



Original article

Pulmonary tuberculosis: A comparative study of conventional methods and serological diagnosis

Mohammed Khaleel¹, N. K. Saxena², V. C. Kishore Reddy³

¹Department of Microbiology, Deccan College of Medical Sciences, Hyderabad 500 058, A.P., India.

²Department of Microbiology, Gandhi Medical College, Secunderabad, A.P., India.

³State TB Center, Irrumnama, Hyderabad, A.P., India.

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Corresponding author

Mohammed Khaleel
Department of Microbiology,
Deccan College of Medical Sciences,
Kanchanbagh, Hyderabad 500058,
Andhra Pradesh, India.
Email: drmdkhaleel2006@gmail.com
Phone: +91 9550261140

Abstract

The objective of the study was to evaluate mycobacterial identification and isolation rates obtained by different conventional methods. Sputum and serum samples were taken from 80 clinically diagnosed pulmonary tuberculosis (PTB) cases. Smear microscopy alone showed an efficacy of 85%, culture 90% and serology 72.5%, in diagnosis of PTB. The efficacy of smear microscopy increased by examining more than one sputum sample to about 98%. The correlation between smear, culture and serology was assessed. Further investigation of smear-negative cases with culture examination was done. Concomitant x-ray studies delineated the location and extent of the lesion, mostly exudative type confined to upper zone. The culture isolates subjected to drug susceptibility test, showed 27% multi-drug resistant strains.

Key words: tuberculosis, Lowenstein Jensen medium, smear, chest x-ray, drug susceptibility, ELISA

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Pulmonary tuberculosis (PTB) is a bacterial infection caused by *M. tuberculosis*, spread by inhaling droplets of mucus that have been expelled by an infected person. The tuberculosis epidemic has been receiving more attention in the last decade of which about 50 million are likely to be infected with drug resistant strains. Globally PTB is the leading cause of death among infections, killing 3 million people every year. Poverty and tuberculosis go together in developing countries like ours, which is classified along with the sub-Saharan African countries, among those with a high prevalence. Tuberculosis is India's worst scourge as it bears one-third of the entire world's tuberculosis burden¹. Every second, an Indian over

20 years of age is infected, with 1.8 million people acquiring it and 0.41 million people dying of it, annually. Some epidemiologists forecast a rise in incidence of 20% in the next 20 yrs for India, with a cumulative rise of 46 million cases of tuberculosis during that period, largely as a consequence of Human Immunodeficiency Virus (HIV) epidemic².

With improvement in standards of living tuberculosis had declined rapidly in affluent countries, but the emergence of HIV has paved the way for the resurgence of *Mycobacterium tuberculosis* infection-pulmonary as well as extra-pulmonary more so in India. As a result, the World Health Organization (WHO) declared this disease as a global emergency in 1993³.

Drug resistant and multidrug resistant (MDR) forms of TB have also become prevalent, and the HIV epidemic having significantly contributed towards this scenario.

Robert Koch (1882) aptly said that, '*in the future battle against this plague of mankind (tuberculosis), it will not be just an uncertain something but a tangible parasite, about whose characteristics a great deal is known and much can be explored*'.

The present study was aimed at finding out the efficacy of different conventional laboratory methods for the diagnosis of TB. This study also attempts to correlate the findings of different conventional methods with each other and with serological diagnosis and radiological status of the disease. It also sought to explore correlation with the drug susceptibility pattern of sputum isolates.

Materials and methods

Subjects and samples

Eighty sputum and blood samples were collected from patients presenting with symptoms and signs strongly suggestive of pulmonary tuberculosis and were referred to tuberculosis clinic. All sputum samples were investigated with conventional methods namely smear microscopy after staining and culture & drug susceptibility. Also patient's sera were subjected to detection of IgG antibodies. The study was approved by Institutional Ethics Committee and informed consent was obtained from all the subjects.

Microscopy and staining

Sputum was collected in a sterile wide mouth 'universal' container. Smear is made from the purulent part of the sputum which is likely to have more number of bacilli. Smear was heat fixed and stained using Ziehl-Neelsen technique which identifies Acid-Fast bacilli. Recording and grading of results was done as per the International Union Against Tuberculosis and Lung Disease (IUATLD)⁴.

Culture and drug susceptibility

The most reliable way of establishing confirmatory diagnosis of any infectious disease is to isolate and identify the causative organism in culture⁵. Isolation, identification and sensitivity-testing of *Mycobacterium tuberculosis* were done as per the guidelines of National Tuberculosis Institute, Bangalore⁴.

Mc Cartney bottles were used to facilitate long incubation period and due to slow growth of organism, cultures were incubated in a walk-in incubator.

The most widely used medium for isolation of *M. tuberculosis* - egg-based Lowenstein-Jensen (LJ) medium was used for growth; and media with 500 µg of PNB per ml^{4,6} to differentiate human and bovine tubercle bacilli; and rate of growth, growth at 25°C, 37°C, 42°C for other species identification. All the samples in the study were processed using Nassau's swab technique^{4,7,8}. Strict adherence to aseptic technique was observed, and samples were carried in the lamination hood throughout the processing. Colonies on LJ medium were regarded as typical tubercle bacilli colony if they had moderate or coarsely granular mat surface and were buff colored⁸.

Drug susceptibility was done to find out whether the organisms isolated in the culture were sensitive or resistant to anti-tuberculosis drug. The drugs used were streptomycin, isoniazid, rifampicin and ethambutol. The isolates were exposed to series of appropriate concentration of each drug. The drugs used were of same brand and same company to ensure uniform quality. H 37 Rv was used as standard control. The minimum inhibitory concentration (MIC) of drugs was estimated using resistance ratio method. The concentration of drugs slopes in which less than 20 colonies or no growth is seen, is taken as MIC of the drug^{4,8}. Multi-drug resistant tuberculosis (MDR-TB) is essentially a bacteriological diagnosis and it implies that the bacilli have been proved to be resistant to atleast Rifampicin and Isoniazid by *in-vitro* culture and sensitivity testing⁹.

Serology

Enzyme linked immuno-sorbent assay (ELISA)¹⁰ with microtitre plate system was used, and read on the ELISA reader (BIORAD, Germany) for sero-diagnosis of tuberculosis. OMEGA Pathozymemyco (Qualigens, UK) kit was used for the detection of immunodominant and highly specific recombinant 38 kDa antigen, seen in patient's sera in response to infection with *Mycobacterium* species¹¹.

Chest x-ray

On the day of sputum collection, a chest x-ray (Posterior-anterior view) was also taken. Radiological findings were later correlated with the smear microscopy and culture results.

Results

Age specific analysis shows clinical diagnosis of PTB is highest in the age group 31-60 yrs (Fig 1). Acid-Fast staining of sputum smear using Ziehl-Neelsen technique shows 85% positivity, culture showed 90% positivity, IgG antibodies detection by

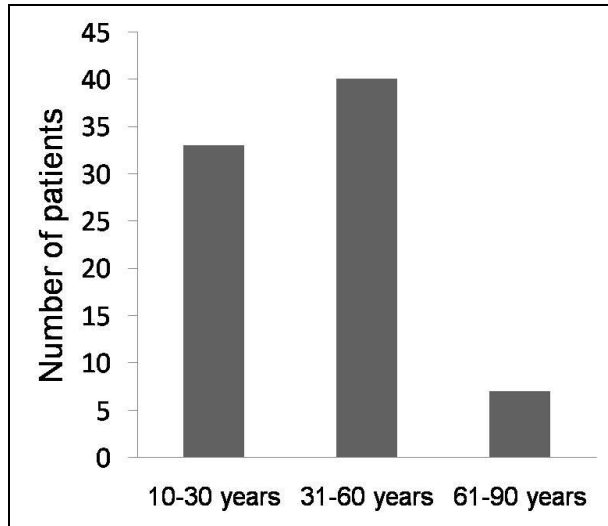


Fig 1. Age-wise distribution of patients

Table I. Comparison of the results of all the three tests

Test	Test samples (n=80)	
	Positive	Negative
Acid-Fast staining	68	12
Culture on L J medium	72	8
ELISA	58	22

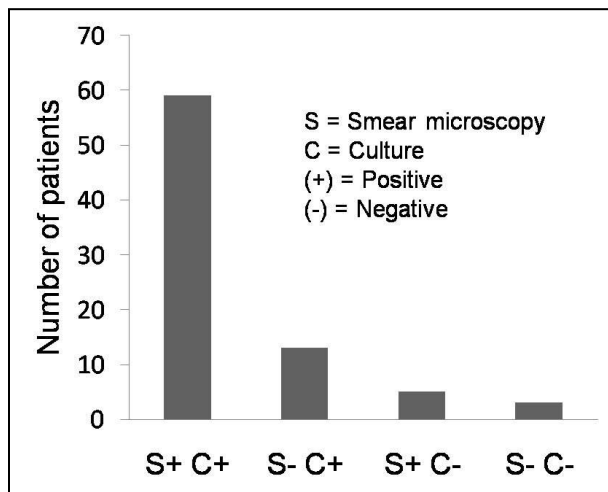


Fig 2. Evaluation of culture and smear results

ELISA shows 72.5% positivity in diagnosing PTB (Table I). Out of 80 patients, 58 (72.5%) tested positive for PTB with all the three tests. The findings of combined smear microscopy and culture examination are depicted in Fig 2.

The comparative study of ELISA IgG to that of Acid-Fast staining shows a sensitivity of 79.4%, specificity of 66.67%, positive predictive value of

93.10%, negative predictive value of 36.3% and accuracy value of 77.5%. The kappa coefficient is 0.36% which shows a good agreement between the two tests (Table II).

Table II. Result of ELISA and Acid-Fast staining of test samples

ELISA	Acid -Fast staining		Total
	Positive	Negative	
Positive	54	4	58
Negative	14	8	22
Total	68	12	80

The comparative study of serology by ELISA IgG to that of the culture shows a sensitivity of 77%, specificity of 75.5%, positive predictive value of 96.55%, negative predictive value of 27.27% and accuracy of 77.5%. The kappa coefficient is 0.3% which shows a good agreement between the two tests (Table III).

Table III. Result of ELISA and culture of test samples

ELISA	Culture on LJ medium		Total
	Positive	Negative	
Positive	56	2	58
Negative	16	6	22
Total	72	8	80

The common finding in the chest x-rays was exudative and fibrocavitary lesion in the upper zone (Fig 3 & 4), less commonly other type of lesions (Fig 5). Table IV shows correlation of locations of lesions on chest x-ray with smear microscopy and culture results.



Fig 3. Exudative lesion with miliary mottling

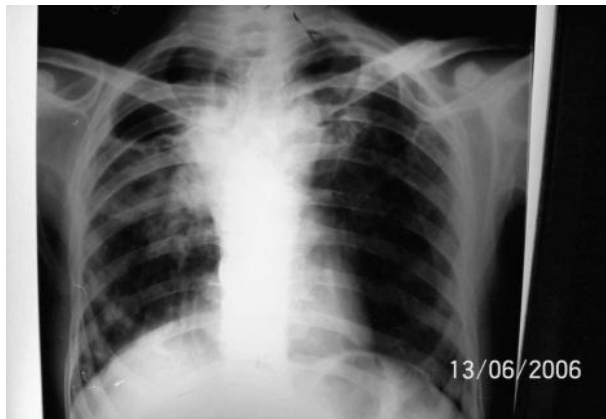


Fig 4. Fibrocavitary lesion

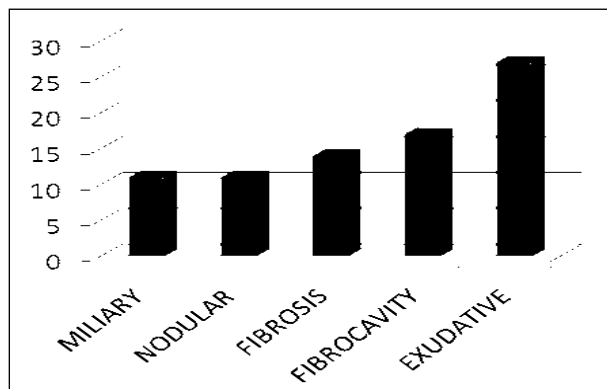


Fig 5. Types of radiological lesion

Table IV. Correlation of smear and culture results with chest x-ray findings

Lesion in chest x-ray	Smear and culture results			
	S+ C+	S- C+	S+ C-	S- C-
Upper zone	34	9	2	2
Mid and lower zones	17	3	2	1
Miliary	8	1	1	0
Total=80	59	13	5	3

S = Smear, C = Culture, (+) = Positive, (-) = Negative

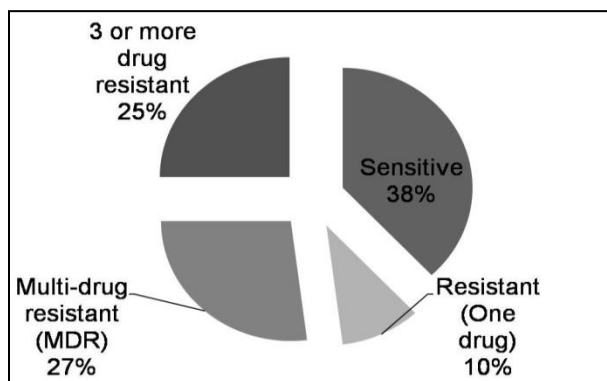


Fig 6. Drug susceptibility results

The drug susceptibility test revealed that most of the organisms are drug resistant strains. Isolates from 8 (10%) patients were single drug resistant, from another 22 (27%) patients were resistant to both isoniazid and rifampicin (MDR) and from other 20 (25%) patients were resistant to more than two drugs (Fig 6).

Discussion

In the present study, 80 individuals were subjected to different tests, conventional and sero-diagnostic. Out of 80 patients, 58 (72.5%) tested positive for PTB with all the three tests.

The rapid presumptive diagnosis of tuberculosis is smear examination, and culture being more sensitive which confirms the causative agent *Mycobacterium tuberculosis*¹². Our study revealed that 24% of smear-positive cases fall in the age group 31-60yr. While a single smear of sputum has a sensitivity of only 22-43%, the detection rate goes up considerably when multiple specimens are examined⁵. Our study has shown that a sensitivity of 85% with single sputum sample increased to 98% when three sputum specimens were examined.

In our study culture sensitivity was 90% and specificity 90%, and hence culture is still the most sensitive method as it detects 10-100 viable organisms / ml of sputum^{12,13}. It therefore seems that for the final diagnosis of tuberculosis, both the sensitivity and specificity are far better with this test, besides this is also essential for surveillance of drug resistance.

Rao et al (1966) in their analysis of many published studies showed that culture examination, followed by tests for identifying the bacilli, is recognized as the most accurate and reliable method¹⁴. In epidemiological community surveys (Indian Council of Medical Research), it has been found that culture positives that were also smear positives varied from 73% to 87%¹⁵. In our study, 59 (74%) patients were both culture smear positive.

Anti-body detection by ELISA turned out to be least sensitive and hence sero-diagnosis by ELISA for IgG can be used as an adjunct or as a supplement. In many previous studies, all three immunoglobulins-IgG, IgM and IgA have been estimated, but IgG estimation was preferred by us, using 38 kDa antigen which is immunodominant and highly specific.

Introduction of radiography as a diagnostic tool was a landmark in the knowledge of the natural history of tuberculosis. However, practical experience with many studies has shown that, many diseases of lung show a similar radiographic appear-

ance and easily mimic tuberculosis. It can only localize the abnormality in the lung, therefore maybe considered unreliable. In tuberculosis patients with a relatively intact immune system, the radiographic findings were predominantly upper lobe lung lesions, cavitation and fibrosis¹⁶. Our results are also in accordance with such lesions.

Our study showed a prevalence of 27% MDR-TB and another 25% were resistant to three or more drugs. MDR-TB is amongst the most worrisome elements of the pandemic of antibiotic resistance. Globally about three percent of newly diagnosed patients have MDR-TB¹⁷. This rising drug resistance in tuberculosis has become a threat to TB-control programmes⁶.

Conclusion

To conclude, diagnostic process of tuberculosis initiates with a high clinical suspicion, and is supported through the use of various diagnostics. The only rapid test for presumptive diagnosis of tuberculosis is smear examination and culture remains the gold standard for final confirmatory laboratory diagnosis of tuberculosis. Sero-diagnosis may be used as a supplement to smear and culture examination. In developing countries like India, expensive diagnosis by molecular methods may not replace the smear microscopy and culture, in practice because of high reliability in terms of sensitivity and specificity.

Conflict of interest

The authors have no conflict of interest to disclose.

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