Research Article

TO EVALUATE THE EFFICACY OF THE MANTOUX TEST AND SPUTUM SMEAR EXAMINATION AS A DIAGNOSTIC AID IN TUBERCULOSIS

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ABSTRACT

Introduction: Mycobacterium tuberculosis is the causative agent for tuberculosis. It spread when people with tuberculosis cough, sneeze or spit which spread through air. Despite being a preventable and curable disease, 1.5 million people die from TB each year – making it the world's top infectious killer. Most of the people who fall ill with TB live in low- and middle-income countries, but TB is present all over the world. About half of all people with TB can be found in 8 countries: Bangladesh, China, India, Indonesia, Nigeria, Pakistan, Philippines and South Africa.

Material and Method: A prospective observational study was conducted among 100 patients with suspected tuberculosis. The patients were subjected to all three diagnostic tests including the Mantoux test, sputum examination, and CB-NAAT. CB-NAAT was considered as the gold standard for TB diagnosis. Results of the Mantoux and sputum tests were compared with CB-NAAT to assess the sensitivity and specificity of individual tests.

Result: Mantoux test was positive in 39% and negative in 61%, in sputum examination 41% patient tested positive whereas 59% were negative, and in CBNAAT 78% samples were positive and 22% were found to be negative. Sensitivity analysis of Mantoux test and sputum examination were compared with CB-NAAT result which considered as the gold standard for tuberculosis diagnosis. In the Mantoux test 35% were true positive while 6% whereas false positive, 19% were true negative while 40% were false whereas in sputum examination 37% were true positive while 5% were false positive, 21% were true negative while 37% were false negative.

Conclusion: The combination of the Mantoux test, sputum smear, and CB-NAAT test when performed together acts as an aid in diagnosing tuberculosis and also help in assessing the immunity status of the persons with latent tuberculosis. It all helps in follow the treatment protocol to cure the diseased case and to prevent the progress of the latent case to disease.

INTRODUCTION

Mycobacterium tuberculosis(MTB) is a causative agent for tuberculosis (TB).[1] Tuberculosis is a public health problem worldwide, especially in developing countries despite global endeavors to eradicate tuberculosis.[2] There have been continuous efforts over the past several decades to achieve an efficacious treatment of tuberculosis.[3] The goal is to reduce the spread of infection for this the most effective way is to avoid transmission and treatment of patients suffering from an infection.[4] the patients may have developed drug resistance and remain infectious for a long time if the outcome of treatment failed which becomes a public health risk.[5] The drug-resistance bacteria, patient characteristics, patient actions, and quality of health are affecting the treatment of tuberculosis, therefore the treatment is done as a Directly Observed Treatment Shortcourse (DOTS) strategy.[6] The

results of the follow-up, using a microbiological examination at the end of the two-month intensive phase of treatment, indicate whether the therapeutic regimen can be shifted to the perpetuation phase, or whether the patient should be given an extra month of intensive phase treatment. However, it has been reported that the results of sputum smear-positive pulmonary tuberculosis obtained from patients, at the end of two months of anti-tuberculosis treatment, can predict unexpected outcomes in terms of the increased failure of treatment or relapse rates of tuberculosis.[7], [8], [9] Awareness of the factors related to persistent sputum positivity at the end of two months of anti-tuberculosis treatment might, therefore, help clinicians to better manage their patients and improve outcomes.

The prevalence in India of the active disease in adults is 18 per 1000 population.[10] Although pulmonary tuberculosis is the main variant, around 25–35% of cases have an extrapulmonary presentation. The ratio of pulmonary to extrapulmonary

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tuberculosis is 3:1.[11] Main symptoms of tuberculosis include low-grade fever for >2 weeks, loss of appetite, poor weight gain, recent weight loss, night sweats, dry cough of >2 weeks, and significant lymphadenopathy. Although tuberculosis is a common infection, diagnosis is still a tough task; especially in children. Therefore, an early and reliable diagnostic test with good predictive value is essential to curb the disease in its initial stages.

The tuberculin skin test (TST) also known as the Mantoux test was introduced in 1890 and is widely used as a diagnostic aid for patients initially suspected of tuberculosis and also to detect latent infection.[12] Based on CDC guidelines for the classification of TST reactions, an induration of 0–4 mm is considered negative and an induration of 5 mm or above is considered positive.[13] Approximately 10- 25% of patients with microbiologically diagnosed active tuberculosis disease do not respond to tuberculin and have negative TST results because it is based on the diameter of skin induration which is dependent on the cell-mediated immune response to the tuberculin antigens.[14] The 2% error in measurement reduces the accuracy of the Mantoux test by 25% and the impact exceeds 50% for 5 % error since the measurement and interpretation of the results is user-specific.[15] The standard protocol for the detection of tuberculosis has been the microscopic and acid-fast bacilli (AFB) analysis of sputum as well as non-respiratory samples. Due to the low sensitivity and increased number of smear-negative tuberculosis, it results in missing out on a large number of positive cases.[16]

WHO recommended cartridge-based nucleic acid amplification test (CB-NAAT) to be used as a diagnostic aid for TB infections, in December 2010 whereas in 2013 endorsed Xpert Mycobacterium Tuberculosis (MTB)/ resistance to rifampin (RIF) to be used as the diagnostic test in all adults with presumptive TB and multidrug-resistant TB.[17] Xpert MTB/RIF is an automated, semi-nested real-time polymerase chain reaction that detects MTB and tests every positive sample for rifampicin sensitivity using molecular beacons.[18] CB-NAAT has high specificity (100%) and a good predictive value. However, it is not readily available in all clinical settings. It is essential to test the sensitivity, easy-to-use, and cost-effective diagnostic tests such as the Mantoux test and sputum smear examination for the presence of AFB. Because of this, the present study was carried out to evaluate the efficacy of the Mantoux test and sputum smear examination as a diagnostic aid in latent TB.

MATERIALS AND METHODS

A prospective observational study was conducted among 100 patients with suspected tuberculosis. All patients reporting to the Department of Microbiology with suspected mycobacterial tuberculosis (positive contact history or low weight for age or persistent fever for more than 2 weeks or cough for more than 2 weeks or significant lymphadenopathy) irrespective of age and sex were included in the study.

Patients with a previous history of tuberculosis and those who were previously treated for the same were excluded from the study.

After obtaining informed consent, the patients were subjected to all three diagnostic tests including the Mantoux test, sputum examination, and CB-NAAT. CB-NAAT was considered the gold standard for TB diagnosis. Mantoux test was performed

by injecting the test dose (0.1 ml PPD-RT23; 5 tuberculin units) and control dose (0.1 ml normal saline) intra-dermally over the flexor aspect of the left and right forearm, respectively.[13] Using the Sokal method, the transverse diameter of the induration was measured after 48–72 h by a single observer in all patients.[19] Direct microscopic examination of the sputum samples of patients was carried out to test the presence of AFB by Ziehl–Neelsen staining. The slide was examined under oil immersion and the presence of curved rods in red color over green background was considered as AFB positive. Simultaneously, the sputum samples were sent to a different center for CB-NAAT testing. Results of the Mantoux and sputum tests were compared with CB-NAAT to assess the sensitivity and specificity of individual tests.
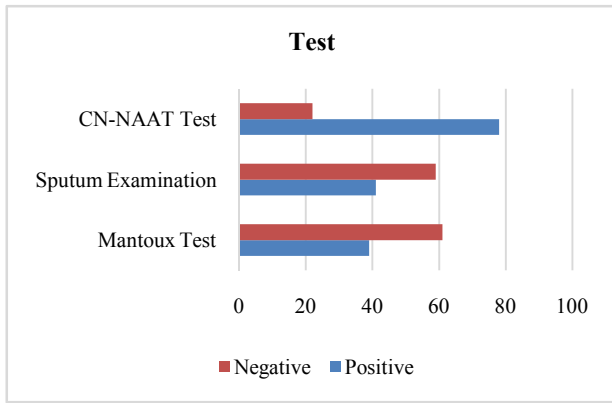
RESULTS
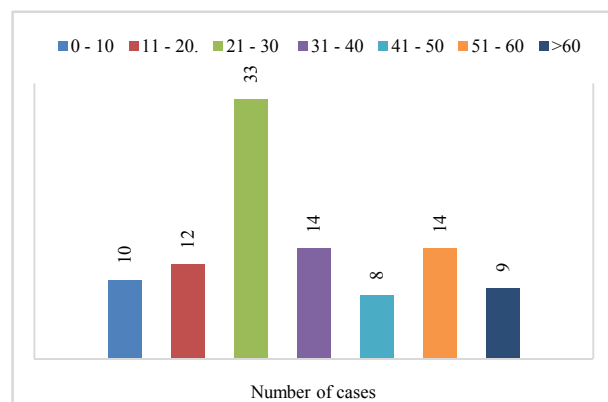
Age (yr)	Number of cases
0 - 10	10
11 - 20.	12
21 - 30	33
31 - 40	14
41 - 50	8
51 - 60	14
>60	9
TOTAL	**100**

Graph & Table 1: Distribution of cases based on age wised
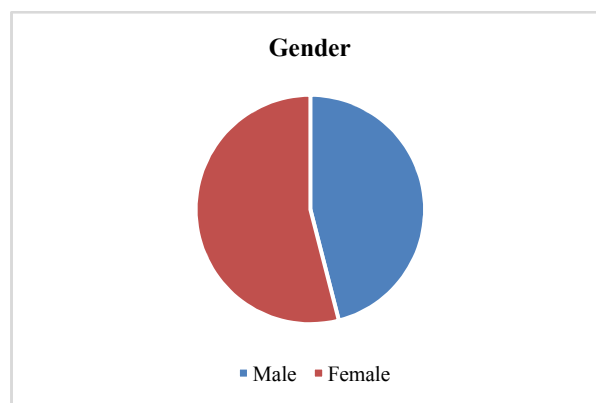
Gender	Number of Cases
Male	46
Female	54
TOTAL	**100**

Graph & Table 2 Distribution of cases based on sex wised

Test

Test	Positive	Negative	Total
Mantoux Test	39	61	100
Sputum Examination	41	59	100
CB-NAAT Test	78	22	100

Graph & Table 3 Inter-comparison of the diagnostic test for tuberculosis

Sensitivity Analysis

Parameter	Mantoux Test	Sputum Examination
True Positive	35	37
False Positive	6	5
True Negative	19	21
False Negative	40	37
TOTAL	**100**	**100**
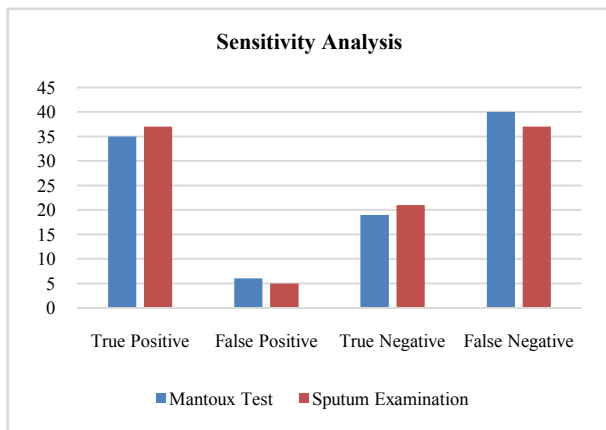
Graph & Table 4 Sensitivity analysis of Mantoux and sputum examination

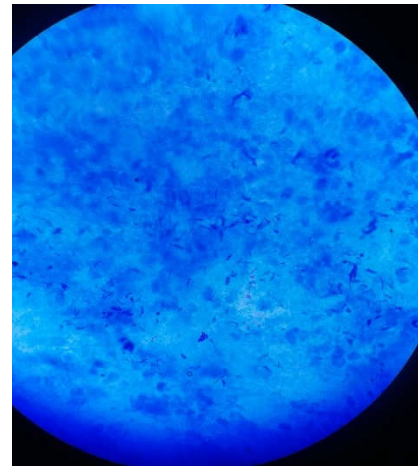**Fig 1** Presence of induration measuring around 20mm

Fig 2 Acid-fast bacilli in ZN stained smear (3+) in oil immersion

In our study, most of the patients that are 33% belong to the age group of 21 – 30 years (Graph & Table 1) whereas the female (54%) is slightly predominance compared to the male (46%) population (Graph & Table 2). Mantoux test was positive in 39% and negative in 61%, in sputum examination, 41% of patients tested positive whereas 59% were negative, and in CBNAAT 78% samples were positive and 22% were found to be negative (Graph and Table 3) Mantoux test interpretation is based on the size of induration in mm, Fig. 1 shows the presence of induration measuring around 20mm.Sensitivity analysis of the Mantoux test and sputum examination were compared with CB-NAAT result which is considered as the gold standard for tuberculosis diagnosis. In the Mantoux test, 35% were true positive while 6% whereas false positive, 19% were true negative while 40% were false whereas in sputum examination 37% were true positive while 5% were false positive, 21% were true negative while 37% were false negative (Graph & Table 4)

DISCUSSION

"END TB" strategy by 2035, the mission of World Health Organization given that it is important to deal with latent tuberculosis as it increases the disease burden and it is important to emphasize early case detection. [20]Recent reviews of the WHO strategy "END TB" specify the priority tasks such as the development of biomarkers for the detection and diagnosis of TB including systematic screening for active diseases and detection of latent TB infection to reduce the pool of individuals with latent infection. [21]

An incidence of tuberculosis highest occurs during 25–34 years of adult life as this age group ventures out for work or socialize, they have a much higher risk of exposure to MTB from the surroundings [20] whereas in our study, 33% of the patients belonged to the age group of 21–30 years.

Previously male predominance was observed, especially in head and neck tuberculosis. [22] In our study, although the sex distribution was almost equal slight female predominance was noted (54%).

CB-NAAT (GeneXpert) is an automated cartridge-based molecular technique that not only detects MTB but also rifampicin resistance within 2 h and has been endorsed by the WHO as an initial diagnostic test in children suspected of having TB both in pulmonary and specific forms of extrapulmonary TB.[23] In a study done by Sharma *et al*. [24], Xpert MTB/RIF had a high sensitivity of 95.7% and a

specificity of 99.3% for detecting MTB in pulmonary samples of patients with TB. According to Sachdeva *et al.*, CB-NAAT has high specificity (100%) and a good predictive value.[22] Hence, this was considered a gold standard for the diagnosis of TB in our study. In the present study, the diagnostic efficacy of readily available Mantoux test and sputum smear examination was tested with CB-NAAT. A correctly applied Mantoux test can be invaluable in the assessment of a child with suspected TB. The interpretation of the result, however, is often difficult, with different workers using different induration sizes to indicate a positive reaction.

The review of national policies on the management of latent TB infection of 98 countries identified that tuberculin skin testing was the most frequently recommended diagnostic tool. The test is comparably cheaper compared to other molecular tests. However, the cutoff points used in different countries and authors vary and no international agreement on cutoff values for the definition of a positive tuberculin reaction has been identified.[25] The choice among commonly used cutoff values depends on an individual's risk factor profile for TB. Usually, a lower cutoff value of ≥5 mm is used for individuals at higher risk of TB and a higher cutoff value of ≥10 mm is applied for individuals at lower risk of TB.[26] In a study by Kiwanuka, a reaction of ≥ 5 mm was considered positive. They reported that around 52% of cases were negative while 48% of patients were positive.[27]Similarly, using a cutoff value of 5 mm in our study, we observed that the Mantoux test was positive in 39% of patients and negative in 61% of patients. Although there was no correlation between the size of induration and the likelihood of current active TB disease, this may indicate the future risk of developing TB disease.

Similarly, AFB analysis of sputum is also considered a standard protocol for the detection of TB. However, the previous studies have suggested that sputum smear-negative patients were detected positive with clinical examination and had a better response to TB therapy.[28,29] This is because of the low sensitivity and increased number of smear-negative TB, a large number of positive cases are missed.[16] In a study by Saktiawati *et al.*,[24] 32% were smeared positive. In our study, sputum was positive in 41% of patients and negative in 59% of patients. The previous studies have suggested that combinations of investigations are better as compared to individual tests.

Saktiawati *et al.*[24] suggested that a combination of clinical evaluation with sputum microscopy and chest radiography provided high sensitivity and specificity in diagnosing TB in lung clinics; in only 4.4%, the diagnosis was incorrect. Similarly, we believe that a combination of the Mantoux test, sputum smear examination, and CB-NAAT will have better results than the individual tests. Smaller sample sizes and single-center studies limit the generalizability of the results. Moreover, mycobacterial cultures were not done to confirm the diagnosis. We further suggest larger multi-centric studies to test the predictive value of these routinely used tests.

CONCLUSION

Our study results suggest that a combination of the Mantoux test, sputum smear, and CB-NAAT test when performed together acts as an aid in diagnosing tuberculosis and also helps in assessing the immunity status of the persons with latent tuberculosis. It all helps in following the treatment protocol to cure the diseased case and to prevent the progress of the latent case to disease.

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