Original Article

TANAFFOS

Detection of Antibodies Against 6, 16 and 38 kDa Antigens of *Mycobacterium tuberculosis* as a Rapid Test for Diagnosis of Tuberculosis

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Background: Serological assays for diagnosis of tuberculosis (TB) are very attractive because they are inexpensive, non invasive and simple. Present study was conducted to evaluate the tuberculosis rapid test device in Iran.

Materials and Methods: The tuberculosis rapid test device based on detection of IgM, IgA and IgG antibodies against 6, 16 and 38-kDa antigens of *Mycobacterium tuberculosis* via chromatography was used in 96 cases of pulmonary and extra pulmonary TB. Fifty four patients with conditions other than TB were selected as the control group. Tuberculin skin test (TST) was performed in two groups. None of the patients were immunodeficient. All of them were evaluated in terms of presence of BCG scar.

Results: Tuberculosis rapid test was positive in 75 cases (78.1%) and 15 controls (27.8%). This difference was statistically significant (P-value < 0.001). TST was positive in 66 patients (68.8%) with tuberculosis and 10 (18.5%) controls with no statistically significant difference (P-value = 0.065).

Sensitivity, specificity, positive and negative predictive values of the tuberculosis rapid test for diagnosis of tuberculosis were 78.1%, 72.2%, 83.3% and 65%, respectively.

These parameters for TST were 31.3%, 81.5%, 75%, and 40%, respectively.

Conclusion: Tuberculosis rapid test has better sensitivity than TST and may be helpful in diagnosis of tuberculosis as a complementary test or in epidemiological investigations.

Key words: Tuberculosis, Rapid diagnosis, Serological assays, Skin test

INTRODUCTION

Despite global activities for decreasing the burden of tuberculosis (TB), it is still a public health hazard especially in developing countries. Earlier diagnosis of active or latent infections especially in close contacts is very important for disease control in the community (1).

Tuberculin skin test (TST) is the most popular and oldest assay for diagnosis of mycobacterial infection which is being used since 1910 worldwide (2). Due to the low sensitivity of this test and its special mechanism, TST may not diagnose latent tuberculosis infection in some high risk groups, especially immunocompromised patients for whom chemoprophylaxis is necessary (3). On the other hand, specificity of TST is low and it may become falsely positive due to vaccination with Bacillus Calmette-Guerin (BCG) containing more than 200 antigens shared with protein-purified derivative (PPD) which is used in TST. Also, TST may be positive in the infection with non-tuberculous mycobacteria which is another drawback. (4)

Experienced technicians are required to perform TST and minimally we should wait forty eight hours to read the response. Due to these limitations, a new generation of immune based blood tests was created for diagnosis of infection with *Mycobacterium tuberculosis*. World health organization (WHO) has recommended these rapid assays for earlier diagnosis of disease in recent years (5). The base of these rapid tests is detection of anti-mycobacterial antibodies.

Antigen 5, also known as the 38 kilo Dalton (kDa) antigen, is a major protein present in culture filtrate of *Mycobacterium tuberculosis*. Seroassays targeting antibodies against 38 kDa antigen have been studied frequently (6).

Alpha-crystalline is a 16 kDa cell wall protein of *Mycobacterium tuberculosis*, structurally homologous with proteins belonging to the family of low molecular-weight heat-shock proteins, containing B-cell epitopes specific for *M. tuberculosis* complex (7).

The 6 kDa early secreted antigenic target from *Mycobacterium tuberculosis*, ESAT-6, is the prototype of a family of small proteins produced by Actinobacteria. The frequent recognition of ESAT-6 during TB infection has stimulated great interest in the potential of this antigen for diagnostic use (8).

We performed the present study to evaluate sensitivity and specificity of a rapid serologic assay for diagnosis of tuberculosis based on detection of IgM, IgA and IgG antibodies against 6, 16 and 38 kDa antigens of *Mycobacterium tuberculosis* via chromatography and compared it with TST as the routine assay in Iran.

MATERIALS AND METHODS
Setting

The present study was conducted at National Research Institute of Tuberculosis and Lung Disease (NRITLD), Masih Daneshvari Hospital, located in northeastern Tehran, Iran, as a tertiary referral care center for TB and lung disease. This research was reviewed and approved by the Ethics Committee of the center. Before recruiting patients, a detailed explanation was given and an informed consent was obtained from them.

Study Subjects

Case group included 96 consecutively admitted new pulmonary or extra pulmonary TB cases. Bacteriological or pathological confirmation was considered as the gold standard for diagnosis of tuberculosis. Therefore, pulmonary cases were confirmed by positive sputum smear or culture for *Mycobacterium tuberculosis* and extra pulmonary TB patients were confirmed by pathologic study.

Fifty four patients were selected as the control group among sequential patients with respiratory diseases other than TB admitted to the internal ward. None of them were known cases of immunocompromised state, auto immune disease or under immunosuppressive therapy. Cases and controls were older than 16 years of age.

Tuberculin Skin Test

TST was performed for all cases and controls with 5-tuberculin unit (TU) dose of PPD (0.1 milliliter of solution) according to the protocols outlined by the World Health Organization (WHO) (9).

The reaction was read after 48 to 72 hours. A positive test was defined by the diameter of induration more than 10 millimeters.

Serological Test

We used "ACON Tuberculosis Rapid Test" manufactured by Acon® Laboratories. It is a qualitative, solid phase, two-site sandwich immunoassay for detection of anti-TB antibodies. There is a membrane pre-coated with TB recombinant antigens on the test line region of the device. The specimen migrates upward on the membrane chromatographically by capillary action. The anti-TB antibodies, if present, react with these antigens and generate a colored line that means positive result.

As a technical control, colored line will always appear in the control region indicating that proper volume of specimen has been added and membrane wicking has occurred.

The test can be performed with three drops of any kind of these specimens including whole blood, serum or plasma. Whole blood may be supplied by venipuncture or finger stick and addition of buffer solution is necessary.

We preferred venipuncture and 0.5 cc venous blood samples were collected from each patient, three drops of

each sample were used for the test. The result should be read at 10 minutes time. If two distinct colored lines appeared, the result would be considered positive and if only one colored line appeared in the control region, it was interpreted as negative. In situations where the control line failed to show, the test would be repeated.

Statistical Analysis

After collecting demographic information (age, sex, and nationality), results of TST and serologic rapid tests, data were analyzed using SPSS software (version 15.5, SPSS, Chicago) and sensitivity and specificity of the rapid test were calculated and compared to those of TST. Chi- Square test was used for nominal variables.

RESULTS

The study was performed on 96 admitted cases of tuberculosis including 88 pulmonary and 8 extra pulmonary cases and 54 admitted patients without tuberculosis as controls. There were a total of 70 males (46.7%) of both cases and controls, and 133 (88.7%) were Iranian. The mean age of both groups was 51.33 yrs (± 21.79). All patients were HIV negative and they did not have any type of immunodeficiency (Table 1).

Table 1. Demographic characteristics, presence of scar of BCG vaccination and results of TST and tuberculosis rapid test in the two groups

| | | Tuberculosis | | | | | |
| | | Yes | | | No | | |
		Count	Row %	Column %	Count	Row %	Column %
Sex	Male	50	71.4%	52.1%	20	28.6%	37.0%
	Female	46	57.5%	47.9%	34	42.5%	63.0%
Nationality	Iranian	82	61.7%	85.4%	51	38.3%	94.4%
	Afghan	12	80.0%	12.5%	3	20.0%	5.6%
	Other	2	100.0%	2.1%	0	.0%	.0%
BCG scar	Yes	53	61.6%	55.2%	33	38.4%	61.1%
	No	42	68.9%	43.8%	19	31.1%	35.2%
	Doubtful	1	33.3%	1.0%	2	66.7%	3.7%
TST	Positive	30	75.0%	31.3%	10	25.0%	18.5%
	Negative	66	60.0%	68.8%	44	40.0%	81.5%
Rapid serologic assay	Positive	75	83.3%	78.1%	15	16.7%	27.8%
	Negative	21	35.0%	21.9%	39	65.0%	72.2%

Scars of BCG vaccination were found in 86 patients including 53 cases (61.6%) and 33 controls (38.4%) and in 3 patients, scarring was doubtful. There was no significant difference between the two groups concerning BCG scar (P-value = 0.23); therefore, BCG vaccination had no intervention in this study.

Among 96 TB cases, tuberculosis rapid test was positive in 75 (78.1%) and negative in 21 patients (21.9%). The test was negative in 39 patients in the control group (72.2%) and positive in 15 of them (27.8%). This difference between the two groups was statistically significant (P-value < 0.001).

TST was positive (induration more than 10 millimeters) in 66 patients (68.8%) with tuberculosis and was negative in 30 patients (31.3%). Among the control group, TST was negative in 44 (81.5%) and positive in 10 (18.5%). There was no statistically significant difference between the two groups concerning TST (P-value = 0.065) (Table 1).

Based on our findings, sensitivity of the rapid test for diagnosis of tuberculosis was determined to be 78.1% and specificity was 72.2%. Positive predictive value of this test was 83.3% and negative predictive value was 65%.

Sensitivity and specificity of TST were 31.3% and 81.5%, respectively. Positive predictive value of TST was 75% and negative predictive value was 40% (Table 2).

Table 2. Comparison between TST and rapid serologic assay

	Sensitivity	Specificity	PPV[*]	NPV[†]
TST	31.3%	81.5%	75%	40%
Rapid serologic assay	78.1%	72.2%	83.3%	65%

* PPV: Positive predictive value

† NPV: Negative predictive value

DISCUSSION

Timely diagnosis of tuberculosis is very important as it provides early initiation of treatment and limits further spread of infection. In recent decades, new tests such as PCR-DNA amplification or interferon-gamma assay were introduced but they are too expensive and dependent to modern laboratory equipments and expert technicians (10).

Serological assays for diagnosis of tuberculosis are very attractive because they are inexpensive, non invasive, simply performed and do not require high level of expertise.

Accordingly, many studies were conducted on the serodiagnosis of tuberculosis (11-13). The specificities of these serological assays have improved by using highly purified or recombinant antigens specific for the TB complex (14). Many mycobacterial antigens have been identified and evaluated for serodiagnosis of TB infection or disease, such as 12, 14, 16, 19, 23, 38, 65 and 71-kDa proteins. The 38-kDa protein is the most extensively studied antigen (15). Also, multiple methods such as ELISA, agglutination test and immunochromatographic assay were examined for detection of antibodies against these antigens (16-19).

In different studies, a wide range of sensitivity and specificity was mentioned for these tests. Senol and their colleagues found the sensitivity, specificity, positive and negative predictive values of ELISA assay for measuring IgG against 16 and 38-kDa mycobacterial antigens to be 52.5%, 93.3%, 95.9% and 39.7%, respectively (15).

Hauer and their coworkers considered the tuberculin rapid assay from Diva Vita of limited value because of low sensitivity (approximately 60-80%) despite its high specificity (over 95%). They mentioned that the maximum value of the test seems to be in bacteriologically confirmed TB, for which however additional diagnostic evaluations may not be necessary (20).

In one study in Turkey, an immunochromatography assay was evaluated for routine diagnosis of tuberculosis in 72 patients with active pulmonary (19 smear negative) and 8 extra pulmonary TB cases along with 54 controls. The sensitivity, specificity and negative predictive value were 33.3%, 100% and 52.9%, respectively (21).

Reddy et al. in India evaluated another immunochromatographic serological assay based on detection of antibodies against 38, 63, 64, 14 and 59-kDa antigens of *Mycobacterium tuberculosis*. The specificity of the test was 99.42 with sensitivity of 98.52% (19).

In the present study, "ACON Tuberculosis Rapid Test" was evaluated in our center. It is a rapid qualitative test for detection of antibodies against 6, 16 and 38 kDa antigens of *Mycobacterium tuberculosis* in whole blood, serum or plasma. The test is inexpensive (less than 0.5 US$), easy to perform and very safe without any risk of mycobacterial contamination. Also, experienced personnel and laboratory equipments are not necessarily required.

The sensitivity of the rapid test for diagnosis of tuberculosis was 78.1% with 72.2% specificity. Positive predictive value of this test was 83.3% and negative predictive value was 65%. For TST, the sensitivity and specificity were 31.3% and 81.5%, respectively with positive predictive value (PPV) of 75% and negative predictive value (NPV) of 40%. When compared, it is concluded that tuberculosis rapid test was more sensitive than TST with comparable specificity, PPV and NPV.

Detection of IgG and IgA against the mycobacterial antigen 60 (A60), the main thermo-stable component of PPD, may allow an increase in diagnostic accuracy of extrapulmonary tuberculosis (22, 23). A systematic review of 21 studies showed that at present, commercial antibody detection tests play no roles in diagnosis of extrapulmonary tuberculosis (24).

In the present study, 8 cases of tuberculosis were extra-pulmonary; thus, evaluation of accuracy of this test was not performed in this small subgroup separately.

At present, there is no serologic test to replace sputum smear microscopy (24), but one study demonstrated that a screening approach can be shaped by integrating a serological assay with microscopic examination of acid fast bacilli (25).

Our study had some limitations. First of all, it was conducted in a referral center and our patients may have more severe type of disease compared to other patients elsewhere. The second limitation as mentioned earlier was low number of extrapulmonary cases of tuberculosis. We recommend conduction of another study on this subject with a larger number of patients divided into smear positive and smear negative and pulmonary and

extrapulmonary TB groups in order to evaluate the accuracy of the test separately.

In conclusion, we found that tuberculosis rapid test has a considerably better sensitivity than TST and may be helpful for diagnosis of tuberculosis as a complementary test but not as a substitute for mycobacterial study. Also, it may be useful for epidemiological investigations. However, more studies are required in this respect.

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