An Evaluation of QuantiFERON-TB Gold In-Tube and Immunological Tests for TB Diagnosis in Iraqi Patients

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Authors' contributions

Author MM designed the experiment,wrote the protocol,supervised and guided AN and read and approved the final manuscript. N M carried out the experiment and wrote the first draft of the manuscript.

ABSTRACT

Aims: Tuberculosis is an infectious disease caused by the bacillus *Mycobacterium tuberculosis* and other related species. It typically affects the lungs (pulmonary TB) but can affect other sites (extra-pulmonary TB). A profusion of articles have been published on the accuracy and uses of interferon-gamma releasing assays for detection of this disease.

Experimental design: A prospective study.

Place and duration of study:This study was done in Kirkuk city between November 2012 to February 2013.

Methodology: The present study included 50 individuals (40 suspected tuberculosis patients and 10 healthy controls). The patient were examined for the presence of TB by using QuantiFERON-TB Gold In-Tube(QFT-GIT) assay, polymerase chain reaction (PCR) and compared them with certain new and routine tests like AFB smear, *OnSite* TB rapid test, erythrocyte sedimentation rate and chest X-ray.

Result: The present study showed a relation between QFT-GIT and *OnSite* TB rapid test, and they were positive in 25(86%) at the same time; QFT-GIT positive and *OnSite* TB rapid test were negative in 4(14%) of patients; QFT-

GIT negative and *OnSite* TB rapid test positive were seen in 5(45%); while QFT-GIT and *OnSite* TB rapid test were negative in 6(55%) of patients. In the control group only one QFT-GIT positive but it was *OnSite* TB test negative. 9(100%) of individuals for both tests were negative, 29 were males and 21 were females.

Conclusion: The study highlighted the sensitivity of IGRAs for diagnosis of active TB in combination with the rapid IgM/lgG tests for TB.The QFT assay appeared to be a more specific indicator of latent TB infection than TST. The association with blood groups and vaccination is also significant.

Key words: QuantiFERON-TB Gold,Vaccination,BCG,TST, *OnSite* TB IgG/IgM,Blood groups,Tuberculosis,Iraq

1.INTRODUCTION

Mycobacterium is a genus of Gram-positive bacilli that demonstrates the staining characteristic of acid-fastness. Its most important species, *Mycobacterium tuberculosis*, is the most common etiologic agent of tuberculosis[1]. Other species like *M.bovis*, *M. microti, M. canettii, M pinnipedii and M. Caprae* also reported to be causative agents of various types of tuberculosis[2]. Tuberculosis causes ill-health among millions of people each year and ranks as the second leading cause of death from an infectious disease worldwide[3]. Iraq shows its dedication and commitment to prevent and control TB. Between 2003 and 2012, the TB case detection rate gradually and consistently increased to reach thousands cases in 2012 particularly pulmonary type [4].Other works on this disease have been carried out that showed different frequencies of incidences utilizing onsite serolological rapid tests[5,6].Since 1980,Iraq is passing through a series of wars that might influence the status of infectious diseases including TB.There are two main types of TB; one is the latent TB, which means a person carries the

TB germ but is not sick and cannot pass the germ on to other people and the other type is active TB [7].

Latent tuberculosis infection (LTBI), a non-communicable asymptomatic condition, persists in some, who might develop tuberculosis disease months or years later. The main purpose of diagnosing LTBI is to consider medical treatment for preventing tuberculosis disease [2]. The tuberculin skin test (TST) is widely utilized for detection of *M. tuberculosis* infection, but this test has important limitations. The TST can cross-react with non-tuberculous mycobacterial (NTM) species or Bacille Calmette Guerin (BCG) vaccine, thereby complicating the interpretation of TST results especially in BCG-vaccinated individuals from TB-endemic settings. These limitations may reduce TST specificity, and may reduce patient and provider confidence in TST results. Interferon-gamma release assays (IGRAs), such as the commercially available QuantiFERON-TB Gold-In Tube (QFT-GIT, Cellestis, Ltd, Carnegie, Australia) test, has the potential to overcome some of TST's limitations [8]. The availability of *Mycobacterium tuberculosis* antigen specific interferon-gamma (IFN-γ) release assays (IGRAs) represents a significant advance in the field of TB diagnosis [9].

In 2001, the QuantiFERON-TB test (QFT) became the first IGRA approved by the food and drug administration (FDA) as an aid for diagnosing *M. tuberculosis* infection [10,11]. In 2005, the QuantiFERON-TB Gold test (QFT-G) became the second IGRA approved by FDA as an aid for diagnosing *M. tuberculosis* infection. The U.S. Centers for Disease Control and Prevention (CDC) published guidelines for using QFT in 2003 and for using QFT-G in 2005 [12]. The QuantiFERON-TB Gold In Tube test is a test for cell mediated immune (CMI) responses to peptide antigens that simulate mycobacterial proteins [13,14,15].

Since QFT-GIT is a quantitative blood test, its results are less subjective than those of TST. Recently, CDC provided guidance that IGRAs are an acceptable alternative to TST for the detection of *M. tuberculosis* infection, and are the preferred option in some circumstances including testing of BCG-vaccinated populations [(16)]. Several new serological tests for the diagnosis of tuberculosis have been developed in the last decade. Purified antigens and the

use of monoclonal antibodies have begun to overcome the problem posed by the broad cross reactivity of crude extracts from *Mycobacterium tuberculosis* [16,17].

2.MATERIALS AND METHODS

2.1 Study Population:

Fifty individuals (forty suspected tuberculosis patients who were referred to the Consultant Clinic for Respiratory Disease for suspected tuberculosis examination in Kirkuk city and ten healthy controls)were enrolled in this study between November 2012 to February 2013. A full history was taken from each patient including the sex, age, residency, occupation, BCG vaccination, history of cough, night sweating, hemoptysiis, fever and loss of weight. Five ml sample of blood was collected by venipuncture using a disposable syringe or vacuum tube needle for each patient enrolled in this study, One ml of whole blood was added to each of the three QuantiFERON tubes: (Nil, TB Antigen and Mitogen) for ELISA usage. Finally, 2 ml whole blood placed in labeled vacuum tubes for blood group typing[18] and then centrifuged and serum separated and transferred into clean test tube and stored at -20°C for further serological testing for detecting specific *OnSite* TB IgG/IgM rapid test cassette. QFT-GIT testing was performed according to manufacturer's instructions[19]. **PCR, AFB, erythrocyte sedimentation rate and chest X-ray were carried out for all the patients studied.**

Samples for QFT-GIT test were stored at room temperature for up to 2 hours at the consultant clinic for respiratory disease in Kirkuk, until transportation to the laboratory. Following incubation and centrifugation, harvested plasmas were stored at 4°C for up to 2 months prior to ELISA testing. Results were calculated and interpreted by the assay software as positive, negative, or indeterminate, according to manufacturer's instructions. Tests were interpreted as indeterminate if the mitogen minus nil was < 0.5, or the nil was > 8.0; tests were interpreted as negative if the TB antigen minus nil was < 0.35, or if the TB antigen minus nil was ≥ 0.35 but was < 25% of the Nil value; tests were interpreted as positive if the TB antigen minus nil was ≥ 0.35 and was ≥ 25% of the nil value [18].

For the TST, 0.1 ml of tuberculin PPD [equivalent to three tuberculin units (TU) of purified protein derivative solution (PPD-S)] was injected

intradermally into the volar aspect of the forearm, and the transverse induration diameter was evaluated at 48-72hours after the injection. The results of the test were interpreted by hospital staff based on the patient's degree of risk, according to current guidelines [20].

2.2 Statistical Analysis

Comparison carried out using chi-square (X^2) and probability (P value). The P value ≤ 0.05 was considered statistically significant, and less than 0.001 considered highly significant and greater than 0.05 considered non-significant.

3. RESULTS

In this study the highest positives of Quantiferon Tb were found within the age group 31-60 years, while in control only one positive was found within age group1-30 years. The present data reported that males were at higher risk of tuberculosis infection. Residence revealed that the highest positive TB cases were found among peoples of urban area (Table 1). Vaccination revealed the highest number and percentage 26(89.7%) was found in the vaccinated patients; while in control the only positive case was found in vaccinated person as shown in Table 2.

In the present study shows a correlation between QFT-GIT and *OnSite* TB rapid test, they were positive in 25(86%); QFT-GIT positive and *OnSite* TB rapid test were negative in 4(14%) of patients; QFT-GIT negative and *OnSite* TB rapid test positive were seen in 5(45%); while QFT-GIT and *OnSite* TB rapid test were negative in 6(55%) of patients. In control group only one QFT-GIT positive but it was *OnSite* TB test negative. 9(100%) of individuals for both tests were negative.statistically,there was a highly significant difference between the two tests($P<0.001$), see Table 3.

The blood grouping (ABO) and rhesus typing were performed in this study with relation to QuantiFERON-TB Gold-In Tube (QFT-GIT). The highest positive result was found in patients of O^+ 13 (45%); B^+ 9(31%); A^+ 7(24%); only one positive result was found in control group of AB^+ 1(100%). While negative results in patients and controls respectively were found in O^+ 4(36%), 2(22%) and B^+ 2(18%), 6(67%) and A^+ 4(36%), (0%) and AB^+ 1(9.1%), (11%). Statistically, there were highly significant

differences(P<0.01) between different blood groups with reference to QFT-GIT, see Table 4.

In the present study as shown in Table 5, three extra-pulmonary TB patients were tested with both TST and QFT-GIT which were positive, 3(100%). Statistically, there was a significant relation between TST and QFT-GIT.The present study showed that almost 70%(21/29) of the patients infected with active Tb.

4. DISCUSSION

The presented results indicate that tuberculosis affect mainly young age group and is more common in males. Schwartzman *et al.*, showed that the mean age of his patients was 29 years and expansion of DOTS program would cost saving for patients and governments [21]. In relation to QFT-GIT and *OnSite* TB IgG/IgM test, they were positive in 25(86%); QFT-GIT positive and *OnSite* TB test were negative in 4(14%) of patients; QFT-GIT negative and *OnSite* TB test positive were seen in 5(45%); while QFT-GIT and *OnSite* TB test were negative in 6(55%) of patients. Kim *et al*. reported that QFT had a significantly higher sensitivity than easy test TB, and concluded that the combination of easy test TB and QFT could be used to aid in a rapid diagnosis and early treatment of TB [22]. The vaccination reported in the present study showed the highest number 26(89.7%) was found in vaccinated patients. Statistically, there was a non-significant (P> 0.05) association with a QFT-GIT test and vaccination. However, Diel *et al.* concluded that the QFT assay was unaffected by BCG vaccination status, unlike the TST. In close contacts who were BCG-vaccinated, the QFT-GIT assay appeared to be a more specific indicator of latent tuberculosis infection than the TST, and similarly sensitive in unvaccinated contacts [23]. The blood grouping ABO and rhesus typing were performed in 50 individuals (40 suspected tuberculosis patients and 10 healthy controls) examined according to QuantiFERON-TB Gold-In Tube (QFT-GIT). The highest positive result was found in patients of O^+ 13 (45%)%); B^+ 9(31%); A^+ 7(24%); and only one positive result was found among control group of AB^+ 1(100%). Statistically, there was a highly significant (P< 0.01) differences. This result almost similar to those of Sybirna *et al.* who reported an increase in the number of persons with blood group O and B and decrease in those with A blood group among the examined patients comparatively with the control group determined. Considerable depression of activity of T-lymphocytes of sick persons with blood group O and B and specific immunity of patients with blood group O in comparison with blood group A was revealed [24]. Three patients had extra-

pulmonary TB tested with QFT-GIT were positive 3(100%) for both TST and QFT-GIT. Statistically, there were a significant (P <0.05) relation between TST and QFT-GIT. Although, IGRA have higher specificity than tuberculin skin testing in BCG-vaccinated populations [15]. Finally this study recommended that QuantiFERON-TB Gold test was a new measure belonging to a new class of immunological tests for LTBI which is based on modern immune assay technology and exhibit several improvements in test format over the TST like: One patient visit, fully controlled, unaffected by BCG vaccination status, high sensitivity and specificity.

Table 1: Distribution of QuantiFERON-TB Gold In Tube positivity according to predisposing factors (age, sex, residence, smoking, diabetes, hypertension and vaccination), of patients and controls tested.

Predisposing factors		QuantiFERON-TB-Gold In Tube			
		Patient		Control	
		+ve		+ve	
		No.	%	No.	%
Age (years)	7-30	6	20.7	1	100
	31-60	15	51.7	0	0
	61-91	8	27.6	0	0
	Total	29	100	1	100
X^2= 3.399, P-value= 0.0724 (Non-significant)					
Sex	Male	16	55.2	0	0
	Female	13	44.8	1	100
	Total	29	100	1	100
X^2=1.182, P-value= 0.612 (Non-significant)					
Residence	Urban	21	72.4	0	0
	Rural	8	27.6	1	100
	Total	29	100	1	100
X^2= 2.414, P-value= 0.712 (Non-significant)					
Smoking	Smoker	12	41.4	0	0
	Non-smoker	17	58.6	1	100

	Total	29	100	1	100
X^2= 0.690, P-value= 0.872 (Non-significant)					
Diabetes	Yes	7	24.1	0	0
	No	22	75.9	1	100
	Total	29	100	1	100
X^2= 0.315, P-value= 0.788 (Non-significant)					
Hypertension	Yes	4	13.8	0	0
	No	25	86.2	1	100
	Total	29	100	1	100
X^2= 0.159, P-value= 0.979 (Non-significant)					
Vaccination	Done	26	89.7	1	100
	Not done	3	10.3	0	0
	Total	29	100	1	100

X^2= 0.115, P-value= 0.921 (Non-significant)

Table 2: Distribution of QuantiFERON-TB Gold In Tube According to Vaccination.

Vaccine	**QuantiFERON-TB Gold-In Tube Test**				**QuantiFERON-TB Gold-In Tube Test**			
	Patient				**Control**			
	+ve		**-ve**		**+ve**		**-ve**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Done	26	90	10	91	1	100	8	89
Not done	3	10	1	9.1	0	0	1	11
Total	29	100	11	100	1	100	9	100

X^2=0.137 P-Value = 0.82 (Non- significant);+ve,positive test;-ve,negative test.

Table 3: The Diagnostic Significance of QuantiFERON-TB(QFT-GIT) Gold-In Tube.

***Onsite* TB IgG/IgM Rapid Test**	**QFT-GIT Test**							
	Patient				**Control**			
	+ve		**-ve**		**+ve**		**-ve**	
	No.	%	No.	%	No.	%	No.	%

+ve	25	86	5	45	0	0	0	0
-ve	4	14	6	55	1	100	9	100
Total	29	100	11	100	1	100	9	100

X^2=24.269, P-Value = 0.0003 (Highly Significant);+,positive test;-ve,negative test.

Table 4: Relation Between QuantiFERON-TB Gold-In Tube of Patients and Controls Tested and Blood Groups ABO.

Blood groups	**QuantiFERON-TB Gold-In Tube Test**							
	Patients				**Controls**			
	+ve		-ve		+ve		-ve	
	No.	%	No.	%	No.	%	No.	%
A+	7	24	4	36	0	0	0	0
A-	0	0	0	0	0	0	0	0
B+	9	31	2	18	0	0	6	67
B-	0	0	0	0	0	0	0	0
AB+	0	0	1	9.1	1	100	1	11
AB-	0	0	0	0	0	0	0	0
O+	13	45	4	36	0	0	2	22
O-	0	0	0	0	0	0	0	0
Total	29	100	11	100	1	100	9	100

X^2=25.71, P-Value = 0.00216 (Highly Significant);+ve,positive test;-ve,negative test.

Table 5: The Positive Result of Tuberculin Skin Test (TST) in Relation to QuantiFERON-TB Gold-In Tube of Extra-pulmonary TB Patients Examined.

QuantiFERON-TB Gold In Tube	Tuberculin Skin Test (TST)	
	Positive	
	No.	%
+ve	3	100
-ve	0	0
Total	3	100

X^2= 6.000, P-value= 0.014 (Significant);+ve,positive test;-ve,negative test.

5.CONCLUSION

Blood group ABO and rhesus typing were found to be the most prevalent in pulmonary TB patients with blood group O +ve. Seroprevalence of *Mycobacterium tuberculosis* antibodies was relatively high in IgG antibody. QuantiFERON TB Gold In Tube high sensitivity and specificity compared to TST, and requires only one visit. In close contacts which were BCG-vaccinated, the QFT assay appeared to be a more specific indicator of latent TB infection than TST.

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CONSENT

All authors declare that prior to recruiting respondents; a written informed consent was

obtained from them after the purpose of the study had been fully explained to them. The respondents were also assured of the privacy of information giving and their voluntary participation in the study. Again, 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report.

ETHICAL APPROVAL

Ethical clearance for the study was obtained from the Committee of Higher Studies in College of Medicine,University of Tikrit. The researcher did not in any way expose participants of the study to physical or psychological harm. Participation in the study was strictly voluntary with the informed consent of participants that guaranteed their right to privacy. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki."

COMPETING INTEREST

Authors have declared that no competing interests exist.

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