Original Article

T-SPOT: A STEP AHEAD!

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ABSTRACT

Objective:

To assess the utility of T-spot based Anti Tuberculosis therapy (ATT) in patients with Tuberculosis.

Design of Study:

Analytical Case Study.

Setting:

Department of Pathology, University Medical and Dental College (UMDC) and Meezan Laboratory, Faisalabad.

Period:

June, 2010 to December, 2010.

Materials and Methods:

All patients presenting to the Pathology Department at UMDC and Meezan Lab for T-spot testing were included in the study. A detailed history was taken and all cases were followed up.

Results:

Twenty patients presented for T-spot testing during the study period. Out of these 11(55%) were positive and 9 (45%) were negative. ATT was started in 10 out of the 11 positive patients. These patients were followed up and showed an improvement in their symptoms.

Conclusions:

T-spot test is a helpful tool for diagnosing the presence of TB infection. It has many advantages over Mantoux test especially in our setting where BCG vaccination is a major confounder.

Keywords: Tuberculosis, TB, T-spot, ATT

INTRODUCTION

In 1993 World Health Organization (WHO) declared Tuberculosis (TB) a global emergency. The WHO estimates that one third of the world population is infected with Mycobacterium Tuberculosis with 95% of the disease burden in developing countries. Pakistan ranks sixth amongst the countries with a highest burden of TB in the world. TB is the most important single infectious cause of mortality and morbidity worldwide, with approximately 2 million deaths and 9 million new cases reported in 2009[1]. Approximately 5% to 15% of immunocompetent persons with latent tuberculosis infection (LTBI) will develop TB.[2]

Diagnosis of active and latent TB remains a challenge and targeted testing and treatment of individuals with LTBI at increased risk of progression to active disease is a key element

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of tuberculosis control[3]. This becomes even more significant as the number of people living under iatrogenic immunosuppressive conditions for onco-haematological diseases, transplants, anti-TNFα treatment and steroid therapy is constantly increasing. This population has a nine-fold greater risk of developing active TB disease *given the LTBI* than the general population.[4,5]

The classic diagnostic tool for LTBI is the tuberculin skin test (TST), also known as the intradermal Mantoux test since 1910. It is the oldest diagnostic test in use in modern medical practice. This strategy is limited by the poor specificity of the tuberculin skin test in populations vaccinated with Bacille Calmette-Guérin. A new generation of immune-based rapid blood tests for the diagnosis of LTBI seems to be a significant upgrade of the century-old TST.[3,6,7]

Over the last few years, a new approach based on detecting *Mycobacterium tuberculosis*-specific T cells has shown much promise. In particular, there is substantial published evidence showing that the detection of specific T cells using the *ex vivo* enzyme-linked immunospot technique is a marked improvement over the existing tuberculin skin test. This technique, which detects gamma interferon-producing T cells, is now available as the commercial assay T SPOT-TB (Oxford Immunotec, Oxford, UK).[8,9,10]

The blood based test has several advantages compared to TST. Measurement of interferon gamma can be carried out by machines on the next day following the blood draw and objective results are obtained more quickly than with TST. It is not necessary to consider the booster effect in T–spot test PPD is not injected, nor to revisit the doctor.[11]

Higher specificity will reduce or eliminate false-positive test results in BCG-vaccinated people, thus avoiding the costs associated with unnecessary chemoprophylaxis and its associated toxicity. Higher sensitivity would, on the other hand, identify more infected persons among those with a false-negative TST result. More true-positive results in infected people would increase the rate of diagnosis and treatment of LTBI in the most vulnerable populations before progression to active TB[3]. In this study we used T-spot test in patients who were suspected to have tuberculosis but no convincing x-ray findings or lump for histological diagnosis were available.

PATIENTS AND METHODS

After acceptance from UM&DC Committee on Research and Bio-Ethics, the study was conducted in UM&DC and Meezan Laboratory. Twenty cases were selected on the basis that the patients had clinical suspicion of tuberculosis as they were suffering from some of the symptoms of tuberculosis like fever, cough, sputum, haemoptysis, weight loss, generalized weakness, backache, previous history of active TB with suspicion of reactivation or suspicious specks on X-rays. These patients did not have any palpable lymph nodes on which FNAC or excision biopsy and histopathology could have been done.

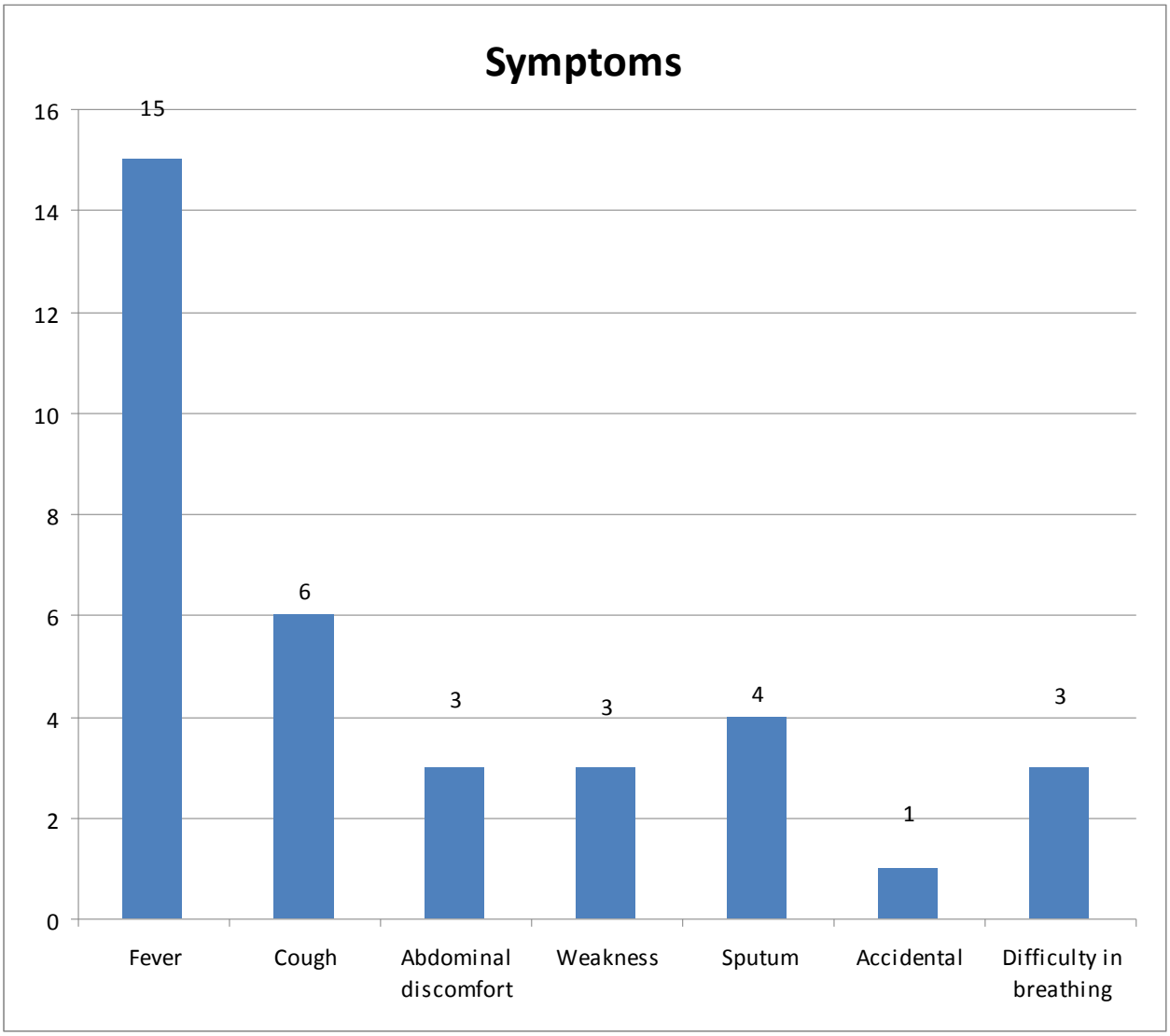
Blood samples for T spot test were taken from the patients at Meezan Laboratory. A proper history was documented and these patients were followed up and interviewed again after the passage of 2–3 months.

The procedure of the test is as follows. The blood sample is drawn and centrifuged to separate the peripheral blood mononuclear cells (PBMC). These are washed and counted to maximize the sensitivity. The PBMCs and specific TB antigens are added to wells pre-coated with antibodies to IFN-χ and are incubated overnight at 37°C in carbon dioxide. IFN-χ is released from the activated T cells. The wells are washed and a secondary conjugated antibody is added. This is incubated for 1 hour. The wells are washed again and substrate is added. This is then incubated for 7 minutes. The reaction is stopped by adding water and the spots are counted using a microscope. One spot is the foot print of a single activated T cell. Six activated T cells (spots) give a positive result.

RESULTS

Most of the tuberculous suspects presented with fever, cough, sputum, weight loss, generalized aches and pains, difficulty in breathing or vague abdominal symptoms. The distribution of symptoms is shown in Figure 1.

Figure 1



The results of T-spot test and the type and result of treatment is given in Figure 2.

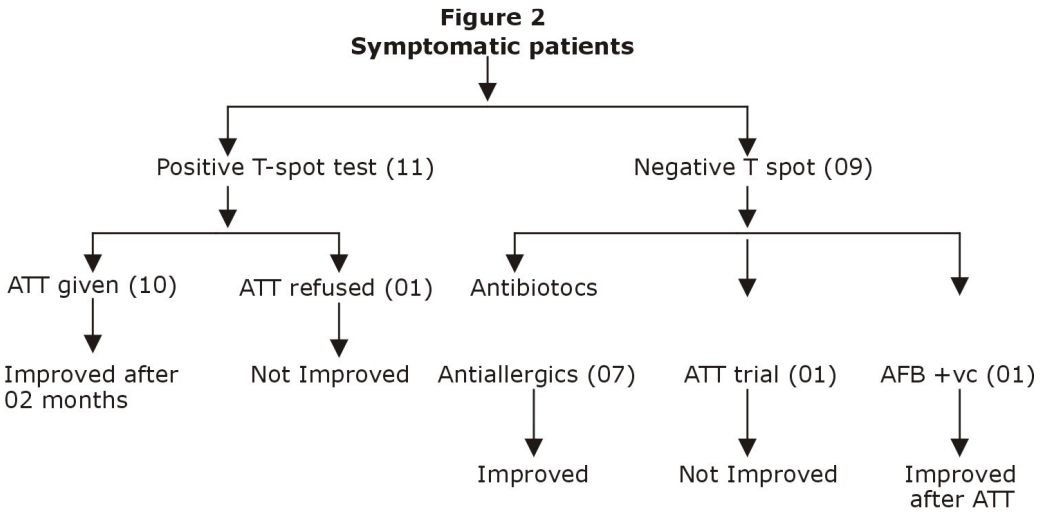
T-spot test was done in 20 cases out of which 11 (55%) cases were positive and 9 (45%) cases were negative.

Out of the 11 cases that were T-spot positive, 10 (90.9%) were given ATT and showed improvement of symptoms after one to two months of ATT. One out of these eleven patients was advised ATT but they refused the treatment. The symptoms of this patient have not improved to date.

Out of the 9 cases that were negative 7 were given antibiotics or anti-allergics and they improved. One was given ATT but she shows no improvement in her symptoms. The sputum of one patient was positive for AFB. He was put on ATT on this basis and has improved.

One patient, who had a positive T-spot test, was discovered accidentally because he was screened on the basis of a suspicious chest X-ray. Following 2 months of ATT there was an improvement in his weakness on exertion and post nasal drip. These symptoms were previously unnoticed by him.

One patient, who presented with dyspnea, had a positive T-spot test. His symptoms improved with anti asthmatics but he was advised proper follow up to rule out latent TB as this might be a possibility.



In two of the cases the patients had TB 5 years and 10 years ago and now had recurrence of the disease again.

DISCUSSION

The classic diagnostic tool for latent Mycobacterium tuberculosis infection is the tuberculin skin test, also known as the intradermal Mantoux test. Developed at the beginning of the twentieth century, this is the oldest unchanged diagnostic test still in use in current medical practice. The tuberculin skin test has two main limitations. First, it is relatively non specific. The main reagent of the tuberculin skin test, the protein purified derivative, is a culture filtrate of tubercle bacilli containing over 200 antigens shared with the vaccine Bacille Calmette-Guerin and most nontuberculous mycobacteria. Thus, individuals vaccinated against tuberculosis but not infected with M. tuberculosis can test falsely positive with the Mantoux test. Second, the tuberculin skin test has an unknown sensitivity for the diagnosis of latent M. tuberculosis infection, since a reliable diagnostic reference standard is lacking[12].

So the help currently given to the diagnosis of LTBI by the degree of positivity of the tuberculin skin test (TST) is limited, both operationally and logistically, in populations vaccinated with BCG or sensitised by atypical mycobacteria, and by its low sensitivity in those immuno-suppressed persons who are at greatest risk of progression. Moreover the TST has other operational limitations linked to return visits, repeat testing causing a boosting effect and subjective interpretation. A new approach that measure the in-vitro production of interferon gamma (IFN-gamma) by the blood mononuclear cells in response to M. tuberculosis specific antigens (ESAT-6 and CFP10) is T-spot test.[13] These tests have additional operational advantages over TST: completed in one visit, results available in 24 hours, absence of inter and intra observer divergence, detection of potential immuno-depression and avoidance of boosting by repeat testing.[12,13]

Moreover Literature review shows a number of research articles and meta analysis comparing the utility and advantages of T-spot test over the Mantoux test and the research also shows greater sensitivity and usefulness of T-spot.[8,9,10,11,12,13,14,15,16]

The advantages of T-spot over Mantoux test which is still the only screening method used in most of the places with a number of false positive cases and other confounding variables is depicted in Table 1.[8.9,10,14,16]

Some of the principal situations where T-spot can be used as a screening tool with a greater reliability that Mantoux are as under.[4,9]

1. People at risk of transmission:
    a) Health care workers
    b) Immigrants
    c) Military
    d) Prisons
    e) Tuberculous contacts
2. TB suspects

3. People with suspicion of reactivation of TB due to impairment of immunity:
   a) Diabetes
   b) Organ transplant
   c) Chronic renal failure
   d) HIV
   e) Haematological disorders
   f) Immunosuppressive therapy

Table 1

	Mantoux test	**T-spot test**
Technology	Over 100 years old	Simplified ELISA spot assay
Patients logistics	Two visits required	One visit required
Time for result	Minimum 2 days	Results in 24 hours
Inter observer & intra observer divergences	Yes	No
Affected by booster dose	Yes	No
Sensitivity	Poor sensitivity in immunocompromised patients	95.6 %
Specificity	Poor specificity due to cross reaction with BCG and most mycobacteria	97.1%

We agree with Richeldi that the introduction of the blood tests like T-spot test in clinical practice would initially increase costs. On the other hand, their higher diagnostic sensitivity, coupled with their higher specificity, could generate cost savings by reducing the future burden of cases of active tuberculosis and decreasing the number of uninfected vaccinated individuals inappropriately treated with chemoprophylaxis.[12]
Although on a limited no of samples yet it's a first study about T-spot from our region and we appreciate future studies to be carried out in order to further validate the presented data.

CONCLUSION

T-spot test is a very helpful, easy and comparatively reliable tool for spotting the patients with TB suspicion. It has many promising advantages over Mantoux especially in our setting where BCG vaccination is a major confounder in correct result of Mantoux test.

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