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Evaluation of T-SPOT® Serology Test for the Diagnosis of Pulmonary Tuberculosis

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Abstract

***Background*:** tuberculosis is a chronic contagious infectious disease which is fatal within 5 years in more than half of cases if not diagnosed. Since the fight against tuberculosis is based on early diagnosis and complete treatment of all TB patients, useful biochemical methods are emphasized to find a more rapid diagnostic method. This study aims to evaluate the impact of diagnostic value of T-SPOT® serology in patients suspected or diagnosed with tuberculosis admitted in Bou-Ali Hospital of Zahedan, Iran.

***Materials and Methods*:** The descriptive-analytic study conducted on 60 patients, 30 of whom had AFB sputum smear positive pulmonary tuberculosis, and 30 patients had AFB sputum smear negative pulmonary tuberculosis. The results were stated as sensitivity, specificity, positive and negative predictive value and likelihood ratio using conventional epidemiological table.

***Results*:** In 23 out of 30 patients with AFB sputum smear positive pulmonary tuberculosis, T-SPOT® serology became positive and in 12 out of 30 patients with AFB sputum smear negative pulmonary, T-SPOT® serology became positive. According to the epidemiological table in this study, sensitivity, specificity, positive and negative predictive value and likelihood ratio of this test were determined respectively 76%, 40%, 56%, 63% and 1.25%.

***Conclusion*:** According to the results, this test is not able to distinguish active pulmonary tuberculosis from latent infection. Moreover, considering high contact of regional people with TB patients and pulmonary involvement of people due to factors other than TB, the test value with this likelihood ratio is low.

Introduction

Three million annual deaths from TB occur in the whole world. According to World Health Organization (WHO), TB will remain at its DALYS rating (ranked seventh) till 2020 [1]. This disease is caused by *Mycobacterium tuberculosis* and the man is known as the only host for this bacterium [2, 3].

The important factors in increase of re-incidence of TB include HIV infection and subsequently AIDS, poverty, homelessness, drug addiction, and uncontrolled migration and marginalization around the cities. Irregular use of anti-tuberculosis drugs has been also effective in the emergence and spread of drug resistant strains and MDR tuberculosis epidemics (multidrug resistant) [3].

Since the fight against tuberculosis is based on early diagnosis and complete treatment of all TB patients, faster and more accurate diagnosis and appropriate treatment of these patients along with useful biochemical methods to find faster diagnostic methods have been emphasized. Tuberculosis diagnostic methods that are currently considered include sputum smear, culture [2, 3] PPD skin test [2, 3] and immunoassay for isolation of Mycobacterium tuberculosis, PCR, Tuberculostearic, phage typing and T-SPOT® serology test [4-8].

Body immune response to *Mycobacterium tuberculosis* infection is specifically dependent on cellular immunity. In a part of this response, T cells will become sensitive to *Mycobacterium tuberculosis* antigens and both cells of T-CD4 and T-CD8 produce cytokines such as Interferon-gamma (IFN-γ) when stimulated by antigens. The use of these *Mycobacterium tuberculosis* complex specific antigens (*Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*) improves diagnostic methods and reduces cross-sectional responses to BCG vaccine or environmental mycobacterium [9, 10].

T-SPOT® is a dependent variable from ELISPOT diagnostic technique (Enzyme-Linked Immuno SPOT) and is capable of separating T-cells stimulated by Mycobacterium tuberculosis specific antigens that produce cytokines such as IFN-γ [9]. There is a genomic region in *Mycobacterium tuberculosis* called RD1 (region of difference 1) which does not exist in Mycobacterium bovis and most environmental mycobacterium. This region is able to produce specific antigens called ESAT-6 (early secreted antigenic target) and CFP10 (culture filtrated proteins) [11, 12]. The studies on humans and mice have shown that T-CD4 cells secreting IFN-γ

secondary to ESAT-6 antigen stimulation has an important role in protection against *Mycobacterium tuberculosis* in the body [13, 14].

This study has been conducted to evaluate the effect of diagnostic value of T-SPOT® serology in the diagnosis of tuberculosis.

Materials and Methods

This descriptive-analytical study was conducted during the period of 2005-2006 on patients with pulmonary tuberculosis and non-pulmonary tuberculosis hospitalized in the infectious disease ward of Bou-Ali Hospital of Zahedan, in which *Mycobacterium tuberculosis* has been isolated from smear or culture sputum of patients with pulmonary tuberculosis. Patients were selected based on positivity of culture or sputum smear in terms of Mycobacterium tuberculosis. Individuals were selected through random sampling. In literature review, sensitivity and specificity of testing each one have been reported 90%. Accordingly, to calculate the sensitivity by considering the amount of *p* to be 0.9 and 5% of the first type error and accepting the value of d to be 11%, sample size required to calculate sensitivity will be approximately 30 people and to study specificity in the same conditions, number of people needed who have pulmonary disease other than tuberculosis will be 30 people. Accordingly, the total number of individuals in this study will be 60.

Data were collected by laboratory measurements and filling forms containing information about weight, height and age. For data analysis, the conventional epidemiological table of sensitivity and specificity was used.

Results

The number of subjects in this study was 60 patients referred to Bou-Ali Hospital of Zahedan for tuberculosis examination, among whom 30 cases had positive AFB sputum smear and 30 other cases had negative AFB sputum smear. T-SPOT® serology test was conducted on all of them. In this study, the average age was 55±14 (between 23-89 years). Gender distribution included 34 females and 26 males. Meanwhile, 9 patients had a previous history of treated TB and 20 patients had a history of definite contact with TB patient (Table 1). Among 9 cases of previous history of TB, 4 cases again became positive AFB and 5 cases became negative AFB. 2 people had HIV one of whom was a 50-year-old male with positive pulmonary tuberculosis and the other one was a 30-year-old female with Miliary tuberculosis and negative AFB. Among 30 patients with positive AFB sputum smears, T-SPOT® serology was positive for 23 cases and negative for 7 others.

Among 30 cases with negative AFB sputum smears, T-SPOT® serology was negative for 12 cases and positive for 18 others. Among 34 patients with PPD of below 10 mm, T-SPOT® serology was negative for 14 cases and positive for 20 others. Also, among 11 patients with PPD of above 10 mm, T-SPOT® serology was negative for 2 cases and positive for 9 others. Among 12 individuals who had contact with a TB patient and had a PPD of below 10 mm, T-SPOT® serology was positive for 4 cases and negative for 8 others. Among 8 individuals who had contact with a TB patient and had a PPD of above 10 mm, T-SPOT® serology was positive for 7 cases and negative for one case (Table 2).

According to these results and use of epidemiological table, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratio of T-SPOT® were studied in the target population which were determined 76%, 40%, 56%, 63% and 1/25%, respectively (Table 3).

Table 1. Surveyed PPD among ELIspot people

ELISPOT	PPD>10mm	PPD<10mm
Negative	2	14
Positive	9	20
Total	11	34

Table 2. Examination of ELI SPOT Test among PPD people and people who have had contact with TB patients

History of Exposure			ELISPOT Positive	ELISPOT Negative
Positive	PPD	>10mm	1	7
		<10mm	8	4
Negative	PPD	>10mm	6	16
		<10mm	1	2
		Total	16	29

Table 3. Evaluation of sensitivity, specificity, positive predictive value, negative predictive value and likelihood ratio of T-SPOT® in the target population

T-SPOT®	Patients	Healthy people
+	23	18
-	7	12

Discussion

Sensitivity T-SPOT® test in the target population was determined 76% which is different from the studies conducted in other countries, because sensitivity of test has been reported to be 85-95% in those studies. Now, if we accept the high sensitivity of the test, we will find that the treatment only based on smear AFB positive is not logical. Test specificity in this study was reported 40% which was much lower than the expected limit in the studies conducted in other countries (100%).

Sensitivity of T-SPOT® testing in a study in Germany in 72 patients with confirmed TB has been reported 97%. Meanwhile, in two patients with confirmed TB, the number of SPOT was below the value of diagnostic test due to taking drugs in these people for more than 30 days [15]. In Zambia, sensitivity of test on 50 patients with confirmed tuberculosis has been reported 92%. 78% of these individuals have had concomitant HIV and less than one month had passed from their treatment [16].

In South Africa, a study was conducted on children the sensitivity of which was reported 80%. Children aged between 22 months to 8 years and less than one month had passed from their treatment. 68% of them had culture and smear positive and 32% of them were only smear

positive, 42 cases were studied in terms of HIV 52% of whom were approved HIV [17].

In a study in Germany TST (tubercle skin test) and T-SPOT® serology test were compared among 45 adult patients who had active tuberculosis, where sensitivity of T-SPOT® serology was reported 100% and TST testing 89% [16]. In a meta-analysis study which had investigated sensitivity of three tests of T-SPOT®, QFT-Gold Test (Quantiferon-TB Test) and TST in tuberculosis, sensitivity of each test was reported 96%, 85% and 70%, respectively [18].

A study has reported sensitivity of T-SPOT® serology in immunocompromised patients for diagnosis of tuberculosis to be 95.3%, which has been much more than all reported cases in QFT-Gold test [19]. The important point in this method is that according to the study conducted on children in South Africa, unlike TST test, malnutrition or immune system problems do not affect the results of T-SPOT® serology [17].

A study in the Britain has reported specificity of T-SPOT® serology in 40 normal people to be 100% and in another study in the same country, the test specificity in 18 healthy people without HIV has been reported 100% [20]. T-SPOT® serology is also used to diagnose latent tuberculosis infection. For example, among 75 adult Zambian with normal CXR and no descriptions of tuberculosis symptoms 76% of whom were BCG vaccinated and 28% had HIV positive, T-SPOT® serology was positive in 69% of HIV negative and 43% of HIV positive among whom 49 people had been TST tested 35 of whom were HIV negative and in 28 patients (80%) TST test was positive, but in 14 cases of HIV positive, only 5 TST tests were positive [9].

In India, 100 healthy people with normal CXR and no description of TB symptoms of and HIV negative, in 80% of cases, T-SPOT®test was reported positive [20]. Another important point is that the test results have a close relationship with call duration and encounter intensity and the studies examined in the United States [21], and Italy [22] have confirmed this relationship. Finally, it should be mentioned that according to a study, disease severity affects the results of T-SPOT® serology target cells will be reduced through the successful treatment [14].

In the first conclusion, we realize that this method is not an appropriate test to determine rejectivity of tuberculosis and cannot distinguish latent infection from active tuberculosis and considering the prevalence of TB in Iran; this test is not able to diagnose tuberculosis in a patient referred with chest wall involvement and doubt between pneumonia and tuberculosis.

However, if we accept high specificity and sensitivity of the test in other studies, since this test is used to examine mycobacterium-specific antigens, and only three species of *Mycobacterium Kansas*, *marinom* and *szulgai* are able to secrete these specific antigens, this test cannot be used to confirm the disease of a patient with positive AFB smear. That's because the person may have non-tuberculosis Mycobacterium infection along with latent infection or even tuberculosis given the high prevalence of tuberculosis in Zahedan. Therefore, it is necessary that considering 76% sensitivity of this test and that 24% of patients with positive AFB smear have had negative T-SPOT® test, smears and sputum culture should be concomitantly performed for patients and careful follow up should be conducted to determine the type of grown AFB and the culture should be also antibiogramed to determine drug sensitivity.

PPV of T-SPOT® test in the target population was determined 56% and if we accept test sensitivity in other studies, in this study, the number of actual cases diagnosed with this test is low which is due to lack of distinction between clinical tuberculosis and tuberculosis infection by T-SPOT ®test which has caused the number of false positive cases of T-SPOT® test to be high. NPV in this study was determined 63% which still shows weakness of T-SPOT® test in lack of distinction between tuberculosis infection and disease, because the Iranian society (especially Zahedan) has high contact with TB patients and TB infection has occurred in them. As a result, the number of people who refer with the actual chest wall involvement for whom T-SPOT® test is negative is very low. Likelihood ratio of the test was determined 1.25 which lowers the examination value of the test in this society according to the epidemiological studies.

Among the subjects with PPD of below 10 mm and contact with TB patient, T-SPOT® Test was positive in 8 patients which have more value in the prophylaxis of individuals compared to PPD. Among 25 subjects who had no contact with tuberculous patients, 18 of them had only 2 patients with PPD of above 10 mm, T-SPOT® test became positive in them this is an warning in the community and the people unconsciously had developed the infection.

Totally, T-SPOT® Test is a qualitative test for diagnosis of latent infection and is unable to distinguish latent infection from TB infection and since latent infection is high in Iranian society, and especially Zahedan, and medication prophylactic does not start for them, performing this test with this high cost has no economic value. On the other hand, it is reported that this test can help to evaluate the therapeutic follow up, because stimulated CD4 cells will be reduced through conducting the treatment and after a month, T-SPOT® test will become negative [14]. However, given the difficulties in performing test and preparation of kits and testing procedures and high costs, it is not affordable in Iranian society. In addition, high costs of kit purchase; testing processes that require specific incubator and centrifuges and testing by an experienced expert in a research center are of the project constraints.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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References

1. Raviglion M, Brien R. Tuberculosis. In: Fauci A, Kasper D, Longo D, editors. Harrison's principles of internal medicine. 16th ed. New York: McGraw Hill; 2005: 953-1035.
2. Daniel F, David H. Mycobacterum tuberculosis. In: Mandel G, Bennet J, Dolin R. Principles and Practice of Infectious Diseases. 6th ed. Philadelphia, Churchil Livingstone: Elsevier; 2005: 2852-2883.
3. Velayati A, Ramezani M. Darsname infectiouse disease. 1th ed. Tehran: Poursina Press; 2002: 967-1040.
4. Hershfield MS, Mitchell BS. Immunodeficiency diseases caused by adenosine deaminase deficiency and purine nucleoside phosphorylase deficiency. In The Metabolic Basis of Inherited Disease. 7th ed. New York: McGraw Hill; 1995: 1725-1768.
5. Zavialov A, Engstrom A. Human ADA2 be longs to a new family of growth factors with adenosine deaminase activity. J Biochem 2005; 391(pt1): 51-57.
6. Conlon BA, Law WR. Macrophages are a source of extracellular adenosine deaminase-2 during inflammatory responses. Clin Exp Immunol 2004; 138(1): 14-20.
7. Miller KD, Barnette R, Light RW. Stability of adenosine deaminase during transportation. Chest 2004; 126(6): 1933-1937.
8. Collazos J, Espana P, Mayo J, et al. Sequential evaluation of serum adenosine deaminase in patients treated for tuberculosis. Chest 1998; 114(2): 432-435.
9. Chapman AL, Munkanta M, Wilkinson KA, et al. Rapid detection of active and latent TB in HIV-positive individuals by enumeration of Mycobacterum tuberculosis-specific T-cell. AIDS 2000; 16(17): 2282-2293.
10. Ewer K, Deeks J, Alvarez L, et al. Comparison of T-cell based assay with tuberculin skin test for diagnosis of Mycobacterum tuberculosis infection in a school tuberculosis outbreak. Lancet 2003; 361(9364): 1168-1173.
11. Behr MA, Wilson MA, Gill WP, et al. Comparative genomice of BCG vaccines whole- genomice DNA microarry. Science 1999; 284(5419): 1520-1523.
12. Andersen P, Munk ME, Pollock JM and Doherty TM. Specific immune-bassed diagnosis of tuberculosis. Lancet 2000; 356(9235): 1099-1104.
13. Andersen P, Andersen AB, Sorensen AL and Nagai S. Recall of long-lived immunity to mycobacterium TB infection in mice. J Immunol 1995; 154(7): 3359-3372.
14. Pathan AA, Wilkinson KA, Klenerman P, et al. Direct ex vivo analysis of antigen-specific INF gamma - secreting CD4 T-cell in Mycobacterium tuberculosis-infected individuals associations with clinical disease state and effect of treatment. J Immunol 2001; 167(9): 5217-5225.
15. Meier T, Eulenbruch HP, Wrighton-Smith P, et al. Sensivity of a new commercial enzyme-linked immunospot asssy (T-SPOT) for diagnosis of tuberculosis in clinical practice. J Clin Microbil Infect Dis 2005; 24(8): 529-536.
16. Liebeschuetz S, Bamber S, Ewer K, et al. Diagnosis of tuberculosis in South Aferican children with a T-cell-based assay. Lancet 2004; 364(9452): 2196-2203.
17. Pai M, Rile L, Colford J, et al. INF gamma assay for the immunodiagnosis of tuberculosis. Lancet 2003; 50:1244-1247
18. Mori T, Sakatani M, Yamagish F, et al. Specific detection of tuberculosis infection: An interferon-gamma-based assay using new antigens. Am J Respir Crit Care Med 2004; 170(1): 59-64.
19. Lalvani A, Nagvenkar P, Udwadia Z, et al. Enumeration of T-cell specific for RD1-encoded antigen suggests a high prevalence of latent Mycobacterium TB infection in healthy urban Indians. J Infect Dis 2001; 183(3): 469-477.
20. Shams H, Weis S, Klucar P, et al. Enzyme-linked immunospot and tuberculin skin testing to detect latent the tuberculosis infection. J Respir Crit Care Med 2005; 172(9): 1161-1168.
21. Richeldi L, Ewer K, Losi M, et al. T-cell-based tracking of multidrug resistant tuberculosis infection after after brief exposure. Am J Respir Crit Care Med 2004; 170(3): 288-95.
22. Dheda K, Lalvani A, Miller RF, et al. Performance of a T-cell-based diagnosis test for tuberculosis infection in HIV-infected individuals is independent of CD4 cell count. AIDS 2005; 19(17): 2038-2041.

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