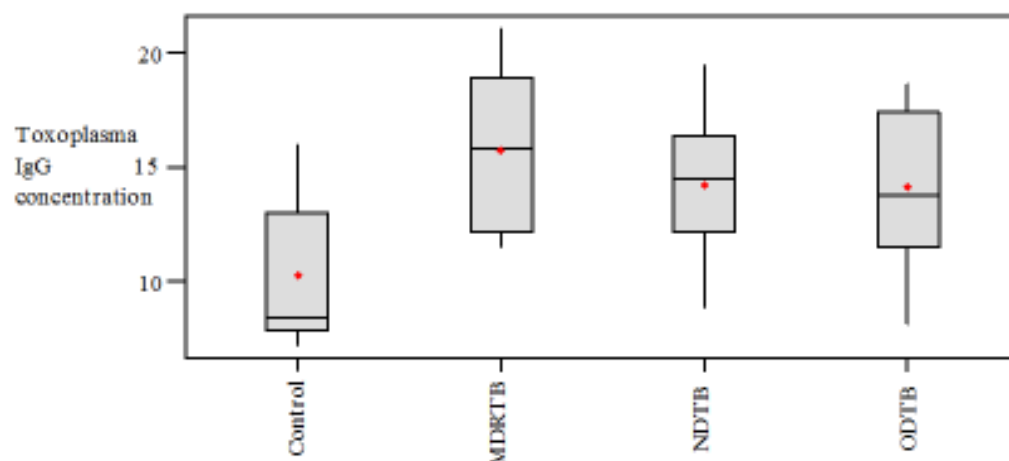


Figure 1: Serum level of Toxoplasma-IgG in control and the sub groups TB patients.

Table 1 : Tuberculosis incidence(%)among study groups in female and males.

Group	Male(%)	Female(%)	Binomial Test	AgeMean±SD
Control (46)	34 (73.9)	12 (26.1)	P=0.001**	39±11.9
MDRTB (34)	24 (70.6)	10 (29.4)	P=0.013**	36.8±8.6
NDTB (58)	42 (72.4)	16 (27.6)	P=0.0005**	36.1±12.1
ODTB (36)	20 (55.6)	16 (44.4)	P=0.308 ^{NS}	39±8.7
Total of patients	86 (67.2)	42 (32.8)	P=0.00007**	P= 0.435
Chi-Square =3.915 P=0.271				

Table 2 : Relationship between clinical signs and study groups.

Group	MDRTB(34)	NDTB(58)	ODTB(36)	Chi-square test
Fever Positive (%)	19(55.9)	43(74.1)	24(66.7)	X ² =3.246P= 0.197
Fever Negative (%)	15 (44.1)	15 (25.9)	12 (33.3)	
Binomial Test	P=0.303 ^{NS}	P=0.0002**	P=0.033*	
Chest X-ray Positive (%)	30(88.2)	56 96.6)	30(83.3)	X ² =4.879P=0.087
Chest X-ray Negative (%)	4 (11.8)	2 (3.4)	6 (16.7)	
Binomial Test	P=0.00001**	P=0.000001**	P=0.0007**	
Weight loss Positive (%)	28(82.4)	43(74.1)	24(66.7)	X ² =2.249P=0.325
Weight loss Negative (%)	6 (17.6)	15 (25.9)	12(33.3)	
Binomial Test	P=0.0002**	P=0.0002**	P=0.0333*	

Table 3 : Serum level of Toxoplasma –IgG in the study groups.

Groups	Toxoplasma IgG concentration IU/ml Mean ±SD	Min-Max	P value
Control (46)	10.35±3.14	7.3-16	1. Between all groups=0.00**
MDRTB(34)	15.756±3.1	11.5-21	2. C&MDRTB=0.00**
NDTB(58)	14.28±3.04	8.9-19.5	3. C&NDTB=0.00**
ODTB(36)	14.14±3.27	8.2-18.6	4. C&ODTB=0.00**
			5. MDRTB&NDTB=0.026*
			6. MDRTB&ODTB=0.037*
			7. NDTB&ODTB=0.85 ^{NS}

Table 4 : Differences between subgroups of patients and the control group in the percentage of infection with toxoplasma

	Control group (C) (46)	MDRTBsubgroup (34)	NDTBsubgroup (58)	ODTBsubgroup (36)
Toxoplasma Positive IgGNo (%)	18 (39.1%)	34 (100%)	50 (86.2%)	33 (91.7%)
Toxoplasma Negative IgGNo (%)	28 (60.9%)	0(0%)	8(13.8%)	3(8.3%)
		Chi square =55.524	P=0.0000	

The semi-quantitative concentration of IgG anti-Toxoplasma was done and expressed as International Units/ml as shown in the table (3) and figure (1) There was significant differences among the groups and control mean±SD 15.756±3.1, 14.28±3.04, 14.14±3.27, 10.35±3.14 respectively. Moreover, Table 4 summarizes a high prevalence of Toxoplasma among subgroups of TB patients compared with control.

Toxoplasma gondii and *Mycobacterium tuberculosis* are an intracellular obligate infectious microorganism that causes toxoplasmosis for 30-50% and 9.0–11.1 million

developed TB disease in 2017 and 23% has a latent TB infection, of the population around the world (Flegr J *et al*, 2014; World Health Organization. Global tuberculosis report, 2018) respectively.

The prevalence of toxoplasmosis disease in several countries reported to be high because it depends on lifestyle, it represent, 61.8% in Cuba, 63.5% in Colombia, 63.9% in Iran, 75.2% in Sao Tome and Principe and 77.5% in Brazil, (Montazeri M *et al*, 2017), while the prevalence of TB disease in many countries were declared in WHO lists of the 30 highest TB burden

countries accounted to be 87% of the world's cases, which include only 3% of the European Region and (3%) of the Americas Region (World Health Organization. Global tuberculosis report, 2018).

This study showed the prevalence of anti-Toxoplasma IgG antibodies in the healthy control group were 39.1%, this is consistent with previous Iraqi studies, which found that a significant proportion of those infected was in the healthy control group 57.1% ,42.5 % Al-Dulaimi S B K *et al*, 2012 and Muhsin S S *et al*, (2018) respectively.

The common important serious health problems in endemic areas in developing countries were co-infections, and there are no adequate studies showing the prevalence of toxoplasmosis in tuberculosis patients as co-infection except very limited studies in Egypt (Mashaly M *et al*, 2017), China (Zhao Y J *et al*, 2017), thus, this study designed to address this issue in Iraqi patients infected with TB.

The prevalence of anti-toxoplasma IgG among TB patients was highly significant than the healthy control group, especially in the MDRTB, NDTB, ODTB subgroups were 100%, 86.2 %, 91.7% respectively may due to the weak immunological status of TB patients, TB and many parasitic diseases overlap with the psychological and political situation, poverty, lifestyle, socio-demographics, and the immunity status of the population where TB and parasites infections occur in the same individual and lead to exacerbating the disease, as in many studies the co-infections between TB and helminths can contribute to the development of TB (Zhao Y J *et al*, 2017; Fang W *et al*, 2017 and Rathnayake R R *et al*, 2010). This study is consistent with the research of Ledru and their colleague (Ledru E *et al*, 1995), who found that there is no significant difference when compared with the healthy control group and differs with the study of Mashaly and their colleague (Mathema B *et al*, 2015), where there was a significant difference with the healthy control group.

The increasing prevalence of Toxoplasma in TB patients may be due to a weak level of hygiene through the preparation of food, during their erroneous dealing with domestic animals such as cats and dogs and poor sanitary habits.

Researchers mentioned that co-infection cause inhibition of the host immunity to a great extent due to many factors previously mentioned which led to coinfections of tuberculosis (TB) with parasitic diseases in human like Hydatid disease, Leishmaniasis, Strongyloidiasis, Giardiasis, Schistosomiasis (Zhao Y J *et al*, 2017; Li XX and Zhou X N, 2013)

CONCLUSION

The present study concludes that the prevalence of anti-toxoplasma IgG among TB patients was highly significant than the healthy control group, and MDRTB subgroup has a high prevalence of Toxoplasma infection than other subgroups due to a weak immunological status, and increase antibacterial tolerance and the deteriorate prognosis of a disease.

Author's contributions

All the authors are equally contributed to complete this research.

Conflicts of interest

There are no conflicts of interest of any sort.

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