Original Article

Use of Mycobacterium Culture in Confirming Diagnosis of Tuberculosis in smear negative pulmonary Tuberculosis Suspects Registered in DOTS Implemented Setting at Lahore

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Objective: Early diagnosis of disease and prompt initiation of treatment is essential for an effective tuberculosis control programme. There is concern that smear negative pulmonary tuberculosis may be over-diagnosed and treated in overburdened and resource poor countries. This study was conducted to determine what proportion of patients being registered for smear negative pulmonary tuberculosis treatment have microbiologically confirmed tuberculosis.

Material and Methods: Subjects of either sex above the age of 15 year with symptoms of and x-ray finding consistent with pulmonary tuberculosis were selected. Sputum specimens of 124 smear negative pulmonary tuberculosis suspects about to be registered for smear negative pulmonary tuberculosis treatment by the national tuberculosis programme were inoculated on LJ culture medium to isolate the organism. The result of smear and culture were then compared.

Results: A total of 124 sputum smear negative cases were subjected to culture on LJ medium. Out of these 18(14.51%) were confirmed positive by culture.

Conclusion: Sputum culture is gold standard the diagnosis of tuberculosis. Complementing smear negative cases with culture may help in reducing over diagnosis of pulmonary tuberculosis.

Keywords: Tuberculosis (TB), Mycobacterium Tuberculosis (MTB), Pulmonary Tuberculosis (PTB), Acid Fast Bacilli (AFB), Ziehl Neelsen staining (ZN), Löwenstein-Jensen medium (LJ).

Introduction

For an effective tuberculosis control programme early diagnosis of disease and prompt initiation of treatment is essential.[1] There is concern that smear negative pulmonary tuberculosis may be over-diagnosed and treated in overburdened and resource poor countries.

Diagnosis of tuberculosis is made by finding acid-fast bacilli (AFB) on direct microscopic examination of sputum smear but it has been observed that microscopic is not very sensitive technique.[2] Half of all cases with tuberculosis can present with negative results.[3,4] These smear-negative cases can be diagnosed by culture of *Mycobacterium tuberculosis* because culture of *Mycobacterium tuberculosis* is the gold standard for diagnosis of tuberculosis.[5] According to WHO report 2004, only 3.9 million cases were sputum positive from of out 8.8 million cases diagnosed in 2002.[6]

DOTS strategy relies mainly on sputum smear microscopy for detection of pulmonary tuberculosis cases. World Health Organization (WHO) recommended strategy of examination of three sputum smears for acid-fast bacilli lacks sensitivity. Studies conducted in different settings have reported an increasing proportion of pulmonary tuberculosis patients with negative smear results. Therefore, one cannot rely on smear examination by ZN staining method only; otherwise significant number of tuberculosis patients will be missed[7] to be initiated on treatment. Such missed cases remain a constant source of infection and threat to community.

Chest x-ray is restricted to diagnosing pulmonary tuberculosis among those suspects whose sputum is negative for AFB.[8] Although pulmonary tuberculosis is usually associated with radiographic abnormalities,[9] the lesions are non-specific and their interpretation depends on many factors.[10] Due to these reasons the proportion of over diagnosing tuberculosis remains high even restricting chest radiograph to smear negative pulmonary tuberculosis suspect.[11]

This study was conducted to determine what proportion of patients being registered for smear negative pulmonary tuberculosis treatment have microbiologically confirmed tuberculosis.

Material and Method

A descriptive observational study was conducted at

outpatient department of Model Chest Clinic Lahore and Punjab Tuberculosis Reference Laboratory, Institute of Public Health, Lahore from January 2012 to December 2012.

124 new pulmonary tuberculosis suspects age 15 years and above of either sex with symptoms and x-ray finding consistent with pulmonary tuberculosis about to start treatment for tuberculosis were selected. Suspects with previous history of tuberculosis and patients currently receiving anti-tuberculosis treatment were excluded from the study.

Sputum specimens of these pulmonary tuberculosis suspects were processed for culture by digestion, decontamination and concentration following modified Petroff`s method and were inoculated on LJ culture media for six weeks to isolate the organisms. Readings were taken every week for eight weeks. The identity of the isolates was made by growth rate and colony morphology. All processing was performed in Bio-safety level-2 cabinet. Sputum specimen were kept in refrigerator at 4-6oC when processing was delayed. Standard strain of H 37 RV was used for the quality control. HIV screening was not performed.

Results

A total number of 213 cases of pulmonary tuberculosis were registered in the DOTS implemented setting. 89 patients had smear positive on microscopy and were registered as smear positive pulmonary tuberculosis. 124 patients were about to register for treatment as smear negative. These smear negative patient submitted three sputum specimens for Mycobacterium Tuberculosis culture. LJ culture media was used for inoculation. Clinically diagnosed cases of smear negative pulmonary tuberculosis belonging to age fifteen and above.

In this study there were 124 smear negative patients, out of which 18(14.51%) were positive on culture while 106(87.84%) patients remained culture negative **(Table-1)**.

Table-1: culture results of smear-negative pulmonary tuberculosis suspects n= 124.

Z-N Staining	Culture Results Positive	Negative	Total
Negative	18 (14.51%)	106 (85.48%)	124 (100%)

Discussion

This study was conducted to observe the use of mycobacterium tuberculosis culture in confirming the diagnosis of tuberculosis in smear-negative pulmonary tuberculosis suspects. In this study sputum samples of 124 patients about to be registered for smear negative pulmonary tuberculosis treatment by the national tuberculosis programme were inoculated on LJ culture medium. Out of these 124 patients, 18(14.51%) patients were positive on culture while 106(85.48%) patients remained negative on culture examination. Some of these culture negative patients may be due to tuberculosis but they are not detected by culture at present.

This phenomenon of culture positivity in smear negative cases is not unusual occurrence. From the literature review it was clear that culture positivity among smear negative cases varies in various studies conducted in different regions of the world. Van Dennl, (2004)[12] reported in his review study 24-62 % positivity rate in different geographical locations.

In this study only 14.51% of those who were presumed to have active PTB and started on anti-tuberculosis treatment actually had TB on sputum culture results.

Additional yield of bacteriological confirmed cases is 20% in this study. In other studies 40%, 27.2% of patients registered for smear negative pulmonary tuberculosis treatment had culture positive results for mycobacterium tuberculosis.[13,14] Wide variability in the results reported by the different researchers and results of present study may be due to various causes.[15]

We are unable to confirm diagnosis in a significant proportion 85.48% of smear-negative pulmonary tuberculosis suspects registered for the treatment of tuberculosis. Some of these cases might be suffering from tuberculosis despite the lack of microbiological confirmation. The results of this study shows that we might be over diagnosing and treating smear negative tuberculosis.

In health care facilities with no access to culture, the services of a thoughtful clinician to interpret the finding secured by all means like good medical history, medical examination and an x-ray film of the chest remains important.

The use of chest x-ray for diagnosis of pulmonary tuberculosis can be compromised by poor quality film, low specificity and difficulties with interpretation.[16] In our country with high disease burden and where tuberculosis control programme is implemented through primary health care system, x-ray reporting is being done by primary care physicians without training in diagnosis of tuberculosis. Chest x-ray remains an important test for the diagnosis of smear negative pulmonary tuberculosis, so

Chemicals used for decontamination and the centrifugation used for concentration of samples during preparation of inoculum have a major impact on the sensitivity of the test. Factors that influence the quantum of viable bacilli in the specimen such as type of collection (spot or overnight) and previous chemotherapy should also be considered.[15,16]

Conclusion

To reduce the over diagnosis of smear negative pulmonary tuberculosis and reducing burden of unnecessary cost of treating individual who may or may not actually having tuberculosis, use of culture is needed.

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