Interferon-gamma Release Assay Agreement With Tuberculin Skin Test in Pretransplant Screening for Latent Tuberculosis in a High-prevalence Country

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Introduction. Tuberculosis reactivation is one of significant complications after transplantation. Tuberculin skin test (TST) has been the major available screening test in end-stage renal disease patients, but it is associated with a low accuracy. Recently, an interferon-γ release assay (IGRA) has been approved as a substitution test in diagnosis of *Mycobacterium tuberculosis* infection. This study aimed to compare the ability of the TST and IGRA in the diagnosis of latent tuberculosis in hemodialysis patients and investigate risk factors of having positive test results.

Materials and Methods. Forty-seven hemodialysis patients underwent the IGRA and TST tests. Demographic data and blood samples were collected and chest radiography was done for all participants.

Result. Abnormal chest radiography was reported in 24% of the study group. The IGRA and TST were positive in 11 (23.4%) and 20 patients (43.5%), respectively. The agreement coefficient (kappa) between the IGRA and TST was 0.31 ($P< .05$). Positive TSTs were significantly associated with male sex and abnormal chest radiography. Diabetes mellitus was a risk factor for a positive IGRA result ($P= .01$).

Conclusions. The IGRA test is not a sensitive test for detection of latent tuberculosisin hemodialysis patients residing in high-prevalence areas. We suggest that assessment of cellular immunity response in end-stage renal disease patient be a priority before reliance on the IGRA test result.

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INTRODUCTION

Tuberculosis reactivation is a significant complication after transplantation. Tuberculin skin test (TST) has been the only available screening test with many false positive and negative results in end-stage renal disease patients in the past. Recently, interferon-γ release assays (IGRAs) have been approved as a substitution in diagnosis of *Mycobacterium tuberculosis* infection. There are limited studies with controversial results which have assessed the efficacy of this new method in hemodialysis patients who are candidates for kidney transplantation. We performed a study to compare the abilities of the TST and IGRA in diagnosis of latent tuberculosis in our hemodialysis patients based on clinical examinations, chest radiography, and some patient's risk factors.

MATERIALS AND METHODS

The study was done from October to December

2012 at Firoozgar Hospital, in Tehran. A total of 47 hemodialysis patients accepted to participate in the study. Demographic data were collected from the patients and blood samples were collected in 1 dialysis session for measurement of serum albumin, 25-hydroxyvitamin D, parathyroid hormone, and phosphorus levels, as well as the IGRA test. The IGRA was measured using the QuantiFERON TB Gold (Cellestis, Carnegie, Victoria, Australia). The test was performed according to the instructions of manufacturer.[1]

All blood samples were taken before dialysis sessions based on Hursitoglu and colleagues' study,[2] which suggests that there is obvious reduction in interferon-γ level after the hemodialysis process. One milliliter of whole blood was drawn in each of 3 separate test tubes: one containing no antigen (nil control), one with mitogen (phytohaemagglutinin; positive control), and one with tuberculosis antigens (ESAT-6, CFP-10, and TB7.7). The three tubes were incubated for 16 to 24 hours at 37°C. Following incubation, the tubes were centrifuged and plasma was removed from each tube and frozen at -20°C. Interferon-γ measurement was subsequently done suing the enzyme-linked immunosorbent assay. Results were expressed in IU/mL. An interferon-γ value of more than 0.35 IU/mL or more than 25% of that in the control tube was considered positive. If the interferon-γ level was less than 0.35 IU/mL in the tuberculosis antigen tube and mitogen control was positive (≥0.5 IU/mL), the test was recorded as negative.[1]

Standardized TST (Tehran Pasteur Institute, Tehran, Iran) was done intradermally by 2 research nurses. A positive TST was defined as induration of more than 10mm in horizontal diameter after 48 hours of injection.[3]

RESULTS

Of 47 participants, 84.8%had vitamin D deficiency. Low socioeconomic status was reported in 25.5% of the patients. Abnormal chest radiographywas reported in 24% of the study group. The IGRA and TST were positive in 11(23.4%) and 20 patients (43.5%), respectively. The agreement coefficient between the IGRA and TST was 0.31 (P<.05). Positive TSTs were associatedwith male sex and abnormal chest radiography (P<.05). Diabetes mellitus was a risk factor for a positive IGRA result (P= .01; Table).

DISCUSSION

Tuberculosis is the prototype of infections that require a cellular immune response for their control.[4] All people have native populations of lymphocytes, mostly CD4 cells, capable of recognizing mycobacterial antigens that have been processed and presented by macrophages in a major histocompatibility complex class II context. In protective immunity to tuberculosis, CD4 T cells as well as the cytokines interferon-γ and tumor necrosis factor-α are essential.[5] However, activation of human monocytes with tumor necrosis factor-α, not interferon-γ, must effectively inhibit

Association of Independent Parameters With Tuberculin Skin Test and Interferon-γ Release Assay*

Parameter	Interferon-γ Release Assay			Tuberculin Skin Test		
	Positive (n = 11)	Negative (n = 36)	*P*	Positive (n = 20)	Negative (n = 27)	*P*
Mean age, y	57.9 ± 15.1	49.5 ± 15.8	.13	52.5 ± 15.6	50.3 ± 16.4	.65
Sex						
Male	8 (72.7)	23 (63.9)		17 (85.0)	14 (51.9)	
Female	3 (27.3)	13 (36.1)	.73	3 (15.0)	13 (48.1)	.02
Low socioeconomic status	3 (27.3)	9 (25.0)	.58	4 (20.0)	8 (29.6)	.50
Serum 25-hydroxyvitamin D, ng/dL	13.1 ± 13.7	13.2 ± 13.5	.98	12.6 ± 11.6	14.2 ± 15.6	.75
Vitamin D deficiency (< 20 ng/dL)	8 (72.7)	20 (55.6)	> .99	14 (70.0)	14 (51.9)	> .99
Diabetes as a cause of ESRD	8 (72.7)	3 (8.3)	.04	10 (50.0)	10 (37.0)	.37
Dialysis duration, mo	14.8 ± 12.2	15.7 ± 20.4	.84	13.5 ± 10.4	17.7 ± 23.5	.46
Postsecondary education	4 (36.4)	13 (36.1)	> .99	8 (40.0)	9 (33.3)	.76
Intact parathyroid hormone, pmol/L	310.5 ± 258.7	339.1 ± 302.8	.74	300.9 ± 310.2	326.0 ± 275.6	.79
Serum phosphorus, mg/dL	5.8 ± 0.9	5.3 ± 1.8	.31	5.5 ± 1.8	5.4 ± 1.5	.83
Serum albumin, g/dL	4.2 ± 0.7	4.4 ± 0.6	.31	4.2 ± 0.7	4.5 ± 0.6	.25
Abnormal chest radiography	5 (45.5)	6 (16.7)	.10	3 (15.0)	3 (11.1)	.03

*Values are mean ± standard deviation for continuous variables and frequency (percentage) for categorical variables.

intracellular replication of *Mtuberculosis*. In humans, an effective response to *Mtuberculosis* tends to follow a T helper-1 pattern with preferential expression of interferon-γ, interleukin-2, and interleukin-12.[6]

There are alterations in innate and adopted immunity in uremic situation. Data have shown impaired T cell activation, increased the ratio of T helper-1 to T helper-2, decreased B lymphocytes,and altered function of antigen-presenting cell in end-stage renal disease patients.[7] Tuberculosis is a common cause of infection in these patient. Early diagnosis and treatment of tuberculosis is a necessity in kidney transplant candidates. The recently introduced IGRA test showed a high specificity in normal population.This test has not validated yetin end-stage renal disease patients with altered immune response. In a study by Triverio and colleagues,[8] positivity rates were 19% for TST, 21% for QFT, and 29% for T-SPOT.TB in 61 hemodialysis patients. Agreement between TST and IGRA was poor (kappa = 0.16). Richardson[9]reported 35.5% and 12.8% test positivity by T-SPOT.TB and TST, respectively,in 203 hemodialysis patients. The agreement between IGRA and TST was also 0.25. Risk factor for positive TST was Bacillus Calmette-Guerin (BCG) vaccination. Birth in endemic countries, radiologic findings, and history of tuberculosis were risk factors of a positive T-SPOT.TB. Sayarlioglu and colleagues,[10] in a study of 89 hemodialysis patients in 2011, found a good agreement between TST and IGRA in non-BCG-vaccinated patients. Soysal and colleagues[11]studied on 411 hemodialysis patients in 2012 and stated that male sex was independently associated with a positive T-SPOT. TB and positive T-SPOT. TB was inversely associated with the presence of BCG vaccine, serum albumin, and dialysis duration. Anibarroa nd associates,[12] in a study among 52 patients in Spain, concluded that the IGRA showed better accuracy for latent tuberculosis diagnosis than TST. In our study, there was also 0.31 agreement between the two tests.

We can propose that this discordance is due to false-positive results in TST. There are 2 important causes of false positive TST: nontuberculous mycobacteria infection and BCG vaccination. Infection with nontuberculous mycobacteria may cause false positive reactions to tuberculin. Estimates of the frequency of false positive TSTs due to nontuberculous mycobacteria range from 1% to 5% of positive tuberculin tests to as many as 50% of 9-mm to 14-mm reactions in the United States healthcare workers.[13] Therefore, the effect is important only if prevalence of true TB infection is low (ie, less than 5 percent). This situation occurs in low-incidence countries with tropical, subtropical, or temperate climates.Vaccination with BCG is a well-known but frequently misunderstood cause of false positive tuberculin reactions. The effect of BCG on TST depends primarily on the age when vaccinated.[2]Vaccination with BCG in the first year of life causes no discernable effect on TST after 10 years or more. Vaccination after the first year of life (such as at entrance to primary school at the age 5 to 6) causes a stronger and longer lasting effect. At this age, 20% of individuals remain positive for 10 years or more after vaccination. In pediatric populations, the interval since vaccination is important since there is progressive waning over the first 10 years after vaccination. In our patients BCG vaccination was performed in the first year of lifeaccording to the national health program.

It does not seem that BCG vaccination causes a false-positive TST. Probably a positive test is due to latent tuberculosis infection. The IGRAs are diagnostic tools for latent tuberculosis infection. They are surrogate markers of *M tuberculosis* infection and indicate a cellular immune response to *M tuberculosis*. In the patients with chronic kidney failure, the level of interferon-γ is low, but maybe the level of other cytokines is sufficient for a positive TST. Further studies are recommended based on assessment of other cytokines level which may affect cellular immune response.

CONCLUSIONS

There was a fair agreement between IGRA and TST in our study (kappa = 0.31). Risk factors for positive tests were male sex and abnormal chest radiography for TST and diabetes mellitus for IGRA. It seems that IGRA test is not sensitive for detection of latent tuberculosis in high-prevalence areas. We suggest that assessment of cellular immunity response in end-stage renal disease patients be a priority before reliance on IGRA test results.

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CONFLICT OF INTEREST

None declared.

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