Diagnosis of Latent Tuberculosis Infection in Candidates for Kidney Transplantation (Comparison of Two Tests)

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Abstract- It is assumed that about 10% of individuals infected with Mycobacterium tuberculosis develop tuberculosis. The rate of tuberculosis in solid organ transplant recipients has been estimated to be 50-fold higher than in the general population. Candidates for solid organ transplantation are routinely screened for latent tuberculosis infection (LTBI). In this study we aimed to compare Tuberculin Skin Test (TST) with QuantiFERON-TB Gold In-Tube (QFT) for the detection of LTBI in candidate for kidney transplantation. Between October 2009 and November 2010, 64 candidates for kidney transplant who referred to the transplant clinic in Imam Khomeini Hospital, were enrolled in the study. Patients were screened for LTBI with both QFT and TST. Concordance between two test results and variables associated with test discordance were assessed. The mean age of patients was 38.5 years (range 16-65); female/male ratio was 26/38. Positive results were found in 21.9% and 21.9%, by TST and QFT, respectively. Four patients (6.3%) had indeterminate QFT. Overall agreement between QFT and TST was 75% (k=0.28 and *P*=0.028). BCG vaccination and past positive TST were not associated with positive QFT result (*P*=0.13 and *P*=0.09 respectively). Overall agreement between test results was fair. Comparison among test results showed that TST andQFT can be used interchangeably for latent TB screening in kidney transplant candidates. The decision to select QFT or TST will depend on the purpose of testing and resource availability.

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Keywords: Kidney transplantation; Latent Tuberculosis Infection; Tuberculin Skin Test; Quanti FERON-TB Gold in-tube

Introduction

Kidney transplantation is the treatment of choice in patients with end stage renal disease (ESRD). Use of immunosuppressors in these patients has led to an increase in both transplant survival and infectious complications (1). The patients with ESRD are 10 to 25 folds more susceptible to active tuberculosis (TB) and are mostly treated for latent tuberculosis infection (LTBI) (2). Hence, the patients waiting for kidney transplantation are usually screened for tuberculosis with tuberculin skin test (TST) (3). Tuberculosis is one of the most common infectious diseases especially in developing countries (4).

In 2007, 13.7 million subjects i.e., 206 people per 100,000 general populations had tuberculosis and the incidence rate was 139 per 100,000 subjects leading to 1.8 million deaths or 27 per 100,000 subjects (5). According to the 2008 WHO report, the prevalence of tuberculosis in Iran is 28 per 100,000, and its annual incidence is 22 per 100,000 (6). Not all patients infected with tuberculosis progress to active infection, and some remain with LTBI. However,up to ten percent may progress to active infection (7). This risk is especially more in cancerous, chronic renal failure and immunosuppressed subjects (8). The association of Human Immunedefieciency Virus (HIV) infection and tuberculosis has led to more problems in control of the patients with LTBI (9).

Although tuberculosis is uncommon among transplant recipients, the risk of tuberculosis among solid organ transplant recipients is 50-fold more

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compared with general population (10,11) leading to the higher mortality rate among these patients up to 40 percent (12). The prevalence of tuberculosis in kidney transplant recipients ranges from 4 to 15 percent and is the main cause of mortality in 20 to 25 percent of patients(13).

The kidney transplant recipients are usually screened for LTBI by TST(14). Recently, the QuantiFERON-TB (QFT) Gold and T-Spot TB tests are used for screening in suspected subjects but the value of these tests in kidney transplant recipients is unclear (3,15). Tuberculin skin test is routinely used in these patients for screening. However, because of anergy and cross-reaction with BCG vaccines, this test is not definite. (2, 15,16). On the other hand, the QFT test is not also a feasible test and may not differentiate between active and latent infection (17). However, it has also been shown that the QFT response would decrease during anti-TB therapy; this dynamic characteristic would show the higher value of this test compared with TST (18).

Considering the endemic status of tuberculosis in Iran and referral status of our tertiary health care center for organ transplantation and importance of early diagnosis of LTBI to treat and reduce the burden of disease and its subsequent active infection, this study was performed to compare the Quantiferon-TB Gold and TST in diagnosis of latent tuberculosis infection in candidates for kidney transplantation.

Materials and Methods

This cross-sectional study was performed on 69 patients candidate for kidney transplantation going to a training hospital in Tehran, Iran from 2009 through 2010. The study was approved by medical ethics committee of Tehran University of Medical Sciences. The patients who signed the informed consent form were consecutively enrolled. The exclusion criteria were active tuberculosis and history of chemoprophylaxis and no re-entry for recording the TST results after five days. In all patients both QFT and TST results were performed and the TST response was evaluated after 48 to 72 hours. For QFT test, three ml blood sample was obtained and stored in three tubes and sent for laboratory up to six hours to be incubated for 24 hours at 37ºC. Then the samples were centrifuged for 15 minutes at 2000-3000 x g and the plasma samples were stored at -70^{o}C before quantification of the gamma interferon levels by ELISA method.

In patients with positive QFT or TST tests, the clinical history was obtained, the clinical examination was performed and chest radiogram was obtained to rule out active infection. The patients with positive TST results without active TB received chemoprophylaxis with isoniazid according to the protocol (300 mg isoniazid plus 40 mg vitamin B6 for nine months). All the patients were followed up throughout the study for clinical symptoms of tuberculosis.

Evaluated variables included age, gender, follow-up duration, chief complaint, etiology of ESRD, the time passed from diagnosis, clinical findings, self and family history of TB, history of anti-tuberculosis therapy and time passed from it, history of anti-tuberculosis chemoprophylaxis therapy and time passed from it, history of BCG vaccination, intravenous opium addiction, history of immunosuppressor medication and its type and dose, previous TST results, history of tuberculosis exposure and the time passed from it, chest radiogram results, CT Scan results, and the HIV evaluation results. Also we looked for the results of TST and QFT tests, receiving the anti-tuberculosis prophylaxis and its adverse effects, cause of no administration of anti-tuberculosis prophylaxis, organ transplantation, and final outcome of the patients.

For TST, 0.1 ml from five-unit tuberculin solution was intradermally injected two to four inches below elbow with five to 15 degrees angle and the induration size was measured after 48 to 72 hours and if ten mm or more in largest diameter was considered positive.

In QFT test, the gamma interferon released from patients' lymphocytes sensitized with two peptides of Mycobacterium tuberculosis including ESAT-6 and CFP-10 was measured in all patients with ELISA method on plamas derived from heparinized blood samples using the kit made by Cellestis Limited (Carnegie Victoria, Australia). The tubes were stored in 4 to 25 ºC The kit was stored in two to eight degrees of centigrade and far from sunray. The test was performed on the three tubes including nil, mitogen and antigen ones. One mL of blood was add to each tube and shook ten times and incubated in 37 ^{o}C for 16 to 24 hours,then the samples were centrifuged at 2000-3000 x g for 15 minutes, then the obtained plasmas were stored in -70 ^{o}C. When ready to perform ELISA, the samples and the kit (except conjugate solution) were leaved in room temperature for 60 minutes. Then 200 µl from each plasma sample was added to each well of the microplate,subsequently, 50 µl from conjugate solution was pipetted to each well of ELISA plate and 50 µl of standard or plasma was added to it. The plates were shaken for one minute , then incubated in room temperature for 120 minutes.

Table 1. Quanti FERON-TB results interpretation.

Negative	Mitogen minus Nil > 0.5	TB_a Ag_b minus Nil < 0.35	Nil≤8.0 IU/ml
		TB Ag minus Nil ≥0.35 and < 25% of Nil value	
Positive	Mitogen any	TB Ag minus Nil > 0.35 and ≥ 25% of Nil value	
Indeterminate	Mitogen minus Nil < 0.5	TB Ag minus Nil < 0.35	
		TB Ag minus Nil ≥ 0.35 & < 25% of Nil value	
	Mitogen any	TB Ag any	Nil>8.0 IU/ml

α-TB= Tuberculosis, *b*- Ag= Antigen

Then the plates were washed six times with buffer solution and 100 ml from Enzyme Substrate solution was added and again shaken and incubated in room temperature for 30 minutes. Thereafter, 50 µl from stop solution wasadded and after shaking the tube, the gamma interferon was measured by ELISA-Reader at 450 nm wavelength within five minutes.

The required results were evaluated by company software as depicted in table 1.

The data were analyzed using SPSS (version 18.0) software [Statistical Procedures for Social Sciences; Chicago, Illinois, USA]. Differences were tested by Chi-Square and Fisher's exact tests and were considered statistically significant at *P*-values less than 0.05. The kappa test was used to analyze agreement between the two tests.

Results

Of 69 patients, four patients were excluded from final analysis due to no re-entry for TST evaluation and one for incorrect QFT procedure and finally 64 patients were evaluated. The mean (± SD) age of the patients was 38.5 (± 12.1) years ranging from 16 to 65 years. Thirty-eight patients (59.4%) were male. The mean follow-up duration was 8.4 months ranging from 1 to 13 months.

None of the patients had positive self history of TB or active infection and only one patient had positive family history of TB. Also none of the patients had positive history of anti-tuberculosis prophylaxis. Fifty-eight patients (90.6%) were previously vaccinated with BCG. One patient had history of positive TST and 43 subjects had negative results and 20 patients have no history of performing TST.

The mean (±SD) time passed from diagnosis was 38.9 (±46.9) months ranging from 2 to 240 months that was less than one year in 24 patients (37.5%). Twenty-six patients (40.6%) were asymptomatic and the most common clinical finding was paleness seen in 30 patients (46.9%). None of the patients had clinical symptoms related to active tuberculosis. The chest radiogram was normal in 96.8%. The etiology of ESRD in patients is shown in Table 2.

The TST was positive in 14 patients (21.9%). The QFT was positive in 14 patients (21.9%), negative in 46 subjects (71.9%), and indeterminate in four patients (6.3%). Overall agreement between QFT and TST was 75% and the total Kappa coefficient of two tests was 0.28 (*P*=0.028) (Figure 1). The Kappa coefficient of two test in diabetic patients was -0.15 (*P*=0.59) and in patients with unknown etiology of ESRD, the Kappa coefficient of two tests was 0.31 (*P*=0.03). In patients with hypertension Kappa coefficient of two tests was 0.54 (*P*=0.17).

Table 2. Etiology of end stage renal disease in the study population.

Etiology	Frequency	Percent
Diabetes Mellitus	11	17.2
Hypertension	5	7.8
Recurrent UTI_a	1	1.6
Unknown	47	73.4
Total	64	100

α UTI= Urinary Tract Infection

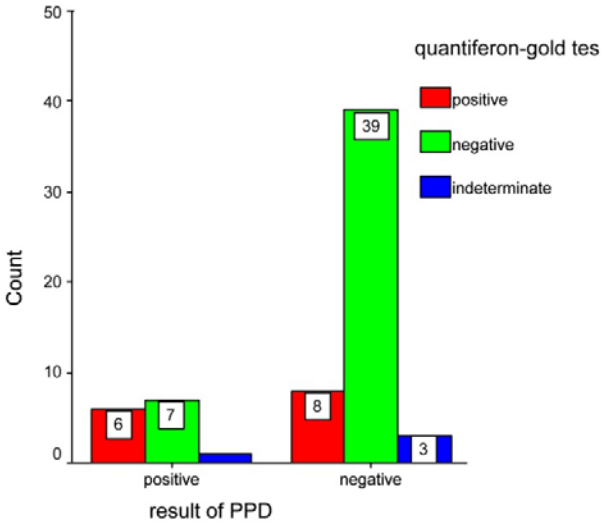

Figure 1. Comparison of results obtained in Tuberculin Skin Test and QuantiFERON-TB tests.

All patients with positive TST received anti-tuberculosis prophylaxis with no adverse effects. None of the patients developed tuberculosis in the follows up. Twelve out of 64 patients (18.8%) underwent organ transplantation and three cases were from alive donors. Three patients died in the follow-up (4.7%) that were due to emboli/cardiac infarction, falling and kidney hemorrhage, and delayed dialysis. There was no association between TST and QTF results and none of the variables including BCG vaccination and past positive TST (P=0.13 and P=0.09 respectively).

Discussion

In this study the QFT and TSTin diagnosis of latent tuberculosis infection were compared in candidates for kidney transplantation. There was a Kappa coefficient of 0.28 that shows a fair agreement. We found that the QFT and TST results were not influenced by any of the clinical and paraclinical items and also the etiology of ESRD such as diabetes and immunosuppressive medication. However some studies have shown that diabetes or immunosuppressive medication results in more inaccurate results (19,20).

In this study, four patients had no re-entry for TST ; this matter is one of the most important constraints for this test as reported by some previous studies (21,22). As reported in previous studies, the patients awaiting organ transplantation are mainly male subjects as seen in our study (7,17,23). This matter may be due to higher prevalence of disease leading to ESRD in male subjects compared with female patients.

The mean age was lower in our study compared with previous reports (23,24) ; this matter may be due to lower life expectancy in patients with ESRD in Iran compared with other regions or may be explained by the different etiologies for transplantation in different countries.

The positive TST results in patients awaiting organ transplantation were seen in nearly 22% of patients in our study; this frequency was reported to be 26 percent and 19 percent in other studies (24,25). However, it has reported to be as high as 62.5% in some studies (2).

The kappa coefficient in our study was 0.28 (p=0.028); the majority of other studies have reported Kappa to be between 0.03 and 0.8. In this study, both tests were positive in six patients (9.4%) and both were negative in 39 subjects (60.9%). In this study, the overall agreement between QFT and TST was 75%.

In the study by Manuel *et al.*, 24.2% had a positive TST and 22.2% had a positive QFT (7). Overall agreement between tests was 85.1% (Kappa=0.60, P<0.0001) but the observed agreement in our study was less. The study by Kim *et al*. (26) demonstrated that agreement between the TST and QFT was moderate (Kappa= 0.57) that is higher than the fair agreement observed in our study. Seyhan *et al.* (3) demonstrated that forty-three percent had a positive QFT and 34 percent had a positive TST and overall agreement between the QFT and the TST was 65% (concordance Kappa=0.26, P=0.01) that is lower than the agreement seen in our study. Bartalesi *et al.* (27) reported that 19% of subjects were TST-positive and 13% were QFT positive and the concordance between TST and QFT results was good (87.7%) that is more than the agreement observed in our study. These differences between various studies may be due to factors such as understudy population, sample size, ethnic variations, different instruments used for the study, and many other unknown factors.

Finally, according to the obtained results in this study, it may be concluded that both QFT and TST may be used to diagnose the latent tuberculosis in patients undergoing organ transplantation according to patients' condition and feasibility. However, further studies should be carried out to determine the tests with higher sensitivity and most permitted specificity.

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