



Tuberculin Skin Test and T-SPOT.TB for Latent Tuberculosis Infection Detection in Healthcare Workers

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Abstract

Healthcare Workers (HCWs) have a higher Tuberculosis (TB) risk than the general population. This study analyzed effectiveness of Tuberculin Skin Test (TST) and T-SPOT.TB for detection prevalence of Latent Tuberculosis Infection (LTBI) among HCWs and the correlation between work locations and HCW duties. A trial study with a cross-sectional study design in Dr. Moewardi Hospital, Surakarta, in December 2018. The sampling technique used consecutive sampling with 30 subjects. Data analysis was by SPSS 21 for Windows. The appropriate levels of TST and T-SPOT indicated substantial TB ($K = 0.603$, $p < 0.001$). The sensitivity and specificity of T-SPOT.TB with close contact was 60% and 86.7%. The sensitivity and specificity of TST with close contact were 33.3% and 93.3%. T-SPOT.TB was a significant correlation between the work location, the pulmonary care ward $r = 0.436$ and $p = 0.008$ ($p < 0.05$). T-SPOT.TB has a slightly better sensitivity than TST.

Introduction

The World Health Organization (WHO) tuberculosis (TB) elimination program is called the End TB Strategy, envisioning a TB-free world (Al Abri et al., 2020). One of the ten End TB Strategy priority indicators is that more than 90% of patients with a latent TB infection (LTBI) receive treatment (Uplekar et al., 2015). Groups that have a high risk of developing LTBI into active TB include children, health care workers (HCW), people with human immunodeficiency virus (HIV) infection, those undergoing treatment with anti-tumor necrosis factor-alpha (TNF- α), and those with silicosis (Erkens et al., 2016). The WHO treats high-risk LTBI sufferers to prevent them from becoming active. The recommended examinations in determining LTBI are a tuberculin skin test (TST) and interferon-gamma release assay (IGRA) (Nayak and Acharjya, 2012).

The Centers for Disease Control and Prevention (CDC) issued guidelines to prevent TB transmission in healthcare facilities. This guideline was issued in response to the rise of TB in the United States in the mid-1980s and early 1990s (Churchyard et al., 2017). There was some documentation of improved TB health care and HIV coinfection, irregular infection control practices, delays in TB diagnosis and treatment, and increased transmission of multidrug-resistant TB strains (Jensen et al., 2005).

The Presidential Decree of the Republic of Indonesia number 22 of 1993, concerning illnesses arising from work, determines infectious diseases caused by viruses, bacteria, or parasites acquired through an occupation has an individual risk of contamination, including TB. The HCW with active TB can be a valuable source of infection in patients providing care

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and with co-workers (Nasreen et al., 2016). The HCW has a higher risk of obtaining TB than the general population. The TB risk of HCW, especially in 22 top-burden countries and low and middle-income states, covers 80% of global TB cases. Increased exposure and less than optimal infection control measures in these countries with limited resources increase the risk of occupational health workers (Horsburgh et al., 2015).

Latent TB infection (LTBI) is an individual exposed to *Mycobacterium tuberculosis* but does not show symptoms or transmit the disease (Setyawati, 2021). Latent TB infection can be reactivated to become active, especially in high-risk groups. One of which is health workers (HCW). The gold standard for LTBI diagnosis currently does not exist. TST and IGRA tests are used to diagnose LTBI. Still, these tests are limited in distinguishing whether the patient has recovered from TB infection, is currently in treatment, or is progressing from latent to active TB. The tuberculin skin test (TST) is a classic immunoassay test that has been used for a long time for the diagnosis of TB with relatively low specificity and accuracy due to the cross-reaction of pure tuberculin protein derivatives (PPD) with those induced by BCG (Getahun et al., 2015).

Recently, T-SPOT.TB has been developed to show higher performance for the diagnosis of Tuberculosis. It is a commercial IGRA assay that uses an ELISPOT format to diagnose LTBI. The T-SPOT.TB assay is a simplified enzyme-linked immunospot (ELISPOT) method designed to detect effector T cells responding to stimulation by specific antigen [6 kDa initial secreted antigenic target (ESAT-6) and 10 kDa protein filtrate culture (CFP10)] for *Mycobacterium tuberculosis* (MTB) (3,5-9). ESAT-6 and CFP10 are only found in MTB, but not in bacilli Calmette-Guerin (BCG) lines. So theoretically, the T-SPOT is assumed. TB test has higher specificity. The results of previous studies indicate that the T-SPOT.TB test can be used as a more accurate indicator of the presence of LTBI and active TB (ATB) in cases of low prevalence (Zhu et al, 2014).

Health workers have a relatively high prevalence rate of LTBI. Therefore, it is essential to analyze the effectiveness of the diagnostic

value of TST and T-SPOT.TB for specific populations in the high prevalence group, namely health workers. There are differences in the specificity and sensitivity of TST and T-SPOT TB in the group with a high prevalence of LTBI, so that is important to analyze the effectiveness of TST and T-SPOT TB to detect the occurrence of LTBI in health workers. This study aims to determine the concordance of the two examination instruments, determine LTBI's prevalence among HCWs and relate it to their work location and length of time as an HCW. We also analyzed the effect of BCG vaccination history as a child on tuberculin test reactions and T-SPOT.

Methods

The Dr. Moewardi Hospital Committee for Research on Human Subjects (Medical) approved this study (No. 3/I/HREC/2019). It is a diagnostic test study with a cross-sectional design, where the independent and dependent variables are measured simultaneously to examine TST and T-SPOT. TB. This study was conducted at the Regional General Hospital Dr. Moewardi (RSDM), in Surakarta, in October 2018 until sufficient study samples were obtained. The target population of this study is the health staff of the RSDM, who have no history or current diagnosis of TB.

The subjects willing to participate in the research are asked to sign the consent form. The research subjects who met the inclusion criteria were educated on the study's purpose. Afterward, we collected the data, including identity, natural history, physical examination, TST examination, and blood samples for T-SPOT. TB examination. The research subjects must return in 48–72 hours for TST readings. The study subjects were divided into two groups: the first group was 15 nurses in the pulmonary ward, and the second group was 15 administrative staff assigned to other communities. The sampling technique using consecutive sampling with a minimum sample size required for this study was 30 subjects. Inclusion criteria: Age above 18 years, willing to participate in research. Exclusion Criteria: HCW with symptoms or clinical signs of TB, HCW with a history of suffering from TB, a history of taking antituberculosis drugs, HCW

with comorbid diseases and immunosuppressed diseases such as diabetes, HIV, kidney failure, and use of corticosteroids. Data analysis was by SPSS 21 for Windows. This study presents the analysis with frequency distribution, and the percentage continues with univariate analysis. The correlation test in this study used a non-parametric test, the contingency coefficient test. Some limitations remain because one subject that did not get a TST examination booster two steps.

Results and Discussion

This study involved 30 health staff of RSDM. TST and T-SPOT. TB examination results detected LTBI for 11 (36.7%) subjects and did not suffer from LTBI for 19 (63.3%) subjects. Positive TST examination results were obtained in 6 (20%) subjects and positive

T-SPOT. TB examination results were obtained in 11 (36.7%) subjects. The characteristics of the subjects of this study are based on gender, age, close contact, length of work, and nutritional status. LTBI is more prevalent in eight female subjects (26.7%) and three (10.0%) male subjects. The subjects in this study aged <30 years were 15(50.0%) subjects and aged >30 years were 15 (50.0%). LTBI was more prevalent in subjects aged >30 years 7 (23.3%) subjects], 15 (50.0%) subjects had close TB contact, and we found LTBI positive TB T-Spot of these subjects were 9 (30.0%) subjects who suffer from LTBI. Most issues had worked there for>5 years. There were 17 (56.7%) subjects, 7 (23.3%) of whom had LTBI. Twenty-three subjects (76.7%) had a normal nutritional status (Table 1).

TABLE 1. Characteristics of Research Subjects

Variables	Total n (%)	TST (+) n (%)	T-Spot (+) n (%)
Gender			
Female	23 (76.7%)	4 (13.3%)	8 (26.7%)
Male	7 (23.3%)	2 (6.7%)	3 (10.0%)
Age (years)			
<30	15 (50.0%)	1 (3.3%)	4 (13.3%)
>30	15 (50.0%)	5 (16.7%)	7 (23.3%)
Close Contact			
Nurse (+)	15 (50.0%)	5 (16.7%)	9 (30.0%)
Admin (-)	15 (50.0%)	1 (3.3%)	2 (6.7%)
Length of work			
<5 years	13 (43.3%)	2 (6.7%)	4 (13.3%)
>5 years	17 (56.7%)	4 (13.3%)	7 (23.3%)
Nutritional status			
Underweight	2 (6.7%)	0 (0.0%)	0 (0.0%)
Normal	23 (76.7%)	5 (16.7%)	9 (30.0%)
Overweight	5 (16.7%)	1 (3.3%)	2 (6.7%)
Total	30	6 (20.0%)	11 (36.7%)

Source: primary data 2018

Based on table 2, there were six positive TST examinations, while positive T-SPOT.TB found 11 positive results. The cross-tabulation results of the positive TST examination with positive T-SPOT.TB of six (20%) subjects. The results of negative TST examination with positive T-SPOT.TB found five (16.7%) subjects and negative TST with negative T-SPOT. TB found 19 (63.3%) subjects. The degree of conformity obtained the kappa value of 0.603. The data show the suitability level $K = 0.603$,

which is substantial ($0.600 < K < 0.800$).

Based on table 3 that showed a close contact examination with T-SPOT.TB gets a sensitivity of 60.0%, which means 60.0% of subjects with positive close contact can be detected by T-SPOT.TB and the specificity value of the T-SPOT measurement obtained in this study was 86.7%. It means that subjects who have close negative contact can probably be removed from subjects who have a positive T-SPOT.TB of 86.7%. Examination of TST in

close contact subjects received a sensitivity of 33.3%, which means that 33.3% of subjects with positive close contact could be detected with TST. The specificity of the measurement of TST obtained in this study was 93.3% meaning it was probable that close contact subjects negative that can be removed from subjects who have a positive TST of 93.3%.

Based on table 4 shows that the correlation between the length of work with the LTBI incidence was bivariate analysis. In this study, the data with a nominal scale that followed the correlation test used a non-parametric test, the contingency coefficient test. The results showed

subjects who had worked at the hospital <5 years tended to have a negative T-SPOT.TB examination (30.0%), whereas subjects with a work history >5 years tend to have a T-SPOT.TB examination (23.3%). The correlation test between the length of work history with the T-SPOT examination. TB in this study obtained $r = 0.106$ and $p = 0.558$ ($p > 0.05$), which means there is no significant correlation, the correlation test between the length of work with the TST examination obtained $r = 0.100$ and $p = 0.580$ ($p > 0.05$) with a very weak correlation value with the two inspection instruments namely $r = 0.106$ and $r = 0.100$.

TABLE 2. Compatibility Level of TST Examination with T-SPOT.TB in Detecting LTBI

		TST		Total	K	p
		Positive	Negative			
T-SPOT.TB	Positive	6	5	11	0.603	< 0.001
	Negative	0	19	19		
	Total	6	24	30		

Note: K = kappa; $p < 0.001$ considered statistically significant

Sources: Primary data, 2018

TABLE 3. Close contact test with T.SPOT.TB and TST Examination

Close contact	Abbreviation (units)	Value	
		T-SPOT.TB	TST
Sensitivity	(%)	60.0	33.3
Specificity	(%)	86.7	93.3
Positive predictive value	PPV (%)	81.8	83.3
Negative predictive value	NPV (%)	68.4	58.3
Positive predictive ratio	PPR (%)	4.5	5
Negative predictive ratio	NPR (%)	0.462	0.714

Sources: Primary data, 2018

TABLE 4. Correlation between Length of Work and Close contact with T-SPOT.TB and TST examination

Examination		Positive	Negative	Total	R	p
T-SPOT.TB						
Length of work	≤5 years	4 (13.3%)	9 (30.0%)	13 (43.3%)	0.106	0.558
	>5 years	7 (23.3%)	10 (33.3%)	17 (56.7%)		
Close Contact	Positive	9 (30.0%)	6 (20.0%)	15 (50.0%)	0.436	0.008
	Negative	2 (6.7%)	13 (43.3%)	15 (50.0%)		
	Total	11 (36.7%)	19 (63.3%)	30 (100.0%)		
TST						
Length of work	≤5 years	2 (6.7%)	11 (36.7%)	13 (43.3%)	0.100	0.580
	>5 years	4 (13.3%)	13 (43.3%)	17 (56.7%)		
Close Contact					0.316	0.068
	Positive	5 (16.7%)	10 (33.3%)	15 (50.0%)		
	Negative	1 (3.3%)	14 (46.7%)	15 (50.0%)		
	Total	6 (20.0%)	24 (80.0%)	30 (100.0%)		

Sources: Primary data, 2018

The results showed that subjects with positive close contact tend to have positive T-SPOT.TB examination (30.0%), while subjects with negative close contact tended to have a negative T-SPOT.TB examination (43.3%). The correlation test between close contact with T-SPOT.TB examination in this study obtained $r = 0.436$ and $p = 0.008$ ($p < 0.05$), which means there is a significant correlation with moderate correlation value ($r = 0.436$), whereas with examination TST results obtained $r = 0.316$ and $p = 0.068$ ($p < 0.05$) which means there is no significant correlation with weak correlation values. Studies comparing IGRA and TST have high rates of HCW at 40%–66%, such as Lien et al. (2009) research in Vietnam, the Rangaka et al. (2015) study in Turkey, and the Murray et al. (2015). Healthcare workers have a greater risk of obtaining TB than the general population. The TB risk of HCW, especially in 22 high-burden countries, and low and middle-income countries, covers 80% of global TB cases. Increased exposure and less optimal infection control measures in these countries with limited resources increase the risk to occupational health workers. HCWs with active TB can be an important source of infection both for patients in care delivery, for colleagues, and the community.

The results of this study found a positive TST examination; there were six subjects, while positive T-SPOT.TB got more results in 11 subjects. The results of cross-tabulation of positive TST examination with positive T-SPOT. TB was six (20%) subjects and positive TST with negative T-SPOT. TB was not obtained (0.0%). The results of the positive TST and T-SPOT. TB-negative tests can be caused by a false positive TST examination that is a reactivation of the previous BCG vaccination or Mycobacterium Other Than Tuberculosis (MOTT) infection. MOTT infection can cause a false positive TST examination in areas that have a low TB prevalence and a high MOTT prevalence. Indonesia has a high TB prevalence, so the results of the positive TST and T-SPOT. TB-negative studies in this study are not false positives due to BCG vaccination and MOTT infections but LTBI-positive (Nasreen et al., 2016).

We calculated the concordance index

using Cohen's kappa coefficient value of 0.603, which is substantial ($0.600 < K < 0.800$). TST examination has several disadvantages compared to T-SPOT.TB can occur with false positives and false negatives, but the results of this study obtained a substantial level of concordance between TST and T-SPOT.TB examination. That means that both TST and T-SPOT. TB examination can be used as an equivalent LTBI diagnostic tool. In our study, TST tests are recommended for detecting LTBI because it is more practical, inexpensive, and widely available in health facilities. A study by Cadena et al. in Germany with 333 people with a TST >10 mm induration found a strong degree of conformity (Cadena et al., 2017). Results with a high degree of conformity were also obtained in the (Rangaka et al., 2015) study in Turkey. TST tuberculin studies of HCW, all of whom use induration limits >10 mm, found the number of booster phenomena is good in countries with a prevalence of low TB, as in Kraut et al. in Canada using a history of BCG vaccination and workers born outside Canada as a strong predictor (Kraut et al., 2004).

The study results found six positive TST examinations and positive T-SPOT.TB got more results; there were 11 subjects. LTBI close contact examination with T-SPOT brings a sensitivity of 60.0%. TST and T-SPOT. TB diagnostic tools have specificity values above 85%, which means they can provide good proportions of healthy subjects who give negative diagnostic results (true negative). Based on the above results, issues with close contact with LTBI obtained many positive effects on the T-SPOT.TB examination compared to the TST examination. This is likely due to the booster phenomenon where the tuberculin test can be negative in infected individuals because the infection time is very long. Still, examining the tuberculin test will stimulate a reaction to the test so that the subsequent results will be positive. The wrong interpretation is said to be a skin test conversion, with the recommendation of the TST 2 test, the step is to repeat 1–3 weeks after the first TST is negative as recommended by PDPI and CDC (Jensen et al., 2005; Nayak and Acharjya, 2012). TST and IGRA examinations have their respective advantages and disadvantages. TST examination is still

the choice for ITBL diagnosis because it is affordable, practical, does not require skilled personnel, and is available in many health facilities compared to the IGRA examination (Setyawati, 2021).

The results of this study note that the place of work (close contact) has a significant correlation ($p = 0.008$), with a moderate correlation value ($r = 0.436$), where subjects with positive close contact tend to be positive with LTBI (30.0%). In contrast, subjects with negative close contact tended to be negative with LTBI (43.3%). The TB contact investigations program or individual investigations that contact active TB sufferers who are effective and, together with the national TB program and other services, can produce a considerable amount of case coverage. Current estimates are in a world with four million people yearly with positive microbiological examination results. Close contact is assumed to be a minimum of three people WHO estimates that 2.5% or 300,000 people are identified as close contact individuals with active TB sufferers (MacNeil, 2019). Twenty-two Molecular research in Mexico and South Africa shows that transmission may occur in social environments such as bars, cafes, and facilities such as hospitals (Mathema et al., 2017). These places are difficult to identify and require knowledge of culture and behavior patterns to focus the contact investigation.

The Centers for Disease Control and Prevention (CDC) recommends LTBI screening in individuals at increased risk of reactivation to active TB diseases, such as close contact with newly diagnosed TB patients, individuals with comorbidities, and those planning to start immunosuppressive medications such as tumor necrosis factor-alpha (TNF- α) blockers or biologics (Wang et al, 2022). The T-SPOT.TB test had a higher sensitivity than the TST. An increased TST spot size was associated with a trend toward an increased rate of T-SPOT.TB positivity. Given the comparable performance, the selection of TST or T-SPOT.TB should instead depend on other considerations, including cost, benefits, and resources (Yang et al, 2019).

The results showed that the period or length of time in which contact occurred

for transmission of each individual was influenced by individual genotype factors and socioeconomic characteristics for the occurrence of Mtb transmission. Over the last decade, TB diagnosis and treatment have shifted from district-level CDC clinics to designated hospitals with state-of-the-art diagnostic equipment. Still, poor access to medical facilities remains a significant risk factor for household transmission of Mtb. Thus, there is still a need to improve the infrastructure and management of medical facilities, especially in areas with a high prevalence of TB (Cui et al, 2019). However, neither is the T-SPOT. Neither the TB test nor the TST was sufficiently accurate to detect active TB disease. The factors of sex, age, length of work, and nutritional status were not significantly related to TST and T-SPOT examination. We suggest that individuals in close contact with a negative TST examination can use the two-stage method: repeat the T ST examination 1–3 weeks later. It is related to the advice from the PDPI and CDC.

Conclusions

There is a substantial level of concordance between TST and T-SPOT.TB examination in detecting LTBI in HCW with a kappa value of 0.603 ($0.600 < K < 0.800$), $p < 0.001$. The results of close contact diagnostic testing with T-SPOT. TB gets a sensitivity of 60.0% and a specificity value of 86.7%, while the results of the close contact diagnostic test with TST get a sensitivity of 33.3% and a specificity value of 93.3%. TST and T-SPOT. TB diagnostic tools have specificity above 85%, so they are good at determining negative (true negative) results in healthy subjects. There is a correlation between the workplace location (close contact) and the T-SPOT.TB examination, the correlation value is moderate ($r = 0.436$) with $p = 0.008$.

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