Utilization of QuantiFERON-TB Gold In-Tube for TB Diagnosis with Reference to other Immunological Tests of Iraqi Patients

Key words

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

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Abstract

A profusion of articles have been published on the accuracy and uses of interferon-gamma releasing assays. This study was done in Kirkuk city between November 2012 to February 2013, to explore immunological facts about tuberculosis and aims to find out possible association between the different blood group ABO and Rh system among TB patients. It included 50 individuals (40 suspected tuberculosis patients and 10 healthy controls), 29 were males and 21 were females, Their age range between 7 to 91 years old and patients of age group 31-60 years were mostly infected with TB. According to vaccination the highest percentage of positive results were found in BCG vaccinated TB patients. The patient were examined for the presence of TB by using QuantiFERON-TB Gold In-Tube assay, Tuberculin skin test(TST), *OnSite* TB IgG/IgM rapid test. We concluded that QFT-GIT implementation for LTBI evaluation in consultant clinic for respiratory disease significantly reduced the proportion of referred individuals in whom LTBI was diagnosed. In close contact that was BCG-vaccinated, particularly if BCG is received after infancy, the QFT assay appeared to be more specific indicator of latent TB infection than TST.

Introduction:

Mycobacterium is a genus of Gram-positive bacilli that demonstrates the staining characteristic of acid-fastness. Its most important species, *Mycobacterium tuberculosis*,

which is the common etiologic agent of tuberculosis. One of the oldest and most devastating of human afflictions, tuberculosis remains a leading cause of infectious disease deaths worldwide today [(1)]. Tuberculosis is a communicable disease caused by infection with *M. tuberculosis* complex organisms (*M. tuberculosis, M. bovis, M. africanum*), which typically spreads to new hosts via airborne droplet nuclei from patients with respiratory tuberculosis disease. A newly infected individual can become ill from tuberculosis within weeks to months, but most infected individuals remain well (curable) if treated [(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. The latest estimates included in 2012 report are that there were almost 9 million new cases in 2011 and 1.4 million TB deaths (990,000 among HIV negative people and 430,000 HIV-associated TB deaths) [(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 57% which is 8,664 TB cases in 2012 [(4)]. Tuberculosis is a disease in which bacteria may invade many parts of the body, such as the brain, the kidney and the spine called (extra-pulmonary TB), TB most common target is the lungs called (pulmonary TB), the TB bacteria damage so much that it is difficult for a person to breath. 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. The other type is active TB, The people with this form of the disease do get sick [(5)].

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 [6]. The availability of *Mycobacterium tuberculosis* antigen specific interferon-gamma (IFN-γ) release assays (IGRAs) represents a significant advance in the field of TB diagnosis [7].

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 [8,9]. In 2005, the QuantiFERON-TB Gold test (QFT-G) became the second IGRA approved by FDA as an aid for diagnosing *M. tuberculosis* infection. CDC published guidelines for using QFT in 2003 and for using QFT-G in 2005 [10]. The QuantiFERON-TB Gold In Tube test is a test for cell mediated immune (CMI) responses to peptide antigens that simulate mycobacterial proteins. These proteins, ESAT-6, CFP-10 and TB7.7(p4), are absent from all BCG strains and from most nontuberculosis mycobacteria with the exception of *M. kansasii, M. szulgai* and *M. marinum* [11].

Numerous studies have demonstrated that these peptides antigens stimulate IFN-γ responses in T-cells from individuals infected with *M. tuberculosis* but generally not from uninfected or BCG vaccinated persons without disease or risk for LTBI [12]. To avoid cross-reactivity, these tests use antigens encoded in the region of difference 1 (RD1), a portion of the *Mycobacterium tuberculosis* genome that is absent from the genome of BCG and many non-tuberculosis mycobacteria (NTM) [13]. The TST assesses in vivo delayed-type hypersensitivity (Type IV), whereas QFT and QFT-G measure in vitro release of IFN-*γ*. The TST and QFT measure response to PPD, a polyvalent antigenic mixture, whereas QFT-G measures response to a mixture of synthetic peptides simulating two specific antigenic proteins that are present in PPD [10].

Since QFT-GIT is a quantitative blood test, its results are less subjective than those of TST. Recently the U.S. Centers for Disease Control and Prevention (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 [14]. 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* [15].

Materials and Methods

Study population:

Fifty individuals (forty suspected tuberculosis patients and ten healthy controls) referred to the consultant clinic for respiratory disease in Kirkuk city, for suspected *M. tuberculosis* infection were enrolled in this study between November 2012 to February 2013. A full history was taken from each patient including the age, residency, occupation, BCG vaccination, history of cough, night sweating, hemoptysis, fever and loss of weight. Five ml sample of blood was collected by vein puncture using 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 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 [16].

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 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 [16].

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 [17].

Statistical Considerations

Computerized statistically analysis was performed using Minitab for data management. Comparison carried out using chi-square (X^2) and probability (P value). The P value $\leq$ 0.05 was considered statistically significant, and less than 0.001 considered highly significant and greater than 0.05 considered non-significant.

Results

In this study the highest positives were found within the age group 31-60 years, while in control only one positive was found within age group1-30 years. According to sex the highest positive was found in male. According to residence the highest positive was found among peoples of urban area. vaccination revealed the highest number and percentage 26(89.7%) was found in vaccinated patients; while in control the only positive case was found in vaccinated person as shown in table 1.

In the present study shows relation 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, see Table 2.

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, see Table 3.

In the present study as shown in Table 4, 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.

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 [18]. 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 [19]. 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 [20]. 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 [21]. In this study because of unavailability and high cost of price of tests only 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. Trajman *et al*. who concluded that both tests are accurate to detect latent tuberculosis. Although, IGRA have higher specificity than tuberculin skin testing in BCG-vaccinated populations [13]. Finally this study recommended that QuantiFERON-TB Gold test was a new measure belonging to a new class of immunological test 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 According to Vaccination.

Vaccine	QuantiFERON-TB Gold-In Tube				QuantiFERON-TB Gold-In Tube			
	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)

Table 2: The Diagnostic Significance of QuantiFERON-TB Gold-In Tube.

Onsite TB IgG/IgM Rapid Test	QuantiFERON-TB Gold-In Tube							
	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)

Table 3: Relation Between QuantiFERON-TB Gold-In Tube of Patients and Controls Tested and Blood Groups ABO.

Blood groups	QuantiFERON-TB Gold-In Tube							
	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).

Table 4: 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)

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