Role of tuberculin skin test (Mantoux test) as an aid in the diagnosis of tuberculosis disease and to identify persons with latent tuberculosis in highly endemic hilly region of Jammu and Kashmir

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ABSTRACT

Background: Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* and one of the major diseases affecting children throughout the world. Mantoux test and direct microscopic examination of acid-fast bacilli (AFB) by Ziehl–Neelsen staining are diagnostic and are widely being used. **Objective:** The objective of the study was 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 suspected cases of TB, over a period of 14 months from May 2019 to June 2020. After obtaining informed consent, patients were subjected to all three diagnostic tests including the Mantoux test, sputum smear examination for AFB, and cartridge-based nucleic acid amplification test (CBNAAT). Results of Mantoux and sputum tests were compared with CBNAAT to assess the sensitivity and specificity of individual tests. **Results:** In our study, most of the patients (32%) belonged to the age group of 21–30 years. There was a slight female predominance in the study population (52% females and 48% males). Mantoux test was positive in 41 (41%) patients and negative in 59 (59%) patients. On sputum examination, 42 (42%) patients tested positive and 58 (58%) patients were negative. On CBNAAT, 74 (74%) samples were positive, 26 (26%) were found to be negative. The sensitivity and specificity of the Mantoux test were 49.35% and 86.95%, respectively, and 53.33% and 92%, respectively, for sputum smear examination. **Conclusion:** Mantoux test, sputum smear, and CBNAAT test when performed together act as an aid in diagnosing TB in patients with latent TB.

Key words: *Cartridge-based nucleic acid amplification test, Induration, Mantoux test, Sputum smear, Tuberculosis*

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* (MTB), is one of the major diseases affecting the population worldwide, especially the developing countries. In India, the prevalence of the active disease in adults is 18 per 1000 population [1]. Although pulmonary TB is the main variant, around 25–35% of cases have an extrapulmonary presentation. The ratio of pulmonary to extrapulmonary TB is 3:1 [2]. Main symptoms of TB 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 TB is a common infection, diagnosis of TB 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.

Since the introduction of the tuberculin skin test (TST) also known as the Mantoux test in 1890, it has been widely used for the initial diagnosis of patients suspected of TB and to detect latent infections [3]. Based on CDC guidelines for the classification of TST reactions, an induration of 0–4 mm is considered negative and induration of 5 mm or above is considered positive [4]. Since the result is based just on the diameter of the skin induration that depends on the cell-mediated immune response to tuberculin antigens, approximately 10–25% of patients with microbiologically diagnosed active TB disease do not respond to tuberculin and have a negative TST result [5]. Moreover, the interpretation of results is user specific, 2% error in measurement reduces the accuracy of the Mantoux test by 25% and the impact exceeds 50% for 5% error [6]. The sensitivity of sputum in the detection of MTB varies from 20 to 80%.

Microscopy and acid-fast bacilli (AFB) analysis of sputum as well as non-respiratory samples has been a standard protocol for the detection of TB. Due to low sensitivity and increased number of smear-negative TB, it results in missing out a large number of positive cases [7]. In December 2010, the WHO recommended cartridge-based nucleic acid amplification test (CB-NAAT) to be used as a diagnostic aid for TB infections. Subsequently in 2013, the WHO endorsed Xpert MTB/resistance to rifampin (RIF) to be used as the initial diagnostic test in all adults with presumptive TB and

multidrug-resistant TB [8]. 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 [9]. The presence of MTB and rifampicin resistance can be detected within 2 h with good sensitivity and specificity. CBNAAT has high specificity (100%) and a good predictive value. However, it is not readily available in all clinical settings. Therefore, it is essential to test the sensitivity of the easy-to-use and cost-effective diagnostic tests such as the Mantoux test and sputum smear examination for the presence of AFB. In view 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 TB at GMC DODA situated in the hilly mountainous region of Jammu and Kashmir for a period of 14 months from May 2019 to June 2020. All patients reporting to the Department of Microbiology with suspected mycobacterial TB (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 TB 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 CBNAAT. CBNAAT was considered as 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) intradermally over the flexor aspect of the left and right forearm, respectively [4]. Using the Sokal method, the transverse diameter of the induration was measured after 48–72 h by a single observer in all patients [10]. 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 CBNAAT testing.

All the recorded data were entered into Microsoft Excel and analysis was carried out using SPSS 19.0 version. Data were presented as frequency and percentages. Results of the Mantoux and sputum tests were compared with CBNAAT to assess the sensitivity and specificity of individual tests.

RESULTS

In our study, most of the patients (32%) belonged to the age group of 21–30 years, while 16% and 15% of patients belonged to 51–60 years and 11–20 years, respectively. The age distribution is summarized in Table 1. There was a slight female predominance in the study population (52% females; 48% males) with a male-to-female ratio of 0.92:1. Mantoux test was positive in 41 (41%) patients and negative in 59 (59%) patients. Interpretation of the Mantoux test based on the size of induration in mm was carried out (Fig. 1). Most of the patients (59%) developed an induration measuring 0–4 mm followed by >15 mm induration seen in 36 (36%) patients in cases. Figure 2 shows the presence of an induration measuring around 20 mm. On sputum examination, 42 (42%) patients tested positive (Fig. 3), 58 (58%) patients were negative. All the sputum samples were subjected to CBNAAT, which were considered as the gold standard for TB diagnosis. While 74 (74%) samples were positive, 26 (26%) were found to be negative (Table 2).

Sensitivity analysis of the Mantoux test and sputum examination was carried out (Table 3). Results were compared with CBNAAT which is considered as the gold standard for diagnosing TB. In the Mantoux test, 38 (38%) patients were true positive while, 3 (3%) patients were false positive, 39 (39%) patients were false negative, and 20 (20%) patients were true negative. In sputum examination, 40 (40%) patients were true positive while, 2 (2%) patients were false positive, 35 (35%) patients were false negative, and 23 (23%) patients were true negative. The sensitivity and specificity of the Mantoux test were 49.35% and 86.95%, respectively, and 53.33% and 92%, respectively, in sputum examination.

DISCUSSION

A higher incidence of TB 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

Table 1: Distribution of the study population

Variable	Age in years	Number of cases (%)
Age in years	0–10	12 (12)
	11–20	15 (15)
	21–30	32 (32)
	31–40	11 (11)
	41–50	6 (6)
	51–60	16 (16)
	>60	8 (8)
Sex	Male	48 (48)
	Female	52 (52)

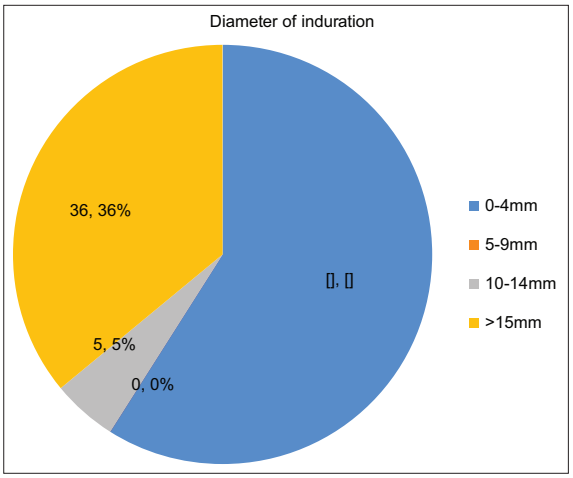

Figure 1: Distribution of patients based on size of induration

Table 2: Diagnostic tests for TB in study population

Test	Positive (%)	Negative (%)	Total (%)
Mantoux test	41 (41)	59 (59)	100 (100)
Sputum examination	42 (41)	58 (58)	100 (100)
CBNAAT test	74 (74)	26 (26)	100 (100)

TB: Tuberculosis, CBNAAT: Cartridge-based nucleic acid amplification test

Table 3: Sensitivity analysis of Mantoux test and sputum examination

Parameter	Mantoux test, n (%)	Sputum examination, n (%)
True positive	38 (38)	40 (40)
False positive	3 (3)	2 (2)
False negative	39 (39)	35 (35)
True negative	20 (20)	23 (23)
Sensitivity	49.35	53.33
Specificity	86.95	92

Figure 2: Presence of induration measuring around 20 mm

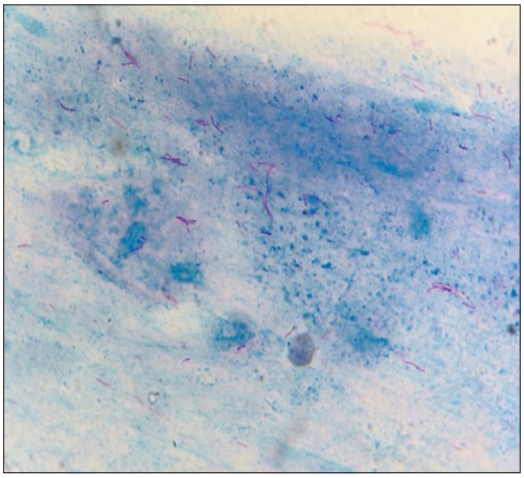

Figure 3: Photomicrograph showing acid-fast bacilli in ZN stained smear (3+) on oil immersion

surroundings [11]. Similarly in our study, most of the patients (32%) belonged to the age group of 21–30 years. Male predominance in TB has been observed previously, especially in head-and-neck TB [12]. In our study, although the sex distribution was almost equal slight female predominance was noted (52%). In view of the WHO End TB strategy by 2035, it is important to deal with latent TB as it substantially adds to the TB disease burden [11]. With an increase in the global burden of TB, current global priorities for TB care and control emphasize early case detection. 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 [13].

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 [14]. In a study done by Sharma *et al*. [15], 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. The sensitivity of Xpert MTB/RIF for detecting smear-negative culture-positive samples was 77.7%; its sensitivity for detecting smear-positive culture-positive samples was 99.2%. According to Sachdeva *et al*., CBNAAT has high specificity (100%) and a good predictive value [12]. Hence, this was considered a gold standard for diagnosis of TB in our study. Around 74 (74%) samples were positive for CBNAAT suggesting TB.

Despite the global trend of using rapid molecular tests is increasing, a considerable proportion of the TB cases reported to the WHO is still clinically diagnosed rather than bacteriological confirmation [14]. Due to the lack of equipment and high cost, Gene-Xpert TB/RIF is not readily available and frequently used [16]. Among the various proposed diagnostic tests, none of them are rapid, affordable, and easy. The use of liquid culture systems and molecular line probe assays to rapidly detect multiresistant TB although is beneficial; however, due to the complexity and cost of the tests, and the need for sophisticated laboratory infrastructure and trained personnel, uptake has been limited in many resource-constrained settings [12]. A good diagnostic test should be able to successfully identify true-negative and true-positive cases. TST-5 as currently recommended in the US Centers for Disease Control and Prevention guidelines might be the best approach [10]. However, it is essential to test the sensitivity of the easy-to-use and cost-effective diagnostic tests. In the present study, the diagnostic efficacy of readily available Mantoux test and sputum smear examination was tested with CBNNAT.

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. Based on the results of Abubakar *et al*., the positive predictive value of TST-5 is 2.2% while that of TST-10 is 2.7%. Since not much difference is seen between both, antigen having 5-tubercular units was used in our study [17]. 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 [18]. 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 [19].

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 [20]. Similarly, using a cutoff value of 5 mm in our study, we observed that the Mantoux test was positive in 41 (41%) patients and negative in 59 (59%) patients, and induration of >15 mm was noticed in 36 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 [21,22]. This is because of the low sensitivity and increased number of smear-negative TB, a large number of positive cases are missed [7]. In a study by Saktiawati *et al*. [15], 32% of were smear positive. Sensitivity and specificity of sputum examination were 81.4% (74–87.5%) and 99.5% (97.2–99.9%), respectively. In our study, sputum was positive in 42 (42%) patients and negative in 58 (58%) patients.

In our study, sensitivity and specificity of the Mantoux test were 49.35% and 86.95%, respectively, and 53.33% and 92%, respectively, for sputum examination. The previous studies have suggested that combinations of investigations are better as compared to individual tests. Saktiawati *et al*. [15] 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 CBNAAT will have better results than the individual tests. Smaller sample size and single-center study limit the generalizability of the results. Moreover, mycobacterial cultures were not done to confirm the diagnosis. We further suggest larger multicentric studies to test the predictive value of these routinely used tests.

CONCLUSION

Results of our study suggest that a combination of the Mantoux test, sputum smear, and CBNAAT test when performed together acts as an aid in diagnosing TB disease and to identify the persons with latent TB infection by assessing the immune status, so that, the treatment can be given to cure the diseased case and to prevent the progression of the latent case to disease.

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