ORIGINAL ARTICLE

Agreement between the results of tuberculin skin test and Interferon-Gamma Release Assays in renal transplant candidates

Shiva Samavat[1,2], Sam Alahyari[3], Ali Sangian[3], Malihe Nasiri[4], Mohsen Nafar[1,2], Ahmad Firoozan[1,2], Fariba Samadian[1,2], Nooshin Dalili[1,2], Fatemeh Poorrezagholi[1,2]

[1]Department of Nephrology, Shahid Labbafinejad Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [2]Chronic Kidney Disease Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [3]Department of Kidney Transplantation of Labbafinejad Hospital, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [4]Department of Biostatics, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Introduction: Identification of latent tuberculosis (TB) infection is important in kidney transplant candidates. Due to the absence of a gold standard, both tuberculin skin test (TST) and interferon-gamma release assays (IGRA) are used to screen patients. The aim of this study was to evaluate the agreement of these two tests in patients undergoing renal transplantation. **Materials and Methods:** Two hundred kidney transplant candidates at a referral center in 2014–2017 were included in this study. TST and Quantiferon-Gold (QFT-G) tests were performed for all patients before transplantation. In case of a positive result in any of the tests, patients were administered a 9-month prophylaxis treatment using isoniazid. Cohen's kappa coefficient (k) test was used to determine the agreement between the two tests. **Results:** The mean age of patients was 40.72 ± 18.33. Nine (4.5%) patients had positive TST and 16 (8%) had positive IGRA. Concordance of the two tests was evaluated as medium (κ = 0.44 and P < 0.001). No association was found between the underlying causes of renal failure and skin test positive or IGRA. The tests showed a poor agreement among diabetics, candidates of re-transplantation, and those who were on dialysis for longer than a year (κ < 0.20). **Conclusion:** TST or IGRA can be used to screen TB in kidney transplant candidates with a moderate agreement. However, we suggest using both TST and QFT-G in diabetics, re-transplant candidates, and those on dialysis for >1 year.

Key words: Interferon-gamma release tests, kidney transplantation, latent tuberculosis infection, tuberculin skin test

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INTRODUCTION

Tuberculosis (TB) is the most common cause of death from infectious disease.[1] It is estimated that in 2017, 1.3 million people died of TB. Mathematical modeling evidence suggests that 1.7 billion people worldwide have latent tuberculosis infection (LTBI).[2] People with LTBI have a 5%–15% chance of developing active TB during their lifetime.[1] Extensive studies have been conducted to determine the role of determinants of the disease, genetic background and host immunity, and environmental effects in LTBI. Patients with active pulmonary TB are the major source of *Mycobacterium tuberculosis* infections, but those with LTBI are the largest reservoir of TB because LTBI may become active TB disease within a few years or decades after initial infection with *M. tuberculosis* which is likely to depend on the level of host immunity.[3] The most important risk factors for active TB infection are immunosuppressive conditions including diabetes, malnutrition, acquired immunodeficiency syndrome, steroids, and the administration of immunosuppressive drugs and chemotherapy medications.[4] Studies have shown that the incidence of active TB in these groups is 20–70 times

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Address for correspondence: Dr. Ali Sangian, Shahid Beheshti University of Medical Sciences, Koodakyar Street, Daneshjoo Blvd, Shahid Chamran, Velenjak, Tehran, Iran.
E-mail: sangian.a@yahoo.com
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higher than the general population and up to 30% higher mortality.[5] The incidence of TB in kidney transplant patients is also higher than healthy people[6] and causes more morbidity and mortality in this group of patients.[7] TB causes rejection of transplanted kidneys in approximately one-third of patients. Most cases of TB in these patients are due to reactivation of the LTBI in the recipient or its transmission from the donors.[8] There is currently no gold standard for LTBI diagnosis. Existing screening tests provide indirect information about the presence of LTBI. In the past, LTBI was diagnosed by a tuberculin skin test (TST). Recently, the interferon-gamma release assays (IGRA) has been introduced to remove some of the limitations of the tuberculin test (cross-reactivity in vaccinated patients and an error in measuring the indurations caused by the skin reaction).[9] Various studies have investigated the results of IGRA test in the diagnosis of LTBI and compared it with the data of TST. These comparisons are made in particular to compare the sensitivity and specificity of the two tests.[10] However; the results of the studies are different.[11] Due to the high cost of IGRA test and lack of consensus to use IGRA or TST for TB screening in kidney transplant candidates, the purpose of this study was to compare the results of two IGRA and TST tests in patients undergoing kidney transplantation for diagnosis of latent TB.

MATERIALS AND METHODS

Patient selection

Between 2014 and 2017, patients undergoing kidney transplantation at a referral center were included. Consecutive sampling method was used. A written informed consent form was obtained from all patients. All patients underwent chest X-rays to screen for evidence of active or latent TB. Exclusion criteria included abnormalities in chest X-ray, active TB disease, history of treatment, or prophylaxis for TB. All patients received an immunosuppressive regimen including calcineurin inhibitors + mycophenolic acid + prednisolone before kidney transplantation. TST was performed on all participants and blood samples were taken for IGRA test to detect latent TB infection. Skin test results were interpreted after 48–72 h. Patients were followed for 1–3 years.

Tuberculin skin test

TST was performed using the 0.1 ml purified protein derivative (PPD) (Pasteur Institute, Tehran, Iran) injection into the anterior aspect of the forearm intradermally in accordance with the Mantoux method.[12] A positive test was defined by the size of induration (not the erythema) induced by PPD 48–72 h after the injection. If the induration size was ≥10 mm, the test was considered positive as recommended by local guidelines (Ministry of Health and Medical Education).

Quantiferon-tuberculosis gold test

Quantiferon-gold (QFT-G) was done at the same visit as TST, but the sample for the test was collected before the injection of PPD. QFT-G test (Cellestis Ltd, Carnegie, VIC, Australia) was carried out according to the manufacturer's instructions. Briefly, whole blood was collected and aliquots were incubated for 16–24 h with TB-specific antigens, including early secretory antigenic target 6-kda protein and culture filtrate protein 10. The concentration of released interferon-gamma was measured by enzyme-linked immunosorbent assay.

Statistical analysis

After data collection, the results were analyzed using the SPSS software (IBM, NY, USA), version 25. Chi-square test was used to investigate the differences between qualitative variables. Numerical variables were reported with mean and standard deviation. Univariate analysis was performed to evaluate the distribution of preliminary results of skin test and IGRA. Fisher's exact test was used to assess the association between the results of the data and active TB disease. Cohen's kappa coefficient (k) test was used to determine the agreement between TST and IGRA by following criteria: $k > 0.20$ (poor agreement); $0.21 < k < 0.40$ (weak agreement); $0.41 < k < 0.60$ (moderate agreement); $0.61 < k < 0.80$ (good agreement); and $k < 0.81$ (very good agreement). $P < 0.05$ was considered statistically significant.

Ethical statement

The study was performed in accordance with the Declaration of Helsinki and was approved by Shahid Beheshti University of Medical Sciences Ethical Committee (ethical code: IR.SBMU.MSP.REC.1396.312).

RESULTS

In this study, 200 renal transplant candidates were studied, of whom 63% (126) were men and 37% (74) were women. The mean age of the patients was 40.72 ± 18.33 years. The mean body mass index was 23.93 ± 4.58 kg/m^2. Table 1 presents patient information and characteristics.

The studied patients were evaluated for dialysis treatment. The results showed that 79% (158 patients) were on dialysis (155 hemodialysis and 3 peritoneal dialysis patients). Of the 158 dialysis patients, 65.8% (104) were men and 34.2% (54) were women. The causes of end-stage renal disease (ESRD) were as follows: 27.5% (n = 55) of hypertension, 15% (n = 30) of diabetes, 11.5% (n = 23) of glomerular diseases, autosomal dominant polycystic kidney disease 10% (n = 20), and 11.5% (n = 23) of nephrotic syndrome. Table 2 shows a univariate analysis of the association of clinical factors and TST and IGRA positivity.

There was no statistically significant difference between the two sexes in any of the studied factors. None of the patients had a history of TB, and all had been vaccinated with Bacille Calmette–Guérin (BCG) according to National Immunization Program (BCG as a single dose at children aged <6 years, shortly after birth or at first contact with the health services). In addition, none of the patients had a history of contact with a person with TB in the past. None of the studied clinical factors had a significant association with positive TST or QFT-G.

Of the 200 patients studied, 4.5% (n = 9) of patients had a positive PPD. Furthermore, 8% (n = 16) had positive QFT-G test and 92% (n = 184) had negative test. According to the results of TST and QFT-G tests, in 181 patients, both the tests were negative, and six patients had both positive results [Figure 1]. The highest discordance between the two tests was reported in TST-/QFT-G + group which were 10 patients and 2 of them were immunocompromised.

As shown in Table 3, concordance between these two tests was evaluated with kappa coefficient $\kappa = 0.44$ and $P < 0.001$ as moderate. Also in this study, the concordance of two tests in immunocompromised patients and patients with transplant history was evaluated which was $\kappa = 0.478$ and $\kappa = 0.143$, respectively.

The tests had a poor agreement among diabetic patients and candidates of re-transplantation. The conformity of the two screening tests in different dialysis durations was also evaluated, as shown in Table 4.

According to the data obtained, it seems that the concordance of the two tests seems to be minimized in patients who have exceeded 1 year of dialysis initiation.

Twenty-one patients were also indicated to receive prophylaxis who were treated with isoniazid over a 9-month period. Patients were followed for 1–3 years. TB was reported in one patient who had negative results for both the tests.

Table 1: Demographic and clinical characteristics of patients

Variable	Patients (n=200)
Age (years)	40.72±18.33
Male/female (%)	126/74 (63/37)
BMI (kg/m^2)	23.93±4.58
History of immunosuppressive treatment (%)	17.5
History of transplantation	8
Dialysis duration (months)	12 (4-27)
Diabetes (%)	30 (15)
Hypertension (%)	55 (27.5)
Nephrotic syndrome (%)	23 (11.5)
Autosomal dominant polycystic kidney disease (%)	20 (10)
Other renal etiologies (%)	72 (36)

BMI=Body mass index

DISCUSSION

TB is considered a serious complication of organ transplantation. Several prognostic factors such as interleukin (IL)-10 and IL-13 play a role in the severity of pulmonary involvement in this disease. Diagnosis and treatment of LTBI is recommended for patients undergoing transplantation.[13-15] Recipients are at increased risk of developing TB disease. Studies show that 1%–15% of patients undergoing organ transplantation develop the disease. Its incidence is estimated at 512 per 100,000 people.[16] Two methods for screening have been introduced, TST and IGRA. Although recommendations for the use of IGRA tests for the diagnosis of TB are increasing,[17] it is still not widely used as a routine test. The reasons for this include lower cost and the availability of skin testing compared to IGRA. IGRA needs specific diagnostic tools that are not available everywhere.[3,5] Comparison of the results of different types of IGRA tests showed that the positivity of QFT was significantly higher than T-SPOT.TB. There was no statistically significant difference in the negative results of the two tests.[18] The aim of this study was to evaluate the

Table 2: Association between clinical factors and tuberculin skin test and interferon-gamma release assays results

Clinical factors	PPD		P	IGRA		P
	Positive	Negative		Positive	Negative	
Age (mean±SD)	40.56±18.34	40.72±18.37	0.98	38.38±21.19	40.92±18.11	0.59
Gender (male/female) (%)	88.9/11.1	61.8/38.2	0.1	56.3/43.8	63.6/36.4	0.56
BMI (mean±SD)	24.11±5.25	23.92±4.57	0.91	22.73±4.58	24.04±4.58	0.31
Dialysis (%)	77.8	79.1	0.93	87.5	78.3	0.38
History of immunosuppressive treatment before transplantation (%)	28.6	17.1	0.28	17.6	17.5	0.98
Hypertension (%)	11.1	28.3	0.26	12.5	28.8	0.16
Glomerular diseases (%)	22.2	11	0.3	12.5	11.4	0.89
ADPKD (%)	11.1	9.9	0.91	18.8	9.2	0.22
Diabetes (%)	0	15.7	0.19	12.5	15.2	0.77

PPD=Purified protein derivative; IGRA=Interferon-gamma release assays; BMI=Body mass index; ADPKD=Autosomal dominant polycystic kidney disease; SD=Standard deviation

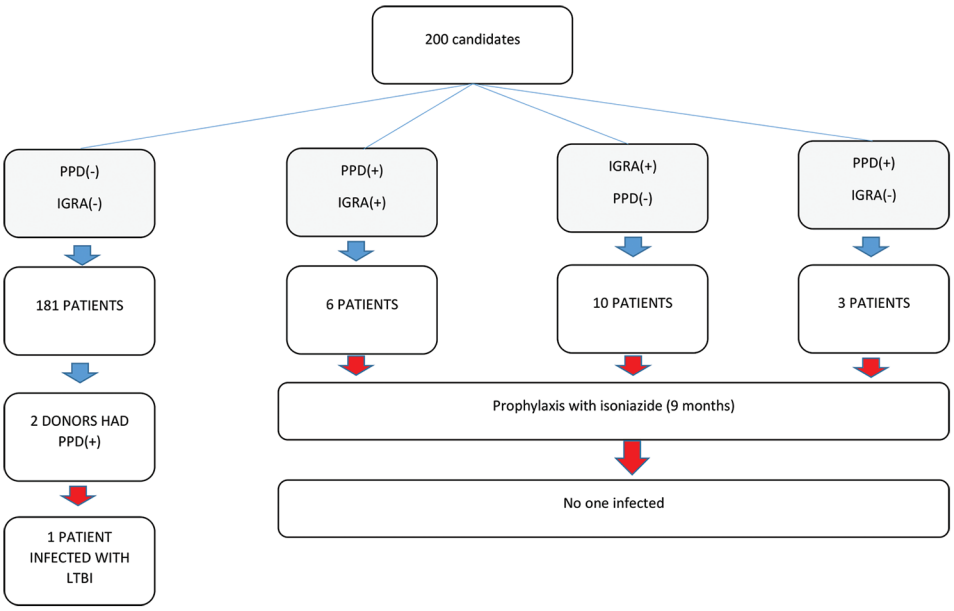

Figure 1: Study flowchart. All patients who had one or two positive screening tests received prophylaxis with isoniazid. In addition, patients whose donor was purified protein derivative (+) received 9-month prophylaxis with isoniazid

Table 3: Concordance of tuberculin skin test and interferon-gamma release assays in different types of patients

	TST+	TST−	κ
All patients			
QFT-G+	6	10	0.44
QFT-G−	3	181	
Diabetic patients			
QFT-G+	0	2	0.00
QFT-G−	0	28	
Hypertensive patients			
QFT-G+	5	6	0.534
QFT-G−	1	55	
Re-transplantation			
QFT-G+	0	1	0.143
QFT-G−	1	6	
Immunosuppressed patients			
QFT-G+	1	2	0.478
QFT-G−	0	32	
Dialysis patients			
QFT-G+	3	7	0.373
QFT-G−	2	145	
Nondialysis patients			
QFT-G+	3	3	0.548
QFT-G−	1	35	

TST=Tuberculin skin test; QFT-G=Quantiferon-gold

compatibility of two tests in the diagnosis of latent TB and offering local indications for choosing one over the other based on patients' characteristics.

In this study, the majority of kidney transplantation candidates were male, similar to other studies.[19] This may be due to the higher prevalence of ESRD among men.

Overall, the concordance of these two tests was evaluated with Cohen's kappa coefficient as moderate (κ = 0.44 and $P < 0.001$), which was similar to the results obtained in other studies.[11] In the study of Kim *et al*., the concordance rate of the two tests was calculated as $\kappa = 0.26$. About 91% of patients had BCG vaccine scar. It was also reported that male sex was associated with a positive result in both the screening tests.[19] In the study by Gonzales *et al*., the concordance of the two tests increased with increasing TST positive threshold from 5 to 15 mm from 0.44 to 0.56. The highest and lowest rates of concordance were among young people and vaccinated people, respectively. In this study, it was concluded that the use of IGRA for BCG-vaccinated patients was superior to TST.[20] In Jambaldorj *et al*. study, among 138 KT recipients who underwent QFT and TST, the QFT tended to be associated with a chest radiographic lesion, history of previous TB, and clinically latent TB despite the absence of statistical significance. However, TST was not associated with clinically latent TB, and agreement between TST and QFT in these 138 KT recipients was poor ($\kappa = 0.327$).[21]

Some studies suggest the association of false-positive TST results with nontuberculosis (NTB) mycobacteria contact such as the BCG vaccine.[22] However, subsequent studies have shown that exposure to NTB mycobacteria is not a significant contributor to the false-positive result in TST, except in cases of very low TB prevalence or high prevalence of NTB susceptibility.[23] In some studies, the positive and negative predictive values of IGRA were higher than the TST, which contradicts the results of the current study.[24] In our study, the discordant results between the two tests were 6.5%, the highest being in the TST-/QFT-G + group. Similar results were found in the

Table 4: Conformity of two tests based on dialysis duration

Dialysis duration	QFT-G	TST+	TST−	*K*
>3 months	Positive	5	7	0.501
	Negative	2	160	
<3 months	Positive	1	3	0.25
	Negative	1	19	
>1 year	Positive	0	2	0.015
	Negative	1	87	
<1 year	Positive	5	8	0.484
	Negative	1	87	

TST=Tuberculin skin test; QFT-G=Quantiferon-gold

study of Kim *et al*.[19] These results were different from other studies in immunocompetent patients in which TST+/QFT-G-group consists of the majority of discordant results.[25] The reason for the high rate of negative TST may be due to false-negative results of skin test. False-negative results may be due to poor cellular immune function due to immunosuppressive conditions.[26]

The tests showed poor agreement among patients with diabetes, candidates of re-transplantation, and those who were on dialysis for longer than a year. Some studies have shown that diabetes increases the risk of a positive IGRA test and this may be the reason for the increasing discrepancy between the two tests.[27] In addition, it has been reported that in dialysis patients, TST is not effective in diagnosing TB and it is better to perform IGRA. Due to the fact that these patients are 10–25 times more likely to develop TB, it was recommended to perform IGRA in this group.[28] Some studies have recommended IGRA to diagnose LTBI in dialysis patients. They concluded that IGRA is more sensitive than TST in these patients.[28] In the present study, similar results were observed for patients who were on dialysis for more than 1 year.

In this study, one patient developed active TB despite the negative results of both the TB tests. These results were lower than data from other studies that reported a TB incidence rate of 0.45%–0.9%.[29] The lower incidence was due to patients receiving drug prophylaxis with at least one positive test.

The most important limitation of this study was the absence of unvaccinated patients, and therefore, it is not possible to compare the two screening tests in these two groups of patients. The other limitation was the lack of high-risk patients among the group, as defined by having a history of untreated TB, known TB contact, alcoholic patients, and radiographic evidence of the previous TB.

CONCLUSION

We found a moderate agreement between two tests of TST and QFT-G. However, when it comes to cost and availability, we recommend using both TST and QFT-G in high-risk patients, diabetics, re-transplant candidates, and those on dialysis for more than a year due to the low agreement of two tests. Further studies are needed to assess the sensitivity and specificity of the two tests in different areas, considering the prevalence of TB, BCG vaccination, and community welfare.

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Conflicts of interest

There are no conflicts of interest.

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