Comparative Sensitivity of Smear Microscopy for AFB versus Gene Xpert/Rif Assay on Endobronchial washings of the Sputum Smear Negative/Sputum Scarce Patients with suspected Pulmonary Tuberculosis

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SKR RU conceived idea, RU SWS SKR drafted the study, SKR SA H collected data, RU H MH statistical analysis and interpretation of data, SKR RU MH critical review manuscript, All approved final version to be published

Declaration of conflicting interests

The authors declare that there is no conflict of interest.

Abstract

Background: Pulmonary Tuberculosis (PTB) is a highly prevalent infectious disease. It can be diagnosed with various tests including sputum ZN staining, Gene Xpert and culture.

Objectives: Objective of this study is to determine the sensitivity of smear microscopy (SM) for AFB and Gene Xpert MTB/RIF assay in diagnosing PTB in sputum negative and sputum scarce patients on endobronchial washings in comparison to culture for mycobacterium.

Methodology: This Cross Sectional Study was conducted at Pulmonology department, Military Hospital Rawalpindi (MH-Rawalpindi). Patients of both gender, aged 18-70 years and sputum smear negative PTB suspects were included in the study. Diagnosed or already on antituberculous treatment patients and Imunocompromised patients (HIV) were excluded.

Endobronchial washings were collected from affected segment from all patients using fiber-optic bronchoscopy. Specimen was divided into 3 parts. Each was sent for ZN-microscopy for AFB, MTB gene Xpert and culture.

Data was analyzed in SPSS version-20. Frequency and percentages were calculated for qualitative variables like gender, sensitivity of SM for AFB/Gene Xpert. Mean ± standard deviation was calculated for quantitative variables like age, duration of illness. . Post stratification Chi-square test was applied.

Results: Mean age was 45.75±17.73 years. 248(74.5%) were male and 85(25.5%) female. Sensitivity and Specificity of BAL smear microscopy was 75.44% and 86.59% while that of Gene XPERT was 94.74% and 80.43% respectively.

Conclusion: Results of this study showed that Gene Xpert is more accurate than BAL smear microscopy in predicting PTB.

Key words: PTB; Gene Xpert; Sputum Microscopy; Blood Culture Bottles; Sterile Syringes; Parapneumonic Effusions

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Introduction

Pulmonary tuberculosis (PTB) is an infectious disease associated with high morbidity and mortality worldwide. Tuberculosis (TB) still remains a major global health problem. In 2012, an estimated 8.6 million people developed TB and 1.3 million died from the disease.[1] It means three deaths each minute due to tuberculosis worldwide.[2] Tuberculosis is also one of the major public health problems in Pakistan. According to report in 2011, incidence of all types of tuberculosis was 231 per 100,000 population.[3] Pakistan ranks fifth amongst TB high-burden countries worldwide. It accounts for 61%

of the TB burden in the WHO Eastern Mediterranean Region. Approximately 420 000 new TB cases emerge every year and half of these are sputum smear positive.[4]

World Health Organization recommends bacteriological confirmation of pulmonary tuberculosis by the detection of acid-fast bacilli (AFB) in respiratory specimens. However about 40-60% of patients with PTB suspected clinically or radiologically may fail to produce sputum, or when it is available, AFB may be negative on repeated smear examination. It means a large population of patients remain smear negative in spite of having pulmonary tuberculosis, which causes unnecessary delay in diagnosis and prompt management, resulting in huge mortality and morbidity of the disease which is completely curable once diagnosed early.

These sputum smear negative patients and those who fail to produce any sputum can be diagnosed by flexible fiber-optic bronchoscopy.[5] Several studies around the world have proven the value of fiber-optic bronchoscopy (FOB) whose pre-bronchoscopic sputum specimens were negative both for smear and PCR analyses.[6] Furthermore, while mycobacterial culture remains the gold standard for laboratory diagnosis of TB, it requires 2-6 weeks to confirm a diagnosis. This results in delays in initiating appropriate treatment while waiting for this confirmation, except for cases where there is strong enough clinical suspicion to initiate a presumptive anti-TB therapy.

Since December 2010, WHO has recommended the Gene Xpert MTB/RIF assay due to its high-quality performance compared to microscopy, and especially in cases of smear-negative specimens.[7] Few studies around the world have compared the diagnostic accuracy of smear microscopy (SM) for AFB to Gene Xpert/RIF assays on endobronchial washings using bronchoscope. A study in France has confirmed the clinical benefits provided by Gene Xpert/RIF assay compared to SM for the early diagnosis of suspected pulmonary TB cases requiring FOB. As compared to culture, sensitivity and specificity values were 80.0% and 98.6% for the Gene Xpert/RIF assay, and 25.0% and 95.8% for SM, respectively. PCR assay enabled pulmonary TB to be diagnosed earlier in 13 more cases, compared to SM.[8] However WHO conditional recommendation on MTB Gene Xpert is only for sputum samples, whereas no specific recommendations for FOB samples have yet been formulated. Finally, there have been very few recent studies that have assessed the Xpert MTB/RIF assay performance using FOB samples for TB diagnosis in high TB-burden countries. There is also no published study in Pakistan at present. We conducted a prospective cross sectional study in which 331 cases of presumptive PTB undergoing FOB to collect washings for SM, Gene Xpert/RIF assay and culture were selected and followed.

Methodology

This cross sectional validation study was conducted at Pulmonology wards/OPD of Military Hospital, Rawalpindi from 20th June 2015 to 19th December 2015. A total of 333 patients were included in the study through consecutive non-probability sampling technique. Sample size was calculated Using WHO sample size calculator keeping confidence level 95%, anticipated population proportion 25 % and absolute precision 10%, sample size came out to be 75 but we included all patients fulfilling the criteria. All patients of either gender who is tuberculosis suspect (including new and old treated cases with suspicion of new relapse of TB) those who are repeatedly sputum smear negative or sputum scarce were included in the study. Patients who are already diagnosed as pulmonary tuberculosis (previously diagnosed and treated cases presenting again with relapse and still undiagnosed), those who are already taking antituberculous treatment and Imunocompromised patients (HIV) were excluded from the study.

After approval from hospital ethical committee a written informed consent was taken from every patient. Demographic characteristics (name, age, sex, residence) were recorded for each patient. All patients who fulfill the inclusion criteria underwent fiber-optic bronchoscopy by a trained pulmonologist. Endobronchial washings were collected using fiber optic bronchoscope after instilling 20-30ml of isotonic saline in affected segment guided by radiological findings. Collected aspirations were divided into 3 specimens and each was sent for ZN microscopy for AFB, MTB gene Xpert/RIF assay and culture for Mycobacterium tuberculosis using either BACTEC or LJ medium. Keeping culture as a gold standard sensitivity of the SM to detect AFB was compared to MTB gene Xpert/RIF.

Data was entered and analyzed in SPSS version 20. Frequency and percentages were calculated for qualitative variables like gender and sensitivity of SM for AFB/Gene Xpert while mean ± standard deviation was calculated for quantitative variables like age and duration of illness. Post stratification Chi-square test was applied. P-value <0.05 was considered significant.

Results

Mean age of patients was 45.75 ± 17.73 years (Table 1). There were 248(74.5%) male and 85(25.5%) female patients (Table 2). Cough was the most common symptom (90 percent of confirmed cases) followed by

fever (75 per cent) weight loss (45 percent) and hemoptysis (17 percent). BAL smear microscopy was positive in 80(24.0 %) and gene Xpert was positive in 108(32.4%) of the patients, while culture was positive in 57(17.1%). Sensitivity and Specificity of Gene Xpert was 94.74% and 80.43%. Sensitivity and Specificity of BAL smear microscopy was 75.44% and 86.59% (Figure 1). There is significant difference between sensitivity of BAL smear microscopy 75.44% and BAL gene Xpert 94.74 %(p-value <0.001).

Table 1. Age distribution of study cases

Total number of cases	333
Mean	45.75
SD	17.73
Minimum	18
Maximum	70

Table 2. Gender distribution of study cases

Gender	Number of patients	Percentage
Male	248	74.5%
Female	85	25.5%
Total	333	100%

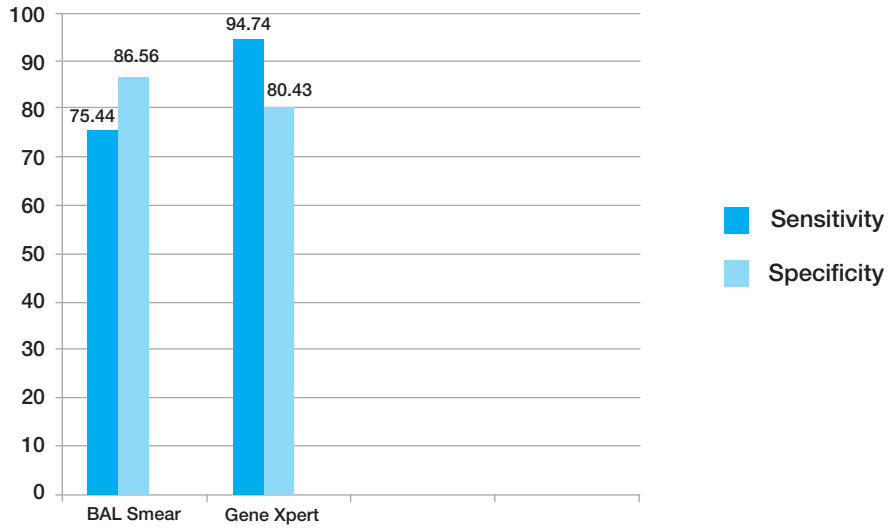

Figure 1: Graph showing sensitivity and specificity of BAL microscopy and gene Xpert

Discussion

World Health Organization recommends bacteriological confirmation of pulmonary tuberculosis (PTB) by the detection of acid-fast bacilli (AFB) in respiratory specimens. However about 40- 60% of patients with PTB suspected clinically or radiologically may fail to produce sputum, or when it is available, AFB may be negative on repeated smear examination. It means a large population of patients remain smear negative in spite of having pulmonary tuberculosis, which causes unnecessary delay in diagnosis and prompt management, resulting in huge mortality and morbidity of the disease which is completely curable once diagnosed early. These sputum smear negative patients and those who fail to produce any sputum can be diagnosed by flexible fiber optic bronchoscopy.[5]

Several studies around the world have proven the value of fiber optic bronchoscopy (FOB) whose pre-bronchoscopic sputum specimens were negative both for smear and PCR analyses.[6] Furthermore, while mycobacterial culture remains the gold standard for laboratory diagnosis of TB, it requires 2-6 weeks to confirm a diagnosis. This results in delays in initiating appropriate treatment while waiting for this confirmation, except for cases where there is strong enough clinical suspicion to initiate a presumptive anti-TB therapy.

Since December 2010, WHO has recommended the

Gene Xpert MTB/RIF assay due to its high-quality performance compared to microscopy, and especially in cases of smear-negative specimens.[7] Few studies around the world has compared the diagnostic accuracy of smear microscopy(SM) for AFB to Gene XPERT. MTB/RIF(XP) assays on endobronchial washings using bronchoscope.

In our study, we determined the frequency of detection of Mycobacterium tuberculosis in BW Gene Xpert in sputum-scarce cases of suspected pulmonary tuberculosis. Many sputum smear negative patients are reported to have progressive disease.[8] Similar results were demonstrated by different researchers, such as Lyer VN et al, reporting detection of TB in 30-48% in smear negative patients.[9] Different studies validated the practical and clinical importance of bronchoscopy in PTB diagnosis. In all these studies bronchoscopies and BW showed increased detection of TB.[10]

Bronchoscopy with BW under local anaesthesia is a relatively safe procedure and well tolerated by most of the patients. BW Gene Xpert can rapidly detect the MTB and rule out rifampicin resistance on the same day, helping in the early diagnosis and management of these patients.[11]

Conclusion

BAL Gene Xpert is more sensitive than SM to detect mycobacterium tuberculosis and rifampicin resistance and can detect drug resistance TB earlier than culture in suspected PTB cases that have either sputum negative or sputum scarce disease.

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