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Detecting Latent Tuberculosis Infection Prior to Kidney Transplantation in a Tertiary Hospital in Saudi Arabia: Comparison of the T-SPOT.TB Test and Tuberculin Test

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Authors' contributions

This work was carried out in collaboration between all authors. Author HAHH designed the study, managed the literature searches, wrote the protocol, and wrote the first draft of the manuscript. Authors MS and AREIH managed the analyses of the study. Author MYE performed the statistical analysis. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Background: Tuberculosis (TB) remains a major worldwide public health problem with over 8.8 million newly diagnosed cases in 2010. Patients with end-stage renal disease (ESRD) who are on hemodialysis (HD) have a significantly higher incidence of Mycobacterium tuberculosis infection or disease than healthy individuals. Most cases of active tuberculosis (TB) in patients with ESRD are due to the reactivation of a latent infection, and this patient group is at roughly 10- to 25-fold higher risk for reactivating TB infection than 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 T-SPOT.TB, for the detection of LTBI in candidates for kidney transplantation.
Methods: Prospective study of 133 HD Patients who did not have a diagnosis of active TB

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diseases or LTBI previously referred, through a 5-month period, to our institutions. Forty four kidney donors without evidence of renal insufficiency or immunocompromising conditions by medical history served as control group. All patients were tested with tuberculin (TST), and T-Spot.TB and the results were compared.
Results: In donors, the concordance between the T-SPOT and the TST was moderate (90.9 %, κ=0.46). Forty of 44 donors (90.9%) had concordance results between the T-Spot TB and TST.In hemodialysis patients, the concordance between the T-SPOT.TB and the TST was poor (60.15 %, κ=0.07). Fifty three of 133 patients (40%) had discordant results between the T-SPOT.TB and TST. Of these, 13 patients had a positive TST but negative T-SPOT.TB and 40 had a positive T-SPOT.TB but a negative TST.
Conclusion: Our data strongly argue against the use of TST in screening of LTBI in HD patients. T-SPOT.TB test in dialysis patients correlated better than TST with the risk of TB infection (e.g. increased age and low body mass index). It is a more reliable and powerful diagnostic tool than TST. However, further studies should be carried out to determine the tests with higher sensitivity and most permitted specificity.

Keywords: T spot test; kidney transplantation; tuberculin test.

1. INTRODUCTION

Tuberculosis (TB) remains a major worldwide public health problem with over 8.8 million newly diagnosed cases in 2010. Saudi Arabia has a low burden of TB with a prevalence of 24 per 100,000, an incidence of 18 per 100,000, causing 1.4 per 100,000 deaths in 2010 [1]. Patients with end-stage renal disease (ESRD) who are on hemodialysis (HD) have a significantly higher incidence of Mycobacterium tuberculosis infection or disease than healthy individuals [2-7]. Most cases of active tuberculosis (TB) in patients with ESRD are due to the reactivation of a latent infection, and this patient group is at roughly 10- to 25-fold higher risk for reactivating TB infection than the general population [2-4]. Moreover, HD units have been shown to be important centers for the spread of infectious TB [8-10]. For these reasons, routine annual TB screening of HD patients has been recommended [8]. In Saudi Arabia the total number of patients on hemodialysis is 11437 with 2846 new cases in 2010 [11]. Until recently, the standard screening tool used to detect latent tuberculosis infection (LTBI) was the tuberculin skin test (TST); however, a large proportion of HD patients exhibit anergy and tuberculin non-reactivity, yielding high rates of false negative results with this method [3,9,12,13].

Indeed, uremia partly inhibits cellular immunity, which leads to false negative skin tests [14]. Moreover, specificity of TST is low, with false positive results due to either BCG (Bacillus of Calmette-Gu´erin) vaccination or exposure to environmental mycobacteria. Over the past 5 years, two *in vitro* T-cell-based assays have been commercialized (QuantiFERON Gold_R in tube, Cellestis Ltd, Carnegie, Australia, and T-SPOT.TB_R, Oxford Immunotec, UK): both measure the production of Interferon-γ (IFN-γ) by peripheral blood mononuclear cells after overnight incubation with antigens of the *M. tuberculosis* complex, which are present neither on the *BCG Mycobacterium Bovis* strain nor on most environmental mycobacteria [15].

The aim of this study was to assess the utility of T Spot TB in these renal dialysis patients as an alternative to skin tuberculin test.

2. MATERIALS AND METHODS

2.1 Study population or Participants

Between January and May 2009, patients with ESRD who had been on HD >6 months and who did not have a diagnosis of active TB diseases (pulmonary and extrapulmonary) or LTBI previously were recruited prospectively. The characteristics of patients including gender, age, BCG vaccine scar status, serum albumin level, serum ferritin level, haemoglobin (Hb) level and hepatitis markers were recorded. Individuals with a history of prior TB or isoniazid prophylactic treatment, those with a malignant disease, patients taking immunosuppressive drugs and age < 18 years were excluded. As BCG vaccination is compulsory in Saudi Arabia, all participants had BCG scars, therefore were considered BCG vaccinated. Patients who were positive on TST or T-SPOT.TB were evaluated for active TB by physical exam and chest x-ray. Isoniazid prophylaxis for nine months was recommended for all patients who were diagnosed with LTBI but no active disease whether T-SPOT.TB or TST were positive. Forty four kidney donors without evidence of renal insufficiency or immunocompromising conditions by medical history served as control group. This study was approved by the ethics committee of our hospital.

2.2 T-SPOT.TB Test

This test was performed according to the manufacturer's instructions (Oxford Immunotec, Oxford, UK) and as previously described (16). Eight milliliters of peripheral venous blood was collected from all the subjects in the study and processed within 2 h. All blood samples were collected before TST to avoid the possible boosting effect of TST on the determination of *M. tuberculosis* specific IFN-γ secreting T-cells. For the T-SPOT.TB test, 250,000 peripheral blood mononuclear cells (for per well)/100 µl AIM-V medium were pipette into four wells (nil–negative control, mitogen positive control, and the two specific antigens, ESAT-6 and CFP-10) per person from a 96-well microtiter plate precoated with monoclonal antibodies directed against IFN-γ. After incubation for 16–20 h at 37ºC in a CO2 incubator, the plate was washed with phosphate-buffered saline (PBS) solution and incubated with conjugate. The reaction was stopped following substrate (BCIP/NBT plus) addition. After drying, the number of IFN-γ-releasing T cells in each well was scored as either positive or negative. Spot forming units (SFUs) were counted manually and the specific antigen-stimulated wells with at least 6 spots more than the negative control (where the negative control had fewer than or equal to 5 spots) were considered positive. If the negative control well had 6 to 10 spots, it was accepted as positive when two specific antigen stimulated wells contained at least twice as many spots as the negative control well [17,18,19]. Tests were considered as indeterminate: (1) if SFUs in the positive control were <20, or (2) if SFUs in the negative well exceeded 10 and both antigen wells had less than twice the number of SFUs of the negative well [20].

2.3 Tuberculin Skin Test

The TST was done by the Mantoux technique after collection of blood samples for the IFN-γ assay, injecting a 2-TU dose of purified protein derivative RT23 (Statens Serum Institute, Copenhagen, Denmark), bioequivalent to five units of the US PPD standard intradermally into the forearm. The positive criterion for TST was ≥10 mm size of induration 72 h after injection [11,21].

2.4 Statistical Analysis (Discrepancy between the Tuberculin Skin Test in Intermediate Burden Country)

All statistical analyses were performed using SPSS for Windows (ver. 16.0). Normally distributed variables are presented as the mean with the standard deviation. Concordance between test results from the TST and the T Spot assay was assessed using κ coefficients (κ > 0.75, excellent agreement; κ < 0.4, poor agreement; and κ between 0.4 and 0.75, fair to good agreement). Instead of calculating sensitivity and specificity of the TST and the T Spot assay, we measured the correlation of the 2 tests with the risk of latent TB infection by estimating the odds ratio (OR) and relating the test results to the likelihood of TB infection. Bivariate and multivariate logistic regression was used to identify which factors were associated with the probability of a positive test. Covariates (age, gender, serum albumin, serum ferritin, Hb,BMI, HCV Ab+ve, HBSAg +ve, diabetus) were included into the models. Analyses were run separately for T-SPOT.TB, and TST. Hosmer and Lemeshow goodness-of-fit test was applied to evaluate model fit: model fit is considered adequate if P >0.05.

3. RESULTS

Fig. 1 illustrates a flow chart for patient inclusion in the study. Of the 190 patients attending HD center at King Fahad specialist hospital in Dammam, from Janurary 1 to May 30, 2009, a total of 157 were eligible to participate in the study. Fourteen patients were excluded due to withdrawal before blood collection or completion of skin testing. Furthermore, ten patients (6.9%) were excluded from further analysis because of indeterminate T SPOT assay test results. One hundred thirty three patients were included in the analysis of tests agreement and interpretation.

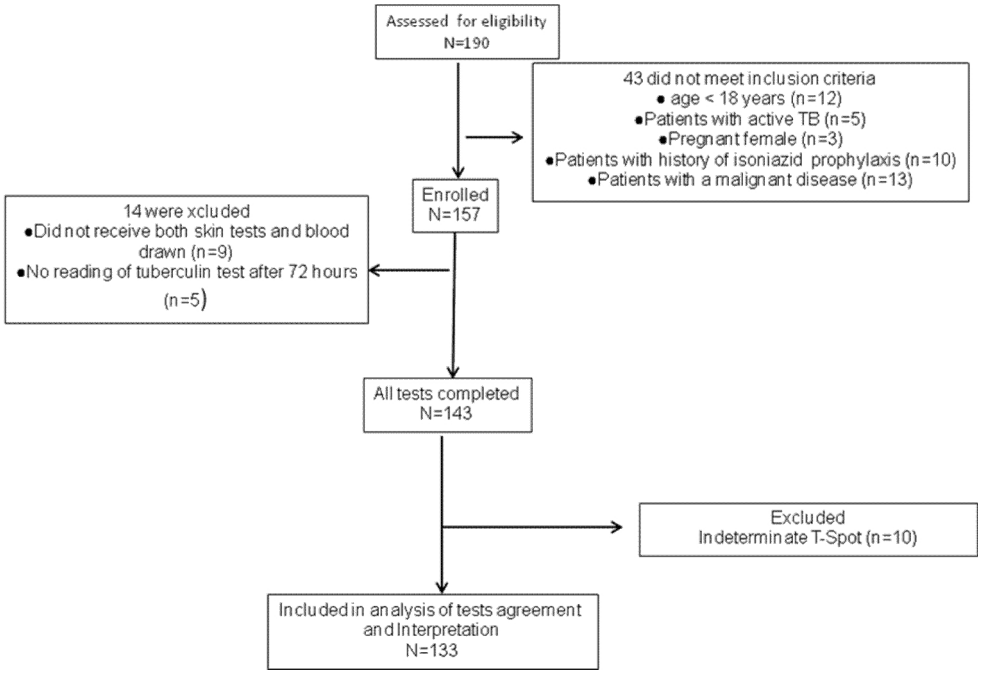

Fig. 1. Flow chart of study comparing the tuberculin skin test with T-SPOT.TB at King Fahd Specialist hospital Dammam

Demographic characteristics and laboratory findings of the enrolled participants are listed in Table 1. Among 44 potential donors, tuberculin test was positive in 13.6% of cases. T SPOT was positive in 4.5 % of cases. Diabetus mellitus was reported in four patients (18.2%). HD patients included in the study were on chronic dialysis. Tuberculin test was positive in 19 % of HD patients. T SPOT was positive in 39 % of cases. Thirty four (25.6%) patients had diabetus mellitus.

Table 1. Demographic and clinical characteristics and laboratory findings of the subjects included in the study

Characteristics	Heamodialysis patients N=133	Kidney donors N=44
Age (years)	41.9±1.5	31.8±7.4
Gender (male/female)	(68M/65F)	(32M/12F)
Albumin (g/dl)	36.9±6.4	41.7±3.7
Ferritin	3.2±3.1	1.7±6.4
Hb	12.1±2.5	14.5±1.5
BMI	24.97±5.9	29.54±6.1
Hepatitis C virus antibody positivity	6.8%	_
Hepatitis B surface antigen positivity	3 %	_
Diabetus (n, %)	34 (25.6%)	4 (18.2%)
Causes of chronic renal failure (n, %)		
Hypertensive nephropathy	47 (35.3%)	
Diabetic nephropathy	34 (25.6%)	
Nephritis	24(18%)	
Congenital causes	20 (15%)	
Obstructive causes	5(0.038%)	
Unknown	3(0.023%)	

N number of cases
Mean±SD
Hb: haemoglobin
BMI: body mass index

3.1 Agreement between TST and T-SPOT.TB

In donors, the concordance between the T-SPOT and the TST was moderate (90.9 %, κ=0.46). Forty of 44 donors (90.9%) had concordance results between the T-Spot TB and TST.

In hemodialysis patients, the concordance between the T-SPOT.TB and the TST was poor (60.15 %, κ=0.07). Fifty three of 133 patients (40%) had discordant results between the T-SPOT.TB and TST. Of these, 13 patients had a positive TST but negative T-SPOT.TB and 40 had a positive T-SPOT.TB but a negative TST. (Table 2).

Table 2. Comparison of the results of tuberculin skin test (TST) and T SPOT.TB for hemodialysis patients and kidney donors

Groups	Hemodialysis patients N=133	Kidney donors N=44	Total
Positive T-SPOT.TB and positive TST	12 (9%)	2(4.5%)	14
Positive T-SPOT.TB and negative TST	40(30%)	0	40
Negative T-SPOT.TB and positive TST	13(10%)	4(9.1%)	17
Negative T-SPOT.TB and negative TST	68(51%)	38(86.4)	106
Agreement, overall %	Poor, 60.15%	Moderate,90.9%	
Κ coefficient	0.077	0.463	
Total			177

N: number
K: concordance

3.2 TST, T-SPOT.TB and Risk Factors

In kidney donors, using univariate and multivariate logistic regression analysis, no association was found between positive TST or T-SPOT.TB and covariates included in the study. In HD patients, a similar pattern was found when tested with TST.

In hemodialysis patients, univariate and multivariate logistic regression analysis showed no significant differences between T-SPOT.TB positive subjects with respect to mean serum albumin level, mean serum ferritin level, mean Hb level, positive HCV Ab and positive HBS Ag. However, T-SPOT.TB positivity was significantly associated with male gender, increased age, low body mass index and diabetes mellitus. (Table 3).

Table 3. Bivariate and multivariate logistic regression analysis for TB risk factors among dialysis patients associated with positive T-SPOT.TB

	Bivariate analysis		Multivariate analysis	
	OR (95% CI)	***P* value**	**OR (95% CI)**	***P* value**
Age	1.04 (1.01-1.07)	0.001	1.06 (1.03 to 1.09)	0.0
Gender	0.38 (0.19-0.79)	0.009	0.46 (0.21 to 0.99)	0.049
Albumin (g/dl)	1.01 (0.96-1.07)	NS	1.02 (0.94 to 1.1)	NS
Ferritin	1.00 (0.99-1.002)	NS	1 (0.99 to 1.0)	NS
Hb	1.10 (0.96-1.28)	NS	1.1(0.91 to 1.34)	NS
BMI	0.96 (0.89-1.00)	0.05	0.92 (0.85 to 0.99)	0.04
HCVAb +ve	1.54 (0.38-6.26)	NS	1.23 (0.27 to 5.53)	NS
HBSAg +ve	0.63 (0.08-4.63)	NS	0.43 (0.05 to 3.46)	NS
Diabetus	0.002 (0.12-0.63)	0.002	0.34 (0.14 to 0.78)	0.01

HCVAb +ve: Hepatitis C virus antibody positive.
HBSAg +ve: Hepatitis B surface antigen positivity.
NS: not significant.

4. DISCUSSION

An accurate diagnosis of LTBI is essential in HD-patients, especially those awaiting transplantation. Several studies [22-24] pointed to the increased risk to develop TB during immunosuppressive thereapy. The current study compared the performance of the commercially available T-SPOT.TB test with TST for the detection of LTBI in end stage renal failure patients under HD in Saudi Arabia, a country with an intermediate burden of TB.

Detection of latent TB has relied mainly on two main techniques, TST and IGRA based assays. One of the pitfalls of IGRA based assays is the indeterminate results. The significance of indeterminate tests is not clear but may represent anergy in cases where there is insufficient response to PHA (positive mitogen control). Indeterminate responses may occur up to 10.2% in routine clinical practice [25] and are associated with elderly and immunocompromised patients, such as those with HIV/AIDS, lymphocytopenia, and hypoalbuminemia [25]. Indeterminate results occurred in only 6.9% of dialysis patients compared with none of the healthy donors in our study. All patients with indeterminate results showed an induration size of less than 5mm with TST. Other studies in ESRD patients found indeterminate results varying from 2.1 to 11% using different IGRA platforms [4,20,26,27]**.** In theory, the T-SPOT.*TB* may be less prone to indeterminate results than the QuantiFERON Gold test because, as an enzyme- linked immunosorbent spot test, it requires the enumeration of T cells before measurement of IFN-γ release so that conditions that result in low T cell counts are controlled for [28].

Due to lack of reference standard for diagnosis of latent TB, some previous studies have used active TB cases as surrogates for positive latent TB status. However in our study active TB cases were excluded because active and latent TB are distinct disease states that elicit different responses from the host immune system and therefore it may be inappropriate to use active TB as an immunologic model for latent TB[29,30]. Several studies have shown that responses to the TST and IGRAs diminish during untreated active TB infection, but rapidly increase after treatment, suggesting that active TB may suppress the host immune response to these tests [31,32].

Our data showed that positivity on the T-SPOT.TB assay was more frequent than was positivity on the TST in HD patients. Fifty two (39%) of the 133 HD patients were positive in the T-SPOT.TB assay. This positivity rate is consistent with previous studies showing positive T-SPOT.TB assay ranging from 30 % [33] to 34 % [21] .In contrast, only nineteen (19 %) of the 133 HD patients were positive in the TST assay, which is comparable with other studies performed on HD patients [2]. Patients in ESRD have high rates of cutaneous anergy when using the TST [34,35].

The American Thoracic Society uses a criterion of ≥5 mm induration for TST positivity in transplant recipients [36]. However, previous studies from countries with intermediate TB burden used a cut-off of 10 mm because of high false-positive rates when lower cut-offs were used [4,21,33,37,38]. Therefore, we defined positivity on TST as an induration size ≥10 mm. If an induration diameter more or equal to 5mm was used, only 4 patients will be added to the positive TST results. These 4 patients showed negative T-SPOT.TB. These data will not affect the overall conclusion of the study. Moreover, in a systematic review to evaluate tests for latent tuberculosis in people with ESRD, their findings were unchanged when they compared studies using a TST cutoff of 5 versus 10 mm (P>0.1) [30]. We did not repeat TST 7–10 days after the first test to evaluate any booster effect. The clinical utility of repeated TSTs in transplant recipients has not been fully established. In a recent study by

Soysal et al, even a two-step TST was not sufficient to detect true LTBI in HD patients [39]. Moreover, repeated testing most likely increases sensitivity at the expense of specificity. Also, repeated testing has operational limitations linked to the need of many return visits [40]. There was poor agreement between the 2 tests (κ = 0.077). In dialysis patients, several studies have found that agreement between the TST and T-SPOT.TB was only poor to fair (0.16 to 0.32) [4,20,27,41]. On the other hand, Winthrop et al. [22] showed that concordance between TST versus T-SPOT.TB was better (71 %).In our study, the agreement was better (fair agreement) in immunocompetent kidney donors compared with HD patients.

There is a 6.9- to 52.5-fold increase in the incidence of TB in dialysis patients compared to the general population, which results from uremic immunodeficiency [7,42-47]. Different studies have evaluated different risk factors for the development of TB in patients on HD. These cofactors include advanced age, reduced body mass index, low serum albumin and anemia. Additionally, diabetes, HCV Ab+ve and HBSAg +ve have been investigated [2,48]. In the current study, when evaluating the TB risk factors that may influence the TST results, no correlation was found between the TST and TB risk factors. Similarly, other authors who evaluated these risk factors in HD patients showed that TB risk factors did not affect the TST results [4,49,50]. Conversely, in the current study, male gender, increased age, low body mass index and diabetes mellitus were independent positive factors significantly affecting the T.SPOT results (*P* value=0.001, 0.009, 0.05 and 0.002). The reported results are concordant with several authors [4, 20, 26,49] who previously found a relationship between TB risk factors and the IGRA results.

Virusspecific CD4 –CD8 T lymphocytes and the liver are responsible in hepatitis C virus (HCV) infection for immune system response [51-53]. A wide range of clinical manifestations are associated with HCV. In a recent study by celikbilek et al [54], TST was not a reliable method to detect latent TB in cirrhosis patients caused by HBV and HCV. In our study, 6.8 % of HD patients were HCV antibody positive. However, when evaluated as a risk factor in HD patients, HCV did not affect the TST or T SPOT results.

The current study was performed in a cohort of HD patients in Saudi Arabia which is a country with intermediate TB burden. The current study showed a positive correlation between positive T SPOT and aging in keeping with the results of Lee et al [48]. Conversely other studies [20, 26] performed in countries with low TB burden found that older patients were more likely to have a negative TST. Our study didn't find a similar pattern in our patient cohort. This may be explained by the preferential acitivity of circulating T cells compared to immunological cells (responsible for mounting immune response during TST) in immunocompromised HD patients. Another explanation of this specific finding may be the superioriority of the T-SPOT test that control the number of T cells included in each reaction irrespective of other potential confounders (e.g age, leucopenia) .

5. CONCLUSION

In conclusion, our data strongly argue against the use of TST in screening of LTBI in HD patients. T-SPOT.TB test in dialysis patients correlated better than TST with the risk of TB infection (e.g. increased age and low body mass index). It is a more reliable and powerful diagnostic tool than TST.

CONSENT

All authors declare that 'written informed consent was obtained from the patients (or other approved parties) for publication of this research.

ETHICAL APPROVAL

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.

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COMPETING INTERESTS

No competing interests exist. No specific funding has been received. The data have been generated as part of the routine work of the hospital.

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