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Comparison of the tuberculin skin test and Quanti-FERON-TB Gold In-Tube (QFT-G) test for the diagnosis of latent tuberculosis infection in dialysis patients

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Summary Dialysis patients are more likely than the general population to develop active tuberculosis (TB). In these patients, the availability of a highly sensitive and specific test to diagnose latent TB will ensure earlier treatment and decreased progression to active disease. In the current study, the Quanti-FERON-TB Gold In-Tube (QFT-G) test was compared with the tuberculin skin test (TST) for the diagnosis of latent tuberculosis infection (LTBI) among 200 hemodialysis patients and 15 confirmed TB disease cases in a tertiary care center in Saudi Arabia. Among the LTBI cases, 26 (13%) were TST positive, and 65 (32.5%) were positive by the QTF-G test, with an overall agreement between the 2 tests of 75.5% ($k = 0.34$) being observed.

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Latent tuberculosis infection

Among the confirmed tuberculosis disease cases, none were positive by TST, and 10 (66.7%) were positive by the QTF-G test, resulting in an overall agreement of 33.3% ($k = 0$). A comparison between the TST and the QTF-G test was performed based on the sensitivity, specificity, and area under the curve (AUC) obtained for the tests. The QTF-G test was more sensitive and less specific than the TST in predicting the confirmed TB disease cases. When we tested the correspondence of the AUC values between the 2 diagnostic modalities, the obtained *p*-value was 0.0003. In conclusion, the AUCs of the examined diagnostic modalities are significantly different in predicting LTBI and tuberculosis.

Introduction

When dialysis patients are infected by bacilli of the *Mycobacterium tuberculosis* complex, they are 10—25 times more likely than immunocompetent people to develop active tuberculosis (TB) [1—5]. In Saudi Arabia, the risk of active TB is estimated to be approximately 50—92 times that in the general population [6—9]. The higher TB incidence observed in patients on dialysis is attributed to the lowered cellular immunity caused by the state of chronic renal failure [10]. Furthermore, the case fatality rate of TB in dialysis patients appears to be higher than that of TB in the general population, ranging from 17% to 75% [2,6,8—15]. This higher mortality rate may largely be associated with late diagnosis and treatment [4], which, in turn, is due to the TB symptoms occasionally being confused with uremic symptoms and the fact that in dialysis patients, TB may present with nonspecific symptoms in approximately 50% of cases [2,8].

The detection and treatment of latent tuberculosis infection (LTBI) in this population are crucial to prevent the progression to active TB and the secondary transmission of the infection to other patients and health care workers (HCWs). Therefore, a test for early detection of LTBI in these patients is essential, and hence, current guidelines advocate screening hemodialysis patients for LTBI [1—3]. The tuberculin skin test (TST) has long been the standard method for detecting LTBI. However, this TST has 2 major disadvantages: a high percentage of dialysis patients show false negative TST results, due to anergy, and false positive TST results, due to cross-reactivity with the Bacille-Calmette-Guerin (BCG) immunization and nontuberculous *Mycobacterium* (NTMB) infection [4—7]. The availability of *M. tuberculosis* (MTB) antigen-specific interferon-gamma (IFN-γ) release assays (IGRAs) represents a significant advancement in the diagnosis of LTBI.

IGRAs are a newly developed type of blood test that measure the production of IFN-a by sensitized lymphocytes after the blood is stimulated with the specific TB antigens culture filtrate protein 10 (CFP-10) and early secretory antigen 6 (ESAT-6). These antigens are encoded in the RD-1 portion of the MTB genome. They are not found in BCG strains or most NTMB and are therefore more specific [8]. Two IGRAs are commercially available: the Quanti-FERON-TB Gold In-Tube (QFT-G) assay (Cellestis Ltd., Carnegie, Australia) and the T-SPOT.TB assay (Oxford Immunotec, Abingdon, UK). Limited data are available on the usefulness of IGRAs in the diagnosis of TB infection in hemodialysis patients. Data from low-TB prevalence countries have indicated that the IGRAs are better diagnostic tools than the TST for LTBI [9—11,13]. Studies performed in high-TB prevalence countries have also recently found that IGRA is a reasonable alternative to the TST in hemodialysis patients [14,15]. The aim of the present study was to compare the performance of the QTF-G test and the TST for detecting LTBI among hemodialysis patients at King Abdulaziz Medical City-National Guard Hospital-Riyadh (KAMC-R) and to investigate the agreement between these 2 tests in the detection of tuberculosis infection in a population showing an intermediate TB prevalence [16].

Subjects and methods

The study participants were hemodialysis patients who were recruited prospectively from the outpatient hemodialysis unit of KAMC-R. We screened all of the hemodialysis patients in our hemodialysis unit for LTBI over a period of 5 months from August to December 2010. Patients who agreed to be enrolled in the study were interviewed by our public health nurse to explain the study's purpose, and these patients all provided written informed

consent. All patients who were diagnosed with confirmed TB disease were also recruited to participate.

The following information was obtained: demographic data, underlying medical problems, contact with the TB index case, history of LTBI or therapy, and documentation of current or previous TB treatments. Blood was drawn for the QTF-G test on the same day that the patients were attached to the dialysis machine. The TST was performed after the initiation of dialysis on the same day and after drawing blood for the QTF-G test.

Tuberculin skin test (tst)

The TST employed in this study was Tubersol — Tuberculin Purified Protein Derivative (Mantoux), 5 TU per 0.1 ml, test manufactured by Sanofi Pasteur Limited, Toronto, Ontario, Canada.

A trained and experienced public health nurse performed all TSTs. Five tuberculin units (0.1 ml) of the purified protein derivative (PPD) were administered via intradermal injection on the volar surface of the forearm that did not have the arteriovenous vessel. The responses were read within 72 h by the same nurse, usually during the next regularly scheduled HD visit. An induration of 10 mm or more in transverse diameter was used as the threshold to classify the test results as positive for LTBI. Patients with an induration of less than 10 mm upon initial testing were considered to be negative and were administered a second TST within 3—6 weeks to elicit a potential booster response [2]. The results obtained from the 2-step testing were used in all further analyses. The TST was considered to be positive if either the 1st or 2nd test showed a response of 10 mm or more.

Quanti-FERON-TB Gold In-Tube (QFT-G) test

Blood was collected before the administration of the TST, and the test was performed according to the manufacturer's instructions [17]. One ml of whole blood was collected in each of 3 separate test tubes: 1 containing no antigen (nil control), 1 with a mitogen (phytohemagglutinin, positive control) and 1 with TB antigens (ESAT-6, CFP-10 and TB7.7). The 3 tubes were incubated overnight for 18—20 h at 37 °C. Following incubation, the tubes were centrifuged, and the plasma was removed from each tube and frozen at −20 °C. Measurement of IFN-γ via ELISA was subsequently performed in batch testing. The results were expressed in IU/ml, as determined from a standard curve run on each plate. According to the manufacturer's instructions, a value of 0.35 IU/ml or more for the relationship ([IFN-γ in the TB antigen tube] − [IFN-γ in the negative control tube]) was considered to be a positive result. If the IFN-γ level was less than 0.35 IU/ml in the TB antigen tube and the mitogen control was positive (≥0.5 IU/ml), the test was recorded as negative.

Radiological assessment

A chest X-ray (CXR) was ordered if one had not been performed within the previous 3 months. All of the radiological reports were then reviewed, and all CXRs that were reported as abnormal were re-examined by an independent pulmonologist who was blinded to the TST and QTF-G results.

The CXR results were divided into suggestive of active tuberculosis, in which case the patient was referred for further investigation, or consistent with a previous TB infection, associated with findings such as upper lobe fibronodular disease, granulomata, calcified mediastinal lymph nodes and/or pleural thickening. Patients with 1 or more additional risk factors for tuberculosis in addition to hemodialysis, such as diabetic nephropathy, previous kidney transplant, use of immunosuppressants during the previous 3 months, a CXR showing changes consistent with untreated, but not active TB or a history of contact with active TB and a BMI < 20, were categorized as showing a high likelihood for LTBI [2].

In this study, the gold standard references for TST and QTF-G sensitivity and specificity were the confirmed TB disease cases, which showed either pulmonary or extrapulmonary TB. Confirmation was based on a positive culture for tuberculosis or granuloma in the biopsy and the response to anti-tuberculosis therapy.

Statistical analyses

Continuous variables, such as age, were reported as the mean ± standard deviation (Mean ± SD), while categorical variables, such as gender and TST, were reported as numbers and percentages (*n* [%]). The overall agreement between the QTF-G test and the TST was evaluated using the kappa (*K*) coefficient and concordance cases. The agreement between the QTF-G test and the TST was stratified by the confirmed treated tuberculosis disease status. The association between the TB disease status and categorical variables, such as gender and the TST and QTF-G test results, was assessed using Chi-square tests. If the expected frequency was smaller than 5, then Fisher's exact test was used as an alternative to the Chi-square test. *p*-Values less than 0.05 were considered to be significant. Finally, to

Table 1 Demographic and clinical characteristics of LTBI and TB disease.

Characteristics	Level	*N* (%)
Demographic, clinical characteristics and diagnostic modalities among 215 patients		
Age (Mean ± SD)		62.27 ± 11.79
Gender	*Female*	112 (52.1)
Documented TB cases[a]	*Positive*	15 (7.0)
Result of first and second TST	*Positive*	26 (12.1)
QTF-G test	*Positive*	75 (34.9)
Demographic, clinical characteristics and diagnostic modalities among 200 patients screened for LTBI (no TB disease)		
Age (Mean ± SD)		58.42 ± 17.65
Duration of dialysis (Mean ± SD)		60.1 ± 175.2
Gender	*Female*	103 (51.5)
BCG vaccine	*Yes*	28 (14.0)
BCG scar	*Yes*	29 (14.5)
TST 1st test	*Positive*	22 (11.0)
Result of first and second TST	*Positive*	26 (13.0)
QTF-G test	*Positive*	65 (32.5)
High likelihood of tuberculosis infection[b]	*Positive*	154 (77.0)
Diabetic nephropathy	Yes	127 (63.5)
Kidney transplant	Failed	21 (10.5)
Immunosuppressant in the last 12 M	Yes	2 (1.0)
Chest X-ray result	Abnormal	10 (5.0)
History of contact with TB patients	Yes	0 (0.0)
BMI	≤20	25 (12.5)

[a] Documented TB cases: defined as patients with confirmed diagnosis of tuberculosis disease based on positive tuberculosis culture or biopsy showing granuloma and good response to anti-tuberculosis therapy.

[b] High likelihood of LTBI: defined as contact with TB case, abnormal chest X-ray, DM, immunosuppressant in the last 12 M, failed kidney transplant or BMI ≤ 20.

evaluate the 2 diagnostic modalities, traditional measures of diagnostic accuracy were used, such as the sensitivity, specificity, positive predictive value, negative predictive value and AUC. Further analyses explored the performance of the 2 diagnostic modalities when predicting the likelihood of LTBI. A high likelihood of LTBI was indicated if any of the following were recorded: diabetic nephropathy, failed kidney transplant, immunosuppressant use in the last 12 M, an abnormal chest X-ray result consistent with changes suggestive of old untreated tuberculosis, a history of contact with an open TB case and a BMI ≤ 20. All statistical calculations were performed with SAS version 9.2.

Results

During the study period, 200 patients were recruited to be tested for LTBI using both the TST and GTF-G test. During the study period, 15 patients presented with a confirmed diagnosis of TB disease. Among the 200 patients screened for LTBI, 127 (63.50%) exhibited diabetic nephropathy; 21 (10.50%) failed kidney transplant; 2 (1%) had been given immunosuppressants in the last 12 months; 10 (5%) displayed an abnormal CXR result consistent with inactive TB; no patient had a history of contact with a TB patient; 25 (12.50%) presented a low BMI (≤20); 154 (77%) exhibited a high likelihood risk for LTBI; and 28 (14%) reported that they had been given a BCG vaccination in the past. Other demographic and clinical characteristics of the cases with LTBI are shown in (Table 1). The TST classifies a patient as being positive for LTBI if the result of either the 1st TST or 2nd TST is ≥10 mm. Of the 200 patients screened for LTBI, 26 (13%) were positive, and 174 (87%) were negative by the TST. The QTF-G test results showed that 65 (32.5%) of the patients screened for LTBI were positive, and 135 (67.5%) were negative. Thus, the percentage of LTBI detected by the QTF-G test was substantially higher than that by the TST (32.5% vs. 13%). Table 2 shows the associations between the demographic and clinical characteristics of the LTBI and TB disease cases and the TST and QTF-G results. The percentages of concordance and Kappa coefficients between the TST and the QTF-G test were 75.5% (fair agreement, $\kappa = 0.34$) in the 200 hemodialysis patients with LTBI and 33.3% (no agreement other than what

Table 2 Associations between demographics and clinical characteristics and TB status.

Characteristics	Level	Tuberculosis status		*p*-Value
		Screened for LTBI (no disease) (*n* = 200)	Confirmed tuberculosis disease cases (*n* = 15)	
Gender, *n* (%)	*Male*	97 (48.5%)	6 (40.0%)	0.525
	Female	103 (51.5%)	9 (60.0%)	
Age (Mean ± SD)		58.42 ± 17.65	62.27 ± 11.79	0.408
TST	*Negative*	174 (87.0%)	15 (100.0%)	0.225
	Positive	26 (13.0%)	0 (0.0%)	
QTF-G test	*Negative*	135 (67.5%)	5 (33.33%)	0.007
	Positive	65 (32.5%)	10 (66.7%)	

Table 3 Agreement between TST and QTF-G among LTBI and TB diseases cases.

	QFT (+ve)* *n* (%)	QFT (−ve)* *n* (%)	Concordance	κ
Patients without TB n = 200				
TST (+ve) *n* (%)	21 (10.5)	5 (2.5)	75.5%	34%
TST (−ve) *n* (%)	44 (22)	130 (65)		
Patients with TB disease n = 15				
TST (+ve) *n* (%)	0 (0.0)	0 (0.0)	33.3%	0%
TST (−ve) *n* (%)	10 (66.7)	5 (33.3)		

+ve = positive; −ve = negative.

would be expected by chance, $\kappa = 0.0$) in the 15 hemodialysis patients with confirmed TB disease (Table 3). Table 4 shows that the TST was negative in all of the confirmed TB disease cases (sensitivity = 0.0%). In contrast, the QTF-G test displayed a specificity of 67.5% and a sensitivity of 66.7%. When we tested the correspondence of the AUC between the 2 diagnostic modalities, the obtained *p*-value was significant ($p = 0.0003$), and a difference was found between the AUC of the TST (AUC = 0.435) and that of the QTF-G test (AUC = 0.671). The TST failed to identify cases with confirmed TB disease, giving a positive predictive value (PPV) of 0.0%, while the QTF-G test had a PPV of 13.3%. The negative predictive value (NPV) of the TST was 92.1%, while the QTF-G test NPV was 96.4% (Table 4). The sensitivity of detecting a high risk for the likelihood of tuberculosis infection of the 2 diagnostic modalities was low for both tests, at 33.1% for the QTF-G test and 12.3% for the TST (Table 4).

Table 4 The performance of the GTF-G test and TST among TB disease and high likelihood of LTBI.*.

Accuracy	GTF-G test	TST
Predicting tuberculosis disease (TB)		
Sensitivity [95% CI]	0.667 [0.428—0.905]	0.000 [0.000—0.000]
Specificity [95% CI]	0.675 [0.610—0.740]	0.870 [0.823—0.917]
PPV [95% CI]	0.133 [0.056—0.210]	0.000 [0.000—0.000]
NPV [95% CI]	0.964 [0.934—0.995]	0.921 [0.882—0.959]
AUC [95% CI]	0.671[0.528—0.814]	0.435 [0.300—0.570]
Predicting of high likelihood of LTBI		
Sensitivity [95% CI]	0.331 [0.257—0.406]	0.123 [0.071—0.175]
Specificity [95% CI]	0.696 [0.563—0.829]	0.848 [0.744—0.952]
PPV [95% CI]	0.785 [0.685—0.885]	0.731 [0.560—0.901]
NPV [95% CI]	0.237 [0.165—0.309]	0.224 [0.162—0.286]
AUC [95% CI]	0.512 [0.437—0.590]	0.486 [0.427—0.544]

Discussion

Tuberculosis remains a serious global health problem, especially among immunocompromised patients, such as those receiving hemodialysis. The early diagnosis and treatment of LTBI in dialysis patients is very challenging, especially in a population that has received the BCG vaccine. False positive TST results may be caused by a previous BCG vaccination or by infection with an atypical mycobacterium. Therefore, more reliable tests are required for detecting LTBI in dialysis patients, particularly as they are at high risk of progression to TB. The QTF-G test has been shown to improve diagnostic accuracy for LTBI. However, limited data are available on how the TST and QTF-G compare among dialysis patients in both LTBI and active TB disease.

In this study, we found that LTBI in the hemodialysis patients was detected by the TST and QTF-G test in 26 (13%) and 65 (32.5%) patients, respectively. The 13% positivity observed for the TST in our patients is lower than the rates reported recently from 6 countries, which ranged from 19% to 40% [10,13—15,18—20]. The low rate of positive TST results found in our population could be due to the presence of a small number of patients with TB disease, excluding TB cases for whom there is no information about TST and QTF-G results, and is most likely due to associated co-morbidities, such as the number of diabetic and immunosuppressed patients, which was much higher in our study than in studies from Korea and Taiwan [14,15]. Furthermore, other researchers have shown that the QTF-G test is more likely than the TST to provide positive results in cases of TB disease [8,18,21]. The overall discriminatory ability between individuals with and without TB disease was not satisfactory, as the sensitivities of the 2 diagnostic modalities were low, at 33.1% for the QTF-G test and only 12.3% for the TST.

In this study, the 2 tests (QTF-G test and TST) were compared and evaluated based on their sensitivity, specificity, positive predictive value, negative predictive value and AUC. The AUC was 0.671 for the QTF-G test and 0.435 for the TST in detecting TB disease, whereas the AUC values obtained for LTBI cases were 0.512 for the QTF-G and 0.486 for the TST. These results indicate that these tests exhibit different accuracies in diagnosed tuberculosis disease and LTBI and that the discriminatory ability of the QTF-G test is superior to that of the TST. The major strength of our study is its reasonably large number of participants. However, one limitation the study presents is that we only considered a size of 10 mm as the cutoff for indicating positive TST, and we did not correlate values less than 10 mm to the QTF-G test. This correlation is expected to be crucial, as some of our TST-negative patients and QTF-G-positive patients with confirmed TB may require a different cutoff point for predicting positivity. Additionally, all of the results that were lower than the positive cutoff value for the QTF-G test were reported as negative. Binary diagnostic tests were considered here; our future research will focus on tests with continuous measures.

In conclusion, screening all dialysis patients using the QTF-G test instead of the TST may be a reasonable method to ensure the early diagnosis of LTBI and, hence, to initiate proper early therapy. In addition, a need exists to explore new diagnostic tools for LTBI and TB disease with better discriminatory power.

Conflict of interest

Funding: No funding sources.
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