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Association between PPD and QuantiFERON Gold TB Test in TB Infection and Disease among HIV-Infected Individuals in Southern Iran

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Abstract

Background: *Tuberculosis* is one of the most common diseases among HIV-infected patients. A person with a positive tuberculin skin test (TST) acquiring HIV infection has a 3-13% annual risk of developing active tuberculosis. The diagnosis of TB in HIV infected patients may be difficult. QuantiFERON-TB Gold (QFT-G) test is a novel method as an aid for diagnosis of *Mycobacterium tuberculosis* infection. We evaluated the association between TST and QFT-G test in latent TB infection (LTBI) and TB in HIV-infected patients.

Methods: One hundred and seventy six HIV-infected subjects from Shiraz Consultation and Behavioral Modification Center (SCBMC) entered our study. The individuals were screened for TST, using 5TU purified protein derivative (PPD). Also, blood sample was provided for QFT, measuring INF-γ response to *M. tuberculosis* antigen.

Results: Of 176 participants, 98.3% returned for evaluation of TST results. Among them, 63% and 37% were negative and positive for TST, respectively. All the participants returned for QFT-G sampling. Of them, 64.8% and 27.8% were respectively negative and positive for the test and 7.4% showed undetermined results. The agreement between PPD and QFT-G in their negative results was 39.9% and 8.1% in their positive results and the overall agreement was 50%. Disagreement of TST^-/QFT^+ was noticed in 19.7% of the subjects and TST^+/QFT^- disagreement in 24.9%. $CD4^+$ count <100 mm^3 was seen in 5.9%, ≥100 and < 200 mm^3 in 17.1% and $CD4^+$ T cell count ≥ 200 mm^3 in 76.9% of subjects.

Conclusion: As the agreement rate between QFT-G and TST in HIV-infected patients was fair, a strategy of simultaneous TST and QFT-G testing would maximize the potential for LTBI diagnosis in HIV-infected subjects.

Keywords: HIV; Tuberculosis; QuantiFERON Gold TB test; PPD

Introduction

Mycobacterium tuberculosis continues to be a major health issue in many parts of the world. Tuberculosis (TB) is one of the most common diseases among HIV-infected patients[1] with estimated 13 million people worldwide currently infected with both HIV and TB.[2]

A person with a positive tuberculin skin test (TST) who has acquired HIV infection has a 3-13% annual risk of developing active tuberculosis. A new TB infection acquired by an HIV-infected individual may evolve to active disease in a matter of weeks rather than months to years.[1] The diagnosis of TB in HIV-infected patients may be difficult not only because of the increased frequency of sputum-smear negativity (up to 40% in culture proven pulmonary cases) but also because of atypical radiographic findings, lack of classic granuloma formation in the late stages, and a negative TST. Delay in treatment may be fatal. Worldwide, approximately one-third of all AIDS-

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related deaths are associated with TB.[1]

Diagnosis and treatment of latent TB infection (LTBI) are essential for TB control and elimination. LTBI has an important role in HIV-infected individuals as they may show a higher rate of progression to an active TB disease than HIV-uninfected subjects, even with the effective use of antiretroviral therapy (ART).[2,3] However, LTBI diagnosis is traditionally based on the TST.[2] Some problems may be noticed with the TST which is limited by the need for two visits, the subjective nature of test interpretation, and false positive reaction from Bacille Calmette-Guerin (BCG) vaccination and non-tuberculous *Mycobacterium* exposure. In addition, HIV-infected patients demonstrate a higher rate of energy, especially with advanced immunosuppression.[4,5]

Newly developed interferon-gamma (INF)-γ release assays (IGRAs) by the use of *M. tuberculosis* specific antigens, early secreted antigens target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10) showed a promise as an alterative diagnostic tool for LTBI.[2] However, there are limited data describing IGRA performance in HIV-infected individuals, whose immunologic impairment may affect the performance in these lymphocyte-based assays.[6]

To better understand the IGRA use in HIV-infection, we compared the performance of QuantiFERON-TB Gold In- tube test QFT with TST for the diagnosis of LTBI and active TB disease in subjects in southern Iran.

Materials and Methods

From January to September 2008, 176 HIV-infected patients who were under follow-up of an infectious disease specialist in Shiraz Consultation and Behavioral Modification Center (SCBMC) in Shiraz, southern Iran were enrolled. The age range of the participants was 18-60 years and they were of both genders.

The inclusion criteria were HIV infection documented with serial ELISA and Western-Blot tests. The exclusion criteria were absence of return of patients in 48-72 hours to have the TST evaluation.

A full physical examination and chest radiography were performed for all the participants. Sputum acid-fast stain and TB culture were done for all the patients suspected with active pulmonary tuberculosis based on clinical symptoms and radiographic examination.

For the patients who were suspected of having extra-pulmonary tuberculosis, invasive procedures including tissue biopsy, acid fast stain and *Mycobacterium* culture of the tissue sample were carried out. The final diagnosis was made on the basis of all clinical, radiological, microbiologic and pathological information, and an active pulmonary TB was confirmed by isolation of *M. Tuberculosis* from sputum samples.

TST was performed for all the patients by an expert, using 5 units of PPD in volar aspect of the forearm. Any induration was measured in millimeter after 48 to 72 hours. We used the 5 mm induration as a positive cut off value for the TST.[1]In QuantiFERON- TB Gold In-Tube (IT), two collection tubes including Nil control and TB antigen were used. For each subject, 1 ml of the whole blood was provided by venipuncture and transferred directly into each of the QFT-Gold IT blood collection tubes. After incubating of the tubes at 37^{o}C, harvesting of the plasma was facilitated by centrifuging tubes for 15 minutes at 2000 to 3000 RCF (g). All the plasma samples and reagents except for conjugate 100x concentrate were brought to room temperature (22^{o}C±5^{o}C) before use and allowed at least 60 minutes for equilibration. The kit (QuantiFERON-TB, Gold, In-Tube Method, Cellestic, UK) was standardized as instructed by the manufacturer.

Statistical analyses were performed, using SPSS software (version 11.5, Chicago, IL). In the subjects with active tuberculosis, the results of PPD and QFT-G were compared with diagnostic Gold standard, and sensitivity and specificity, negative predictive values (NPVS) and positive predictive values (PPVS) were obtained.

INF-γ production in response to antigens stimulation was expressed as trichotomous (positive, negative, indeterminate). The agreement rate between the tests was determined, using Kappa coefficients (K=0.00=poor agreement, K=0.01-0.2=slight agreement, K=0.21-0.4=fair agreement, K=0.41-0.6=moderate agreement, K=0.61=0.8=substantial agreement, K=0.81-1.00=perfect agreement).[7] For the present study, TST results were analyzed in millimeters using <5 mm, ≥ 5 and <10, ≥10 mm as cutoffs. $CD4^{+}$ T cell count was divided into three groups including $CD4^{+}$ cell count <100 mm^3, ≥ 100 and <200 mm^3 and ≥200 mm^3.

Results

The median age was 38 years (range=23-60 years) including 170 (96.6%) men and 6 (4.4%) women. The median T-cell $CD4^{+}$ count was 360 cell/mm^3

(range=34-1300 cell/mm^3) and median T-cell CD4$^+$ nadir was 129 cell/mm^3 (range=34-200 cell/mm^3). A total of 176 subjects underwent TST but 3 subjects did not return for any evaluation within 48 to 72 hours.

One-hundred and nine subjects (63%) had negative TST (PPD<5 mm) with median induration of zero mm (range=0-4 mm), 41 (23.7%) had 5 ≤ PPD< 10 mm and 23 (13.3%) had PPD more than 10 mm. The subjects with positive TST (PPD ≥ 5mm) had median induration of 8 mm (range=5-30 mm).

Table 1 shows the association between CD4 count and PPD results. T-cell CD4$^+$ count result was available for 152 subjects, among whom ninety four subjects had PPD less than 5 mm, 36 had ≥5 and <10, and 22 had ≥10 mm. Table 2 demonstrates the association between CD4 count and QFT-G measures in 152 patients. QFT-G testing yielded negative results in 114 subjects (64.8%), positive in 49 (27.8%) and indeterminate in 13 subjects (7.4%).

Of 176 subjects, 173 had valid TST and QFT-G results, with 109/173 (63%) having PPD <5 mm, of whom 69/109 (63.3%) had negative QFT-G results, 34/109 (31.2%) had positive and 6/109 (5.5%) had indeterminate QFT-G results. 41/173 subjects (23.7%) had PPD ≥5 and <10 mm, with 28/41 subjects (68.3%) having negative, 7/41 (17.1%) positive QFT-G and 6/41 (14.6%) indeterminate results. Of 171 subjects, 23 (13.3%) had PPD ≥10 mm, with 15/23 (65.2%) having negative, 7/23 (30.4%) positive and 1/23 (4.4%) indeterminate QFT-G results. The agreement rate of QFT-G and TST was 0.364 (K=0.039, 95% CI=0-0.2156).

Of 176 subjects, 11 (6.3%) had active TB among them, with 7/11 (63.6%) having negative QFT-G results, 4/11 (36.4%) positive and no one had indeterminate results. Of 176 subjects, 165 (93.8%) were without TB disease. Among them, 107 (64.8%) had negative, 45 (27.3%) positive QFT-G results and 13 (7.9%) had indeterminate results. The agreement rate between QFT-G and TB disease was 0.68 (K=0.03, 95% CI= 0.087%-0.139%). The sensitivity of QFT-G in TB disease was 36.4% and the specificity was 70.4%. The positive predictive value (PPVS) of QFT-G in TB disease was 8.2% and the negative predictive value (NPVS) was 93.9%. Of 11 HIV positive individuals with TB disease, 5 (45.5%) had PPD <5 mm and 6 (54.5%) had PPD ≥ 5 mm. Of 162 subjects without TB disease, 104 (64.2%) had PPD <5 mm and 58 (35.8%) had PPD ≥ 5 mm. The agreement rate between TB disease and PPD was 0.636 (K=0.06, 95% CI=-0.04-0.156). The sensitivity of PPD in TB disease was 54.5% and the specificity was 64.2%. The positive predictive value of PPD in TB disease was 9.4% and the negative predictive value 95.4%.

Discussion

In one study on 294 participants, it was shown that 90% returned for TST evaluation. Among them, 70% had a TST within 48-72 hours, 9.3% positive TST and 90.7% negative TST results.[2] In another study on 160 subjects, 46.3% were HIV infected, among whom 81.8% had negative TST and 9.5% positive TST results.[8] In Kamyar's study, 638 HIV- infected patients were enrolled while 11.4% had active TB disease.[9] In our study, the agreement rate between TST and QFT-G in their negative results was 39.9%

Table 1: Correlation between CD4 and PPD in 152 patients.

CD4 / PPD	<100	³ 100 < 200	³ 200	Total
< 5	8 (8.5%)	19 (20.2%)	67 (71.3%)	94 (100%)
5 ≤ PPD < 10	1 (2.8%)	5 (13.9%)	30 (83.3%)	36 (100%)
PPD ≥ 10	0 (0.00%)	2 (9.1%)	20 (90.9%)	22 (100%)
				152

Table 2: Correlation between CD4 and QFT-G in 152 patients.

CD4 / QFT	<100	³ 100 < 200	³ 200	Total
+	2 (4.5%)	12 (27.3%)	30 (68.2%)	44 (100%)
-	6 (6.2%)	13 (13.4%)	78 (80.4%)	97 (100%)
indeterminate	1 (9.1%)	1 (9.1%)	9 (81.8%)	11 (100%)
Total	9	26	117	152

and 8.1% had agreement in their positive results. The overall agreement was 50%.

TST^-/QFT^+ results were found in 19.7% of the subjects, which may be due to false-positive QFT-G results or to relatively increased sensitivity of QFT-G over PPD. TST^+/QFT^- was found in 24.9% of the subjects while these results may be due to exposure to atypical *Mycobacterium* which cannot be excluded or due to a previous BCG vaccination.[2] In Annie et al.'s study, 85.2% had agreement in their negative and 4.1% in their positive results. TST positive/QFT negative disconcordant results were found in 5.1% of the subjects and TST negative/QFT positive disconcordant results in 45% of the subjects. The overall agreement between TST and QFT was 39.3%.[2] In the absence of a diagnostic gold standard for LTBI, the terms sensitivity and specificity were not applicable for LTBI, but in TB disease we obtained 36.4% sensitivity for QFT-G and 70.4% specificity. The negative predictive value (NPV) of QFT-G was 93.9% and the positive predictive value (PPV) 8.2%. Among 11 subjects with active tuberculosis, 63% had negative and 36.4% had positive QFT-G results and indeterminate results were not observed in any subject with active tuberculosis. The agreement between QFT-G and TB disease was slight (K=0.03, CI 95%=-0.087%-0.139%). In HIV-infected subjects with TB disease, the sensitivity of PPD in 5 mm cutoff was 54.5%, the specificity was 64.2%, the NPV was 95.4% and the PPV was 9.4%. The agreement between PPD and TB disease was slight (K=0.06, CI 95%=-0.04-0.156). So, the sensitivity of PPD in 5 mm cutoff for TB disease was more than QFT-G, but the specificity of QFT-G while excluding indeterminate results was more than the figure for PPD. The NPV and PPV of PPD and QFT-G in TB disease were slightly similar.

In CDC issue guideline on the use of QFT-G in persons with untreated culture-confirmed TB, the sensitivity of QFT-G for detecting *M. tuberculosis* was reported 80% but the sensitivity in specific subgroups of TB patients such as immunocompromised ones is still unknown. For LTBI, the QFT-G sensitivity might be less than that of TST. Identical to negative TST results, the negative QFT-G results alone might be difficult to exclude *M. tuberculosis* infection in immunocompromised persons. QFT-G can be used in all circumstances in which TST is used. In this guideline, the positive QFT-G results regardless of their symptoms or findings, the person should be evaluated for TB disease before LTBI is diagnosed.[10]

In Puneet's study, the sensitivity of QFT-G test was evaluated for the detection of TB among 242 persons in Sanfrancisco, CA and 37 subjects had a culture-confirmed TB. Excluding one of the indeterminate results, 64% of the subjects had positive results, using the QFT-G assay. The 64% sensitivity suggests that QFT-G assay should not be used alone to exclude any active tuberculosis.[11]

Regarding the significant risk of reactivation of LTBI among HIV infected subjects, Annie et al. reported a simultaneous screening for both TST and QFT-G to minimize false-negative tests and the missed opportunities for LTBI therapy.[2] In Young et al.'s study, the sensitivity of QFT-G for active pulmonary TB was 86% and the specificity was 49%. The NPV of QFT-G was 84%, the PPV was 61%, the sensitivity of PPD was 68%, the specificity was 51%, the NPV of PPD was 41%, and the PPV was 55%.[12]

In our study, the $CD4^+$ T cell count<100 mm^3 was 5.9%. 17.1% had $100 \leq CD4^+ < 200$ mm^3 and 77% had $CD4^+ \geq 200$ mm^3. The median $CD4^+$ T cell count was 360 cell/mm^3. In the subjects with $CD4^+ < 100$ mm^3, 12.5% had positive TST and 22.2% had positive QFT-G results. In the subjects with $100 \leq CD4^+ < 200$, 26.9% had positive TST and 46.2% had positive QFT-G results. In those with $CD4^+ \geq 200$ mm^3, 42.7% had positive TST and 25.6% had positive QFT-G results. We showed that a higher $CD4^+$ cell count was correlated with a higher INF-γ release in response to TB antigen[2] and a higher TST reaction. In Annier et al.'s study, the median $CD4^+$ T cell count was 363 and in 10.5% of the subjects with CD4 cell count<100 mm^3, no person had TST and QFT-G positive results. In 37.8% of the subjects with $100 \leq CD4^+ < 350$, 37% of the subjects had positive TST and 24% had positive QFT-G results and in 51.7% with $CD4^+ > 350$, 63% of the subjects had positive TST and 76% had positive QFT-G results.[2]

Our study had several limitations such as subjects with TB diagnosis who received anti-TB treatment and subjects with LTBI who received INH therapy. In addition, the HIV infected persons had a higher rate of anergy particularly with advanced immunocompromision.[4,5] Similar to TST,[4,13,14] QFT-G may be less sensitive in advanced HIV disease. Among our patients with TB disease, the sensitivity of PPD in 5 mm cut off was more than QFT-G but the specificity of QFT-G while excluding undetermined results was more than PPD. As the agreement rate between QFT-G and TST in HIV-infected patients was fair, a strat-

egy of simultaneous TST and QFT-G testing would maximize the potential for LTBI diagnosis in HIV-infected subjects.

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