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Evaluation of performance of quantiferon assay and tuberculin skin test in end stage renal disease patients receiving hemodialysis

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SUMMARY

Purpose: End stage renal disease (ESRD) cases are associated with increased risk of tuberculosis. There is no gold standard method for detecting latent tuberculosis infection (LTBI) in ESRD. The aim of the present study was to analyze the performance of the tuberculin skin test (TST) and QuantiFERON-TB Gold in tube (QFT-G) in cases receiving hemodialysis (HD).
Methods: The TST and QFT-G were prospectively performed in 96 ESRD cases undergoing HD. The agreement of the QFT-G and TST was assessed in two TST cut off values (10 mm and 5 mm) in Bacille Calmette Guérin (BCG) vaccinated and non-vaccinated cases.
Results: Of 96 cases 67 were BCG vaccinated and 29 were BCG non-vaccinated. QFT-G was positive in 39.6% cases and indeterminate in 3.1%. TST was positive in 43.8% of cases in cut off value of 10 mm and positive in 58.3% of cases in cut off value of 5 mm. Agreement between TST and QFT-G results was fair in both BCG vaccinated and non-vaccinated cases in either cut off values, except in cut off value of 10 mm in BCG vaccinated cases in which the agreement was moderate.
Conclusion: The agreement between QFT-G and TST test is fair and there is no significant difference in both cut off values of TST in screening of LTBI in ESRD cases receiving HD.

KEY WORDS: Tuberculosis, Hemodialysis, Quantiferon test

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Tuberculosis (TB) remains a major health problem not only in developing countries but in developed countries as well. The incidence of TB is higher in end-stage renal disease (ESRD) patients owing to impaired host defense mechanisms, particularly immune suppression, which is more important in endemic regions (Hussein *et al.*, 2003, Cengiz *et al.*, 1996, Mitwalli *et al.*, 1991, Andrew *et al.*, Kür at *et al.*, 2001). Diagnosis is difficult and delayed since extra pulmonary involvement is more common than isolated pulmonary involvement, and nonspecific symptoms mimicking uremia are seen frequently. Uremia itself is known to cause impaired cellular immunity, which is the major host defense mechanism against TB infection (Kurtz *et al.*, 1986). Moreover, patients on hemodialysis (HD) are at increased risk of developing active tuberculosis after primary infection, activation of quiescent disease, or reactivation of old TB infection. Nosocomial transmission of TB has also been reported in patients under long-term dialysis (Hickstein *et al.*, 2003).

Diagnosis of TB in immune-compromised patients and patients on HD could be difficult and

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often missed. This is due to vague presentation with inconclusive chest X-rays and negative tuberculin skin tests (TST). The TST remains the standard method for identifying TB infection in screening of LTBI in chronic renal failure patients and in patients receiving hemodialysis. In fact, ESRD is known to be a risk factor for skin test anergy; although the rate of anergy is quite variable, recent reports suggest that about 32% to 40% of HD patients are anergic (Ravi Shankar *et al.*, 2005, Woeltje *et al.*, 1998, Smirnoff *et al.*, 1998). This indicates that additional clinical testing is needed in dialysis patients for the presence of tuberculosis beyond the use of a routine TST. A new diagnostic method, QuantiFERON-TB gold in tube is an in vitro test for Mycobacterium (M) TB infection.

It measures interferon gamma (IFN-γ) secreted by T cells which are stimulated with mixtures of synthetic peptides including the early secretary antigenic target-6 (ESAT-6) and culture filtrate protein 10 (CFP-10) that are specific to M. TB. After incubation of whole blood with the mixture of synthetic peptides, an ELISA detects the release of IFN-γ from lymphocytes. Because the target peptides are secreted by M. TB and M. bovis, but not by strains present in the Bacille Calmette Guérin (BCG) vaccine and by most nontuberculous mycobacteria, QFT-G may correlate better with exposure to M. TB than the TST. In this scenario, blood samples would be taken from the patient and incubated with mycobacterial antigens specific for M. TB complex strains but not for the BCG vaccine strain. T lymphocytes within the blood sample produce IFN-γ as a marker of infection or active TB. Since M. TB is an intracellular pathogen, assessment of whether a patient's T cells have been exposed to and sensitized by antigens specific to M. TB, may provide an alternative approach to diagnosis.

IFN-γ assays have several advantages over TST (Amicosante *et al.*, 2010). Its results can be available in 24 hours after testing and no return visit is required.

Automated testing has the advantage of reducing reader bias as its interpretation is objective. A booster phenomenon does not occur and therefore screening of people who are repeatedly exposed to TB (e.g. healthcare workers) becomes feasible. Patients with impaired immune function are one of the most important targets for the screening of LTBI because of the high risk of progression to active TB. QFT-G tests have been studied in various groups of immunocompromised subjects (HIV infection, immuno-suppressed hematology patients, auto-immune diseases, silicosis) (Jones *et al.,* 2007, Luetkemeyer *et al.,* 2007, Leung *et al.,* 2008, Menzies *et al.,* 2007,Kobashi *et al.,* 2007, Piana *et al.,* 2006.). Although responses to QFT-G tests are slightly reduced in immunosuppressed subjects, when compared with immuno-competent individuals, positivity rate of QFT-G test is substantially higher than that of TST (Menzies *et al.,* 2007). However, there is no sufficient data on QFT-G test use in screening of LTBI in chronic renal failure patients and in patients receiving hemodialysis. There are few studies evaluating the value of QFT-G test in LTBI in these cases and also investigating the agreement between QFT-G test and TSTs.

In this study we aimed to analyze the performance of the TST and QFT-G in cases with ESRD cases receiving hemodialysis and to evaluate the agreement of these tests in BCG vaccinated and non-vaccinated cases.

Patients with ESRD who underwent HD for more than 3 months were enrolled in this study prospectively in Konya, Turkey. Approval of Institutional Review Board was provided and all participants gave written informed consent. Patients' charts were reviewed for demographics (age, and sex), etiology of ESRD, duration of HD and past medical history. Efficiency of dialysis was measured using urea reduction ratio (URR) and depict delivered dose of dialysis (Kt/V). Patients with positive TST or QFT-G test were submitted for evaluation of active TB disease and isoniazid prophylaxis was recommended to those patients in whom no active disease was determined but was positive in either of the two assays.

TST was performed on the volar aspect of the forearm by the Mantoux method using 0.1 ml (5 tuberculin units) of PPD Tuberculin Tween 80 (BB-NCIPD Ltd. Sofia, Bulgaria). Induration was measured after 48-72 hours using the ballpoint pen method. TST response was scored as positive if induration diameter was equal or >10 mm (the recommended TST induration cut-off point among persons with chronic renal failure (American Thoracic Society and the Centers for Disease Control and Prevention). To determine

the development of booster phenomenon, the patients with a reaction of less than 10 mm induration to the initial TST were given a second tuberculin test 1 to 2 weeks later. The booster phenomenon was defined as positive if induration from the second test was 10 mm or more and measured at least 6 mm more than that for the TST-1. These results were defined as TST^1. A secondary evaluation, by accepting TST results as positive if the induration was >5 mm (the recommended TST induration cut-off point for patients with organ transplants and other immunosuppressed patients (American Thoracic Society and the Centers for Disease Control and Prevention)), was done and these results were defined as TST^2.

Interferon-γ release assays, QFT-G test, were performed according to the manufacturer's instructions (Cellestis Ltd., Carnegie, Victoria, Australia). A result of ≥0.35 IU/mL of IFN-γ in the TB antigen tube minus the negative control tube was considered a positive result. If the level was less than this and the mitogen control was positive (≥0.5 IU/mL), a negative result was recorded. If the level in both the TB antigen and mitogen tube was less than the threshold for positive, or the level in the nil tube was >8.0 IU/mL, then an indeterminate result was recorded (according to the manufacturer's instructions).

Results across the two primary modalities in the analysis (QFT- G test and TST) were compared using the Chi-squared test. Multivariate analysis was also performed to analyze the influence of age, vaccination, sex and other confounding factors to the TST and QFT- G test. Agreement between tests was qualified according to kappa statistics. Kappa Agreement was interpreted as follows: κ=<0: Less than chance agreement, κ=0.01-0.20: Slight (poor) agreement, κ=0.21-0.40: Fair agreement, κ=0.41-0.60: Moderate agreement, κ=0.61-0.80: Substantial agreement, κ=0.81-0.99: Almost perfect agreement (Viera *et al.,* 2005). P value <0.05 was considered statistically significant.

A total of 96 (49 male and 47 female) ESRD cases receiving hemodialysis were enrolled to study. The mean age of the patients was 54.81±15.26. The number of cases who were BCG vaccinated was 67 (69.8%) and the median duration of dialysis was 58.50 (3-240) months.

QFT-G test result was positive in 38 (39.6%) cases and it was negative in 55 (57.3%) cases. In 3 (3.1%) cases it was indeterminate. TST was analyzed in two cut off values. Firstly, it was accepted positive if it is equal or above 10 (TST^1). A secondary analysis using a cut-off of 5 mm (TST^2) was done. TST^1 was positive in 42 (43.8%) cases. However, TST^2 was positive in 56 (58.3%) cases. Even though the gender had no effect on the results of QFT-G and TST^1, TST^2 was significantly positive in male cases. QFT-G was significantly more positive in BCG non vaccinated cases, but BCG vaccination did not affect the result of TSTs. Longer dialysis period was associated with positive TST results and QFT-G test was significantly more positive in older cases. However, there was no association between QFT-G test or TST results and parameters such as creatinin, kt/v, urea, ferritine, C-reactive protein and hemoglobin levels of the patients. The results of TST^1, TST^2 and QFT-G with respect to gender and BCG vaccination status were shown in Table 1.

The overall concordance between QFT-G test and TST^1 was 71.9% (κ=0.427) and it was 61.5% between QFT-G test and TST^2 (κ=0.247). In 67 (69.7%) of 96 patients who had been BCG vacci-

TABLE 1 - *The results of TST^1, TST^2 and QFT-G with respect to gender and BCG vaccination status.*

		TST^1 - positive			*TST^2 - positive*			*QFT-G - positive*		
		n	*%*	*p*	*n*	*%*	*p*	*n*	*%*	*p*
Gender	Female	17/47	36.2	0.14	22/47	46.8	0.02	18/46	39.1	0.45
	Male	25/49	51.0		34/49	69.4		20/47	42.6	
BCG	Negative	13/29	44.8	0.53	16/29	55.2	0.42	17/29	58.6	0.01
	Positive	29/67	43.3		40/67	59.7		21/67	32.8	

TST: tuberculin skin test; TST1: positive if it is equal or above 10 mm; TST2: positive if it is equal or above 5 mm; QFT-G: Quantiferon Gold.

TABLE 2 - *The agreement between TST and QFT-G in BCG vaccinated cases and non vaccinated cases.*

	BCG vaccinated			*BCG non-vaccinated*		
	(%)	κ	*95% CI*	*(%)*	κ	*95% CI*
QFT-G vs TST1	74.3	0.432	-0.206-0.657	65.5	0.322	0.007-0.651
QFT-G vs TST2	59.4	0.229	-0.023-0.434	62.0	0.228	0.126-0.582

QFT-G: Quantiferon-TB Gold; κ: kappa statistic; 95% CI: 95% confidence interval; TST: tuberculin skin test TST1: positive if it is equal or above 10 mm; TST2: positive if it is equal or above 5 mm.

nated, TST^1 was positive in 26 (40.6%), TST^2 was positive in 40 (41.7%) and QFT-G test was positive in 21 (35%) cases. In 3 cases with BCG vaccination QFT-G test was indeterminate. When we omit these 3 cases and analyze the agreement between TST^1 and QFT-G, and the agreement between TST^2 and QFT-G in 64 BCG vaccinated cases, we found that the agreement between TST^1 and QFT-G was moderate (κ=0.432), and the agreement between TST^2 and QFT-G was fair (κ=0.229) (Table 2). In non vaccinated 29 cases; TST^1 was positive in 12 (42.4%), TST^2 was positive in 16 (55.2%) and QFT-G test was positive in 17 (58.6%) cases. QFT-G test was not indeterminate in any of these cases. In these 29 BCG non vaccinated cases, the overall agreement between TST^1 and QFT-G, and the agreement between TST^2 and QFT-G were both fair (κ=0.322, and κ=0.228, respectively) (Table 2).

In this study, we found fair to moderate agreements between QFT-G test and TST in either a TST cut off value of 5 mm and 10 mm in cases with renal failure receiving HD. In previous studies determining the performances of QFT-G test versus the TST for detecting LTBI in ESRD cases, the agreement between these tests were variable. However, the agreement was not better than a fair result. In a recent study from Turkey, (Seyhan *et al.,* 2009) reported an overall agreement of 65% (concordance κ=0.26) between the QFT-G and the TST at a cut off value of 10 mm. Lee *et al.* (2009) reported the overall agreement between QFT-G and TST as fair (κ=0.34) when the TST cut off was defined as 5 mm and fair (κ=0.25) when it was defined as 10 mm. They concluded that the QFT-G was a more accurate method for identifying those truly infected with Mycobacterium tuberculosis, even in BCG-vaccinated individuals. Also, the agreement between TST and QFT-G was poor (κ=0.16, p=0.116) in Triverio *et al.* (2009) study. They defined TST positive if an induration diameter was >5 mm and found that QFT-G was twice as effective at detecting probable LTBI (46%) as TST (25%). Another study (Winthrop *et al.,* 2008) tested 100 patients with ESRD who received hemodialysis and they reported that QFT-G and T-SPOT-TB might offer a better method for detecting TB infection in ESRD patients. Inoue *et al.* (2008) reported that the sensitivity of the QFT was 100% and the specificity was 89.7% in hemodialysis patients. In our study, the positivity of QFT-G was similar to these studies (39.6%). However, TST positivity differed according to cut off value. When we defined a positive TST as an induration ≥5 mm, the positivity (58.3%) was significantly higher than QFT-G; but when we defined it as an induration ≥10 the positivity rate decreased to 43.8%. We found that, BCG vaccination had no effect on TST result. However, interestingly QFT-G was significantly more positive in BCG non-vaccinated cases. In previous studies reported from our country revealed a BCG vaccination rate between 77.6% to 90% (Soysal *et al*., 2008, Ozdemir *et al*., 2007, Elbek *et al.*, 2009). In our study the rate of BCG vaccination was 69.7%.

In our study, only age of the patient was associated with positive QFT-G result and dialysis duration was associated with positive TST results. As reported in previous studies nosocomial transmission of TB may occur in patients under long-term dialysis (Hickstein *et al.*, 2003). This may be the reason of positive association of dialysis duration and positive TST. Similar to our study, Passalent *et al*. (2007) found that in patients with end-stage renal disease undergoing dialysis, a positive T-SPOT.TB was strongly associated with age.

There is no diagnostic gold standard for LTBI. So, we could not assess the sensitivity and specificity of QFT-G for this disease state. The only diagnostic standard for LTBI is the eventual development of active TB, and the predictive value for progression of TB can be ascertained only through longitudinal cohort studies that follow clinical outcomes of tested individuals. In the study reported by Diel R *et al.* (2008), they showed that QFT-G is a more accurate indicator of the presence of LTBI than the TST and provides at least the same sensitivity for detecting individuals who will progress to active TB. Also, high sensitivity and specificity of QFT-G tests in active tuberculosis disease is reported. In a meta-analysis done by Pai and O'Brien (2008) revealed that the pooled sensitivity was 78% (CI, 73% to 82%) for QuantiFERON-TB Gold, and 70% (CI, 63% to 78%) for QuantiFERON-TB Gold In-Tube. The pooled specificity for both QuantiFERON tests was 99% among non–BCG-vaccinated participants (CI, 98% to 100%) and 96% (CI, 94% to 98%) among BCG-vaccinated participants. They concluded that the Interferon gamma release assays (IGRAs), especially QuantiFERON-TB Gold and QuantiFERON-TB Gold In-Tube, have excellent specificity that is unaffected by BCG vaccination.

In conclusion, QFT-G test shows fair to moderate agreement with TST and it could be used in screening of LTBI simultaneously with TST; but their relatively high cost and need for laboratory infrastructure is a limitation of QFT-G tests and to analyze the use of it alone in screening of LTBI in cases with ESRD receiving hemodialysis larger long-follow up studies are needed.

The study was conducted in Selcuk University Meram Medical Faculty and in Konya Research and Education Hospital.

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