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DIAGNOSTIC YIELD OF BRONCHOALVEOLAR LAVAGE CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST (CB NAAT) IN SMEAR NEGATIVE PULMONARY TUBERCULOSIS.

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ABSTRACT

Introduction: Tuberculosis (TB) is one of the most common communicable diseases affecting all age groups in low-income countries. According to the WHO Global Report 2015, there were an estimated 10.4 million new (incident) TB cases worldwide, of which 5.9 million (56%) were among men, 3.5 million (34%) among women and 1.0 million (10%) among children. Bronchoscopy with Bronchoalveolar Lavage (BAL) is very useful in patients suspected of pulmonary tuberculosis. Recently, a cartridge system with multicolour real-time PCR capacity for the detection of Mycobacterium tuberculosis (MTB), commonly known as Gene-Xpert, has been developed which detects mutations in the 81-bp Rifampicin Resistance-Determining Region (RRDR) of the rpoB gene within 2 hours, that occurs in 95 - 98% of all rifampin-resistant strains. **Aims and Objectives:** To measure the diagnostic yield of BAL fluid CB NAAT to detect Mycobacterium Tuberculosis and to rule out rifampicin resistance on the same day. **Method and materials:** We performed an analytical study to evaluate pulmonary tuberculosis in the Department of Pulmonary Medicine of our hospital.All the patient included in this study underwent Bronchoscopic evaluation and BAL was taken. A patient was considered a tuberculosis-suspect, on the basis of clinical and radiological features compatible with a diagnosis of pulmonary tuberculosis.A smear-negative case was one in whom two samples, one spot sample and one early morning sample did not reveal acid fast bacilli when examined by microscopy with Ziehl Nelson stain and fluorescent microscopy. **Results:** We evaluate 40 patient with mean age was 38.56 ± 19.045 years. About 25 patients (62.5%) were males while the rest were female patients i.e 15 (37.5%). BAL CB NAAT was positive in 27 (67.5%) and negative in 13(32.5%) patients.BAL positivity by direct smear was present in 13(32.5%) patient and BAL positivity by culture was present in 35 (87.5%). **Conclusion:** Bronchoalveolar lavage gene Xpert(CB NAAT) had a superior diagnostic yield to detect Mycobacterium tuberculosis and rifampicin resistance (with high sensitivity and specificity).This test has the advantages of being inexpensive, requires less manpower and gives results on the same day. Our study highlights that XpertMTB/RIF has high sensitivity and specificity for diagnosis of both smear positive and smear negative PTB cases with high rates of detection of RIF resistance.

KEYWORDS: MTB, Bronchoalveolar Lavage, Gene Xpert, Rifampicin.

INTRODUCTION

Tuberculosis (TB) is one of the most common communicable diseases affecting all age groups in low-income countries. Although TB is preventable and treatable in most cases, according to the WHO Global Report 2015, there were an estimated 10.4 million new (incident) TB cases worldwide, of which 5.9 million (56%) were among men, 3.5 million (34%) among women and 1.0 million (10%) among children. People living with HIV accounted for 1.2 million (11%) of all new TB cases.[1,2] It ranks as the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV).[2] Diagnosis of pulmonary TB in individuals is through clinical presentation, sputum test and bacteriological confirmation in laboratories. Only about 20 - 40% of pulmonary TB patients are smear positive, while rest of the patients had either smearnegative or sputum-scarce disease.[3,4] Bronchoscopy with Bronchoalveolar Lavage (BAL) is routinely performed for these set of patients in a suspected case of pulmonary tuberculosis. These diagnostic tests range from traditional smear microscopy, culture to newer rapid tests like GeneXpert MTB/RIF and line probe assay.

Bronchoalveolar lavage is sent for Acid Fast Bacilli (AFB smear by ZN stain) and mycobacterial cultures. The sensitivity of ZN stain remains low (41%). Mycobacterial cultures, considered as the gold standard (with 86% sensitivity) but they are expensive and results take 6 - 8 weeks for diagnosis.[7]

Complex nucleic acid amplification tests do not have a role in routine diagnosis because of their poor sensitivity and complexity.[8] Recently, a single-tube, single-use sample-processing cartridge system with multicolour real-time PCR capacity for the detection of Mycobacterium tuberculosis (MTB), commonly known as Gene-Xpert, has been developed with the additional feature of detecting mutations in the 81-bp Rifampicin Resistance-Determining Region (RRDR) of the rpoB gene, that occurs in 95 - 98% of all rifampin-resistant strains.[8] This assay is able to detect MTB and rifampicin resistance within 2 hours. The GeneXpert MTB/RIF is an automated cartridge based nucleic acid amplification test that detects Mycobacterial tuberculosis complex DNA and also the bacterial resistance to Rifampicin. It is a rapid screening tool feasible for use in conventional laboratories and requires minimal training. Since it detects Rifampicin resistance, patients can be put on further evaluation of drug resistant TB. Aiming at the rpoB, it helps to deal with all mutations found in more than 99.5% of all rifampicin resistant strains.[2] It does not show cross reactivity to nontuberculous mycobacteria. According to a systematic review, GeneXpert attained a pooled sensitivity of 88% and pooled specificity of 98% when used instead of smear microscopy as an initial diagnostic test. In smear positive, culture positive cases pooled sensitivity was found to be 98%, in smear negative cases it was 68% and 80% in HIV patients. It is also useful in patients who are smear negative cases of presumptive TB. In adults and children suspected of HIV associated TB or MDR TB, it should be used as an initial diagnostic test.[3,4]

The advantages of this test include high sensitivity and specificity, low complexity, low cost, wide availability and less manpower involved.[3,4] This test is found useful in diagnosis and management of suspected cases of pulmonary tuberculosis.

As smear-negative patients form the bulk of cases and delay in diagnosis in this subset often leads to increased morbidity and mortality, so if found superior to mycobacterial cultures that are the gold standard, BAL gene Xpert can rapidly detect the mycobacteria and rule out rifampicin resistance on the same day, helping in the diagnosis and management of these patients.

The aim of this study was to measure the diagnostic yield of bronchoalveolar lavage gene Xpert and compare it with traditional mycobacterial cultures in smear negative and sputum-scarce pulmonary tuberculosis.

AIM

To measure the Diagnostic yield of Broncho alveolar lavage CB NAAT in Smear negative Pulmonary Tuberculosis.

OBJECTIVES

- To measure the diagnostic yield of BAL fluid CB NAAT to detect Mycobacterium Tuberculosis.
- To rule out rifampicin resistance on the same day helping in early initiation of the treatment.

MATERIALS AND METHODS

This was an analytical study, carried out in the Department of Pulmonary Medicine, Dr Panjabrao Deshmukh Memorial Medical College, on 40 patients with suspected pulmonary tuberculosis from 2015 to 2016. Permission was taken from the Hospital Ethics Committee. The financial expenses were borne by the Hospital.

Sample size was calculated using the WHO sample size calculator, using the sensitivity of the Xpert assay being bronchial washing or Bronchoalveolar Lavage (BAL) fluid for the diagnosis of PTB as 81.6%[8] and mycobacterial cultures having sensitivity of 86%.[5] By using the sensitivity calculator and an absolute precision of 0.08, the sample size was determined as 40.

The diagnostic yield was defined and measured in terms of frequency and validity by calculating sensitivity, specificity, positive and negative predictive values. A patient was considered a tuberculosis-suspect, on the basis of clinical and radiological features compatible with a diagnosis of pulmonary tuberculosis.

A smear-negative case was one in whom two samples, one spot sample and one early morning sample did not reveal acid fast bacilli when examined by microscopy with Zeihl Nelson stain and fluorescent microscopy. Patients that had sputum amount less than 1 ml were defined to have sputum scarce disease.

Patients of either gender aged above 18 years of age, that had suspected pulmonary tuberculosis on clinical or radiological grounds and had taken anti-TB medications for less than 1 week, were included in the study. Smear positive cases, those with disseminated or extra pulmonary tuberculosis, HIV positive and immunocompromised patients were excluded from the study. Following written consent for bronchoscopy, demographic and clinical data was collected.

Bronchoscopy was performed by transnasal route and bronchoscope was wedged into the subsegmental bronchus of interest and about 100 ml of Bronchoalveolar Lavage (BAL) was obtained in two aliquots by instillation of sterile normal saline. It was sent for ZN stain and for gene Xpert to detect Mycobacterium tuberculosis (MTB) and rifampicin resistance.

BAL specimens were processed by the laboratory using standardized protocols and quality assurance procedures for the Xpert MTB/RIF assay. The results of all tests were read by a trained technician and reported for detection of MTB and presence or absence of rifampicin resistance. BAL specimens for smear microscopy were evaluated at Dr Panjabrao Deshmukh Memorial Medical College Hospital Laboratory.

SPSS version 16 was used for statistical analysis. Demographic features including age, gender, clinical and radiological features, past history of tuberculosis were recorded. Frequency of AFB smear, gene Xpert assay was calculated by percentages.

INCLUSION AND EXCLUSION CRITERIA
INCLUSION CRITERIA
Patients of either gender aged above 18 years of age with suspected pulmonary tuberculosis on clinical and radiological grounds and are smear negative.

EXCLUSION CRITERIA
• Smear positive
• Patient with co morbidities like diabetes, uncontrolled hypertension, chronic kidney disease, chronic liver disease, HIV seropositive.
• Disseminated tuberculosis or extra pulmonary tuberculosis.
• Poor general condition
• Advanced age.

• REJECTION CRITERIA: Inadequate patient identifiers on specimen.
Unlabelled specimen.
Discrepancy between patient specimen and requisition information.
Improper collection.

ZIEHL NEELSEN 'S STAINING
A typical AFB stain procedure involves dropping the cells in suspension onto a slide, then air drying the liquid and heat fixing the cells.

The slide is flooded with Carbol Fuchsin, which is then heated to dry and rinsed off in tap water.

The slide is then flooded with a 1% solution of hydrochloric acid in isopropyl alcohol (or methanol) to remove the carbol fuchsin, thus removing the stain from cells that are unprotected by a waxy lipid layer.

Thereafter, the cells are stained in methylene blue and viewed on a microscope under oil immersion.

Studies have shown that an AFB stain without a culture has a poor negative predictive value. An AFB Culture should be performed along with an AFB stain; this has a much higher negative predictive value.

GENE XPERT ASSAY(CB NAAT): PROCEDURE
GeneXpert MTB/RIF assay is a rapid diagnosis test of Tuberculosis (TB) and drug resistance. It is revolutionizing TB control with aids in prompt diagnosis and treatment (selection of appropriate TB regimen).

GeneXpert MTB/RIF assay is a nucleic acid amplification (NAA) test which simultaneously detects DNA of Mycobacterium tuberculosis complex (MTBC) and resistance to rifampin (RIF) (i.e. mutation of the rpoB gene) in less than 2 hours. In comparison, standard cultures can take 2 to 6 weeks for MTBC to grow and conventional drug resistance tests can add 3 more weeks.

This system integrates and automates sample processing, nucleic acid amplification, and detection of the target sequences.

The primers in the XpertMTB/RIF assay amplify a portion of the *rpoB* gene containing the 81 base pair "core" region. The probes can differentiate between the conserved wild-type sequence and mutations in the core region that are associated with rifampicin resistance.

MATERIALS/SYSTEM REQUIREMENT
1. GeneXpert System
• equipped with GX2.1 software/computer/printer/barcode wand-reader and operator manual (Cepheid Inc, Sunnyvale, USA).
• It is available in a one, two, four, or 16-module configuration.
2. GeneXpert Cartridge.
• Single-use disposable XpertMTB/RIF cartridges
• Sample extraction, amplification and detection are all carried out within this self-contained cartridge
3. Class II biological safety cabinet (BSC)
4. Sample reagent (provided in XpertMTB/RIF kit), 8ml volume pack per each cartridge. The sample reagent solution is clear, but may range from colorless to golden yellow.
5. Permanent marker pens.
6. Sterile (individually packed) disposable transfer pipettes– with single mark for minimum volume of sample transfer to cartridge (provided in Xpert MTB/RIF kit).
7. Sterile screw-capped specimen collection containers/cups.
8. Discard containers for pipettes and sputum containers

BASIC PROCEDURE
1. Collect sputum sample from the patient with suspected TB.
2. The sputum is mixed with the reagent that is provided with the assay,
and a cartridge containing this mixture is placed in the GeneXpert machine.
3. All processing from this point on is fully automated.

ADVANTAGES OF THE XPERT MTB/RIF ASSAY

1. Time efficient methods for detecting *Mycobacterium tuberculosis* bacteria and mutations isoniazid (INH) resistance.
2. Availability of quick test results leads to improved patient management and outcomes and preventing unnecessary use of resources (avoiding unnecessary treatment, patient isolation).
3. Fully automated system; minimal technical training is required to run the test.
4. Prompt (quick) identification of multidrug-resistant TB (MDR TB) cases as resistance to RIF, in most instances, co-exists with resistance to INH.

Rapid diagnosis of rifampin (RIF) resistance potentially allows TB patients to start on effective treatment much sooner than waiting for results from other types of drug susceptibility testing. If rifampin resistance is detected, confirmation of resistance can be done by DNA sequencing.

*MDR TB is TB that is resistant to both isoniazid (INH) and Rifampicin (RIF).

INTERPRETATION OF GENEXPERT RESULTS:

Results of GeneXpert should be interpreted along with clinical, radiographic, and other laboratory findings. The Xpert MTB/RIF assay does not replace the need for smear with microscopy for acid-fast bacilli, culture for mycobacteria, and growth-based drug susceptibility testing, in addition to genotyping for early discovery of outbreaks.

Results from the Xpert MTB/RIF assay indicate whether or not MTBC was detected in the sample. In some instances, the result is "invalid," whereby the test should be repeated.

If MTBC was detected, the results will also state whether resistance to RIF was

- **Detected**: Mycobacteria have a high probability of resistance to RIF; should be confirmed by additional testing. If RIF resistance is confirmed, rapid molecular testing for drug resistance to both first-line and second-line drugs should be performed so that an effective treatment regimen can be selected.
- **Not detected**: Mycobacteria are probably susceptible to RIF; All tests that are positive for MTBC should have growth-based susceptibility testing to first-line TB drugs.
- **Indeterminate:** the test could not accurately determine if the bacteria are resistant to RIF. Growth-based susceptibility testing to first-line TB drugs should be performed.

LIMITATION OF NUCLEIC ACID AMPLIFICATION (GENEXPERT) SYSTEM

- A negative NAA test result does not exclude the possibility of a positive culture.
- A positive NAA test result does not differentiate between the species of MTBC or determine the presence of other Mycobacteria species.
- Isolates are required for all positive MTB patients to perform culture-based drug susceptibility testing and genotyping.

OBSERVATION AND RESULTS

The mean age of the patients was 38.56 ± 19.045 years. About 25 patients (62.5%) were males while the rest were female patients i.e 15 (37.5%). The common presenting complaints included cough with sputum 40 (100%), fever 36 (90%),wt loss 22 (55%) and hemoptysis 10 (25%).

Radiological features compatible with the diagnosis of tuberculosis included cavity (32.3%), consolidation (34.4%), nodulostriate opacities (20.4%), miliary shadows (2.2%) and others (10.8%).

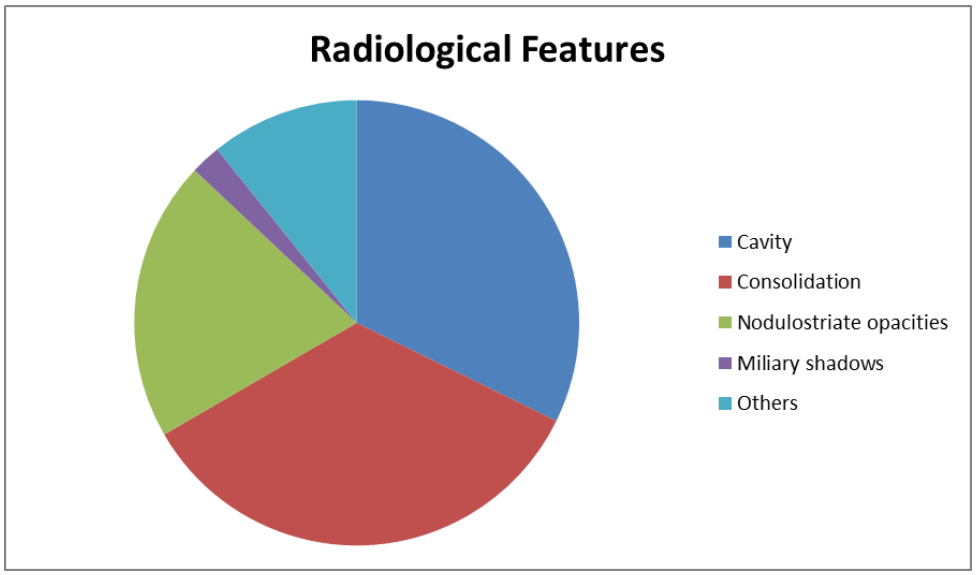


Table I: Age wise Distribution.

Age	No of peoples (%)
20-30	9 (22%)
30-40	13 (32.5%)
>40	18 (45%)

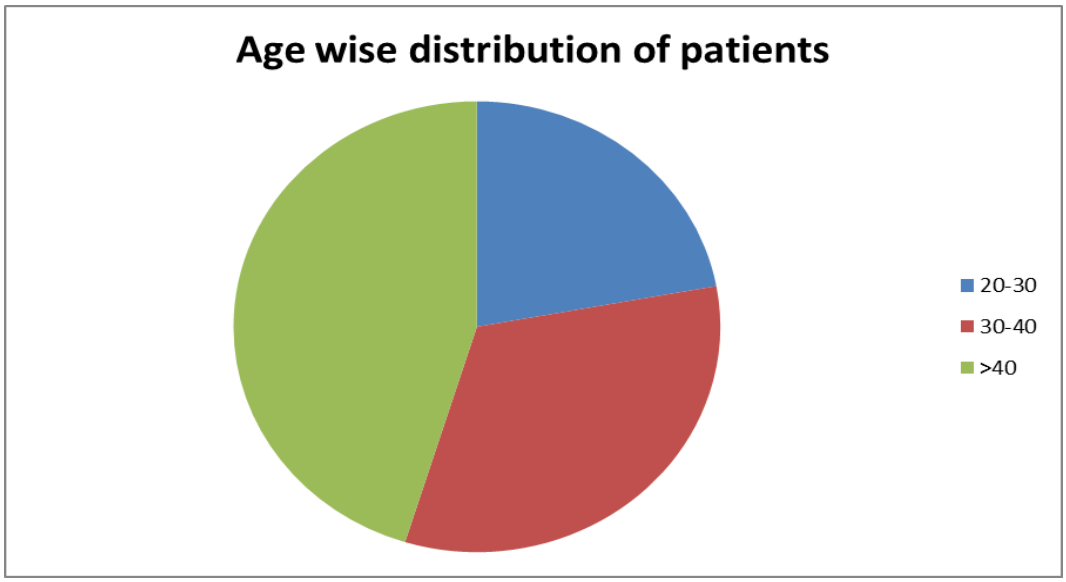


Table II: Gender Distribution.

Sex	No of people (%)
Male	25 (62.5%)
Female	15 (37.5%)

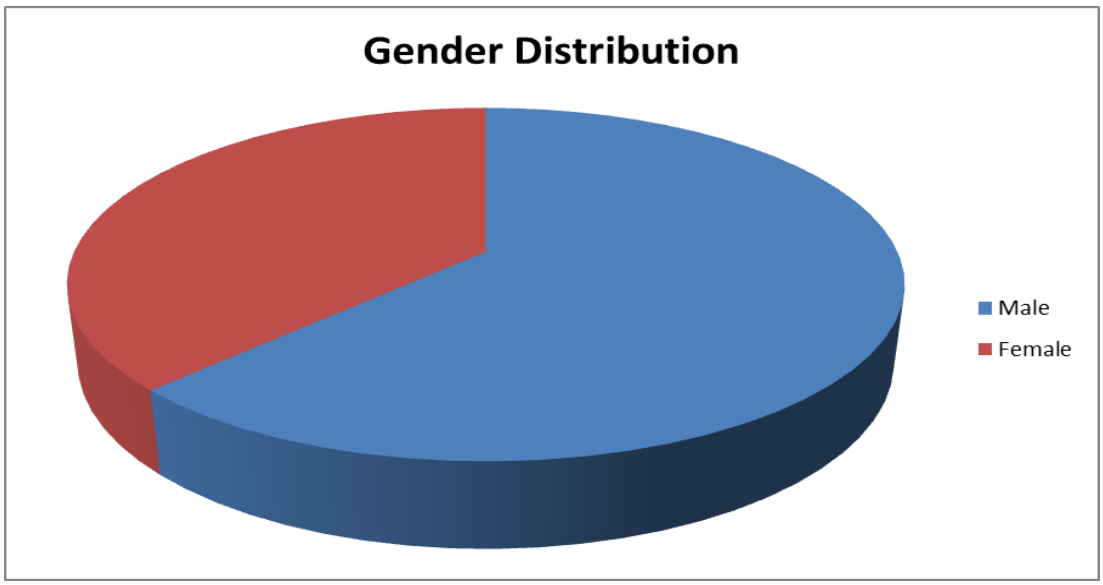


Table III: Chief complaints.

Symptoms	No of people (%)
Cough	40(100%)
Sputum	40(100%)
Fever	36(90%)
Hemoptysis	10(25%)
Weight loss	22(55%)
Dyspnea	08(20%)
Chest pain	10(25%)

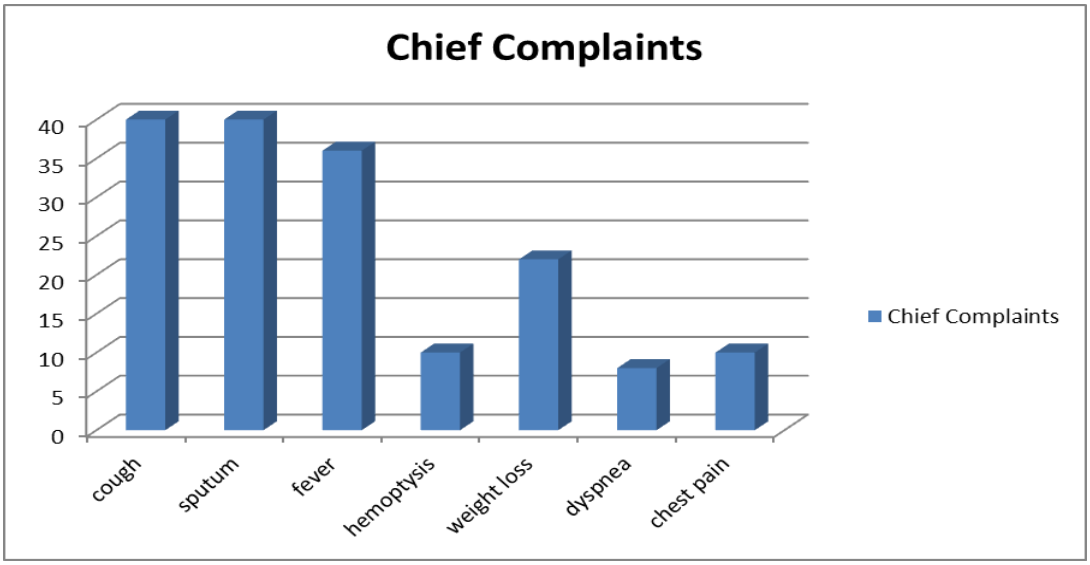


Table 4: Symptoms Duration.

Duration	Number of patients	Percentage
< 4 weeks	**18**	**45%**
4 – 6 weeks	**14**	**35%**
> 6 weeks	**8**	**20%**

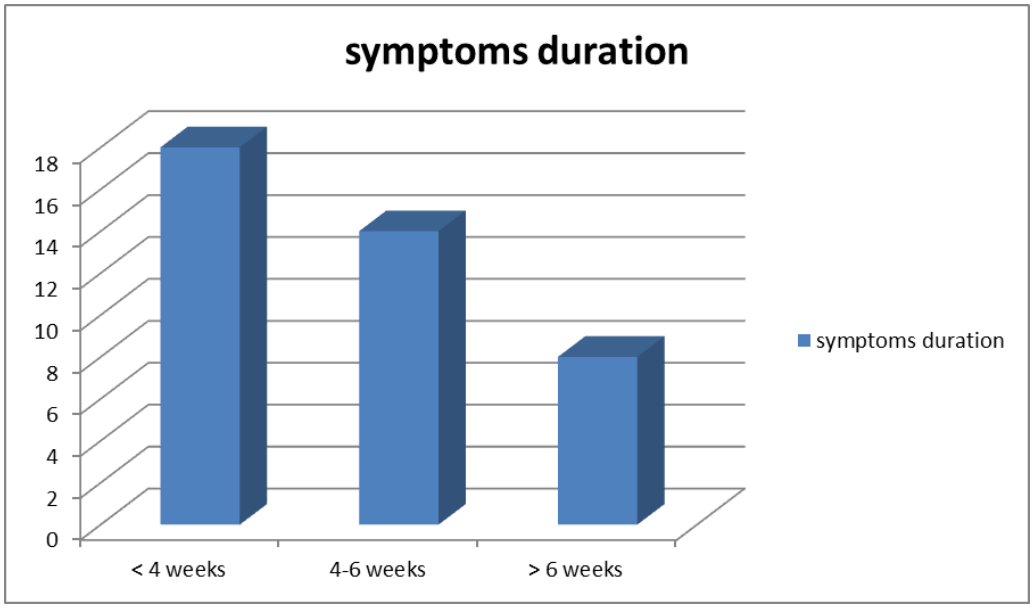


Table IV: Past History.

History	No. of People (%)
Previously Treated	08 (20%)
Never Treated	32 (80%)

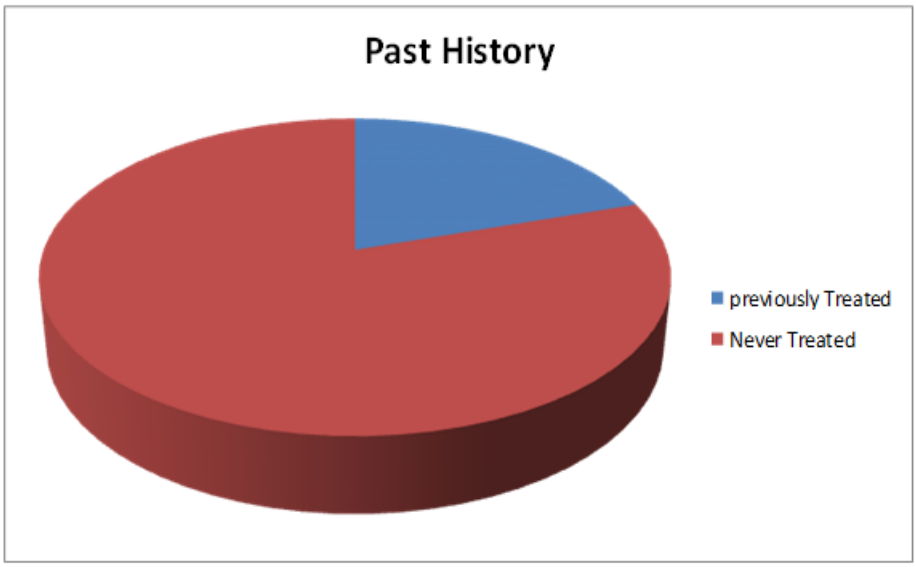


Table V: Vaccination Status.

BCG Vaccination	No. of People (%)
Vaccinated	30 (65%)
Non - Vaccinated	10 (35%)

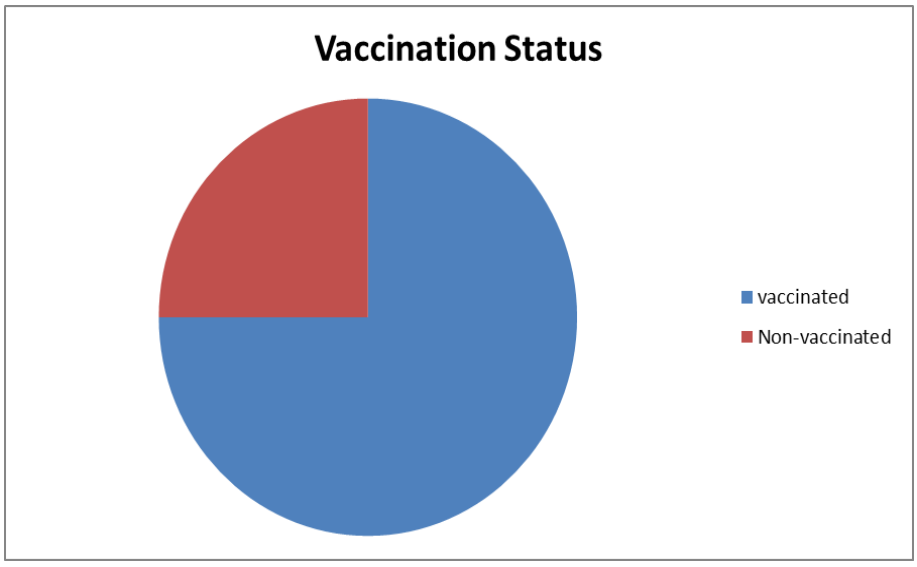


Table VI: Contact History.

H/O Contact	No. of People (%)
Present	14 (35 %)
Absent	26 (65 %)

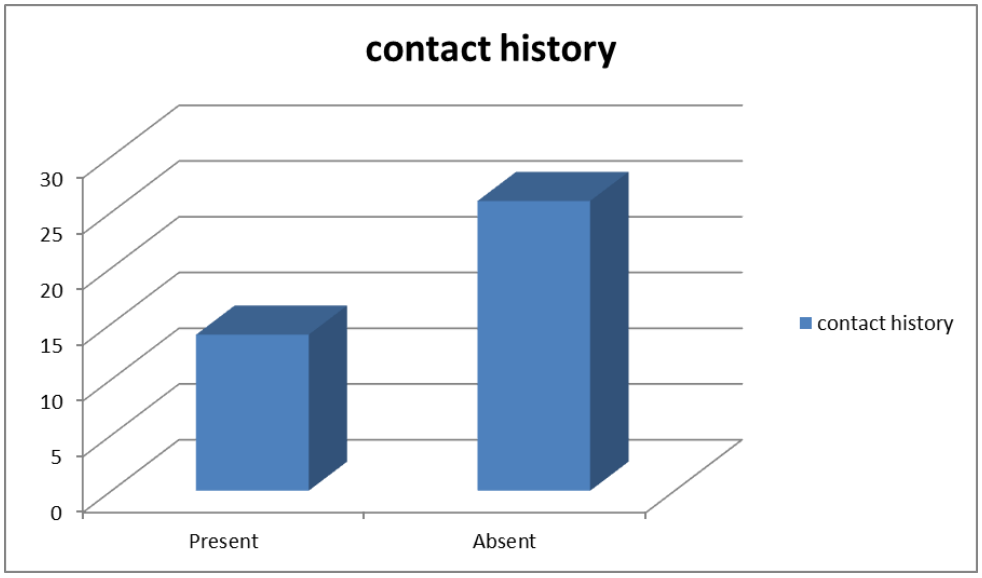


Table VII: Occupational History.

Occupation	No. of People (%)
Labour men	17 (42.5%)
Factory worker	08 (20%)
House wife	15 (37.5%)

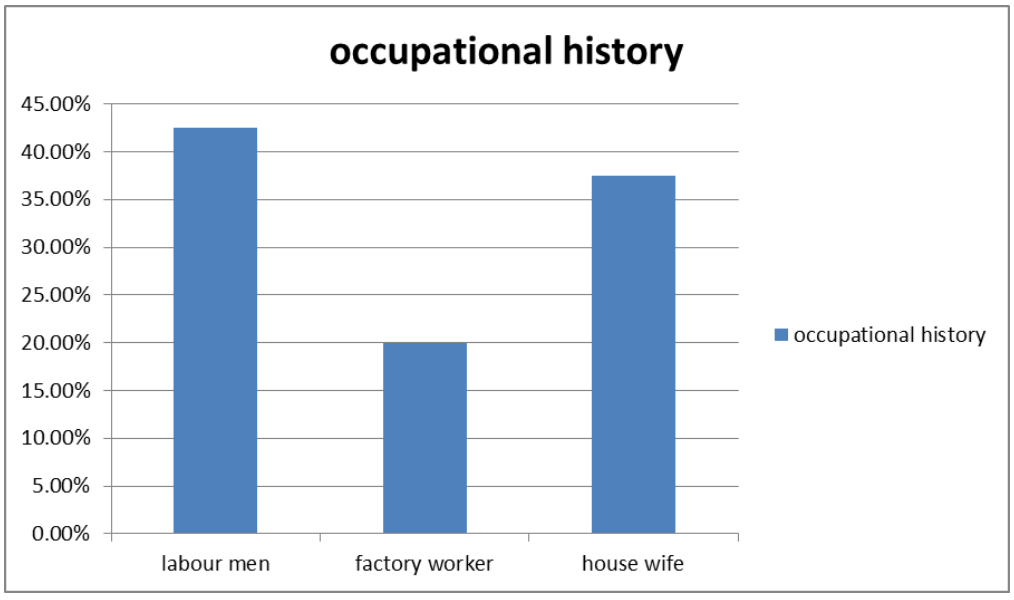


Table VIII: BAL Positivity by Gene Xpert.

BAL (Gene Xpert)	No. of People (%)
Positive	27 (67.5%)
Negative	13 (32.5%)

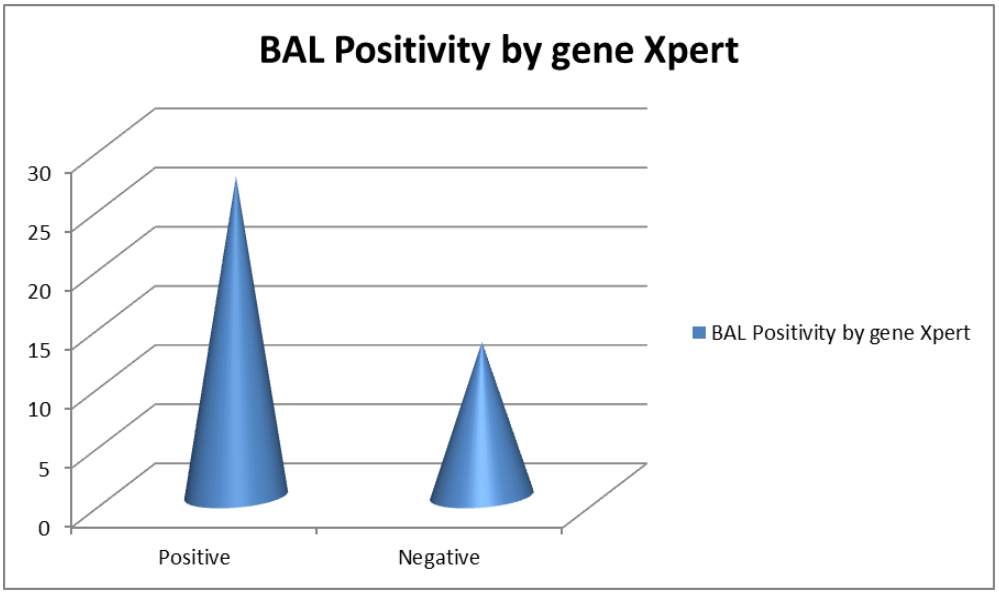


Table X: BAL Positivity by Direct Smear

BAL (Direct smear)	No. of People (%)
Positive	13 (32.5%)
Negative	27 (67.5%)

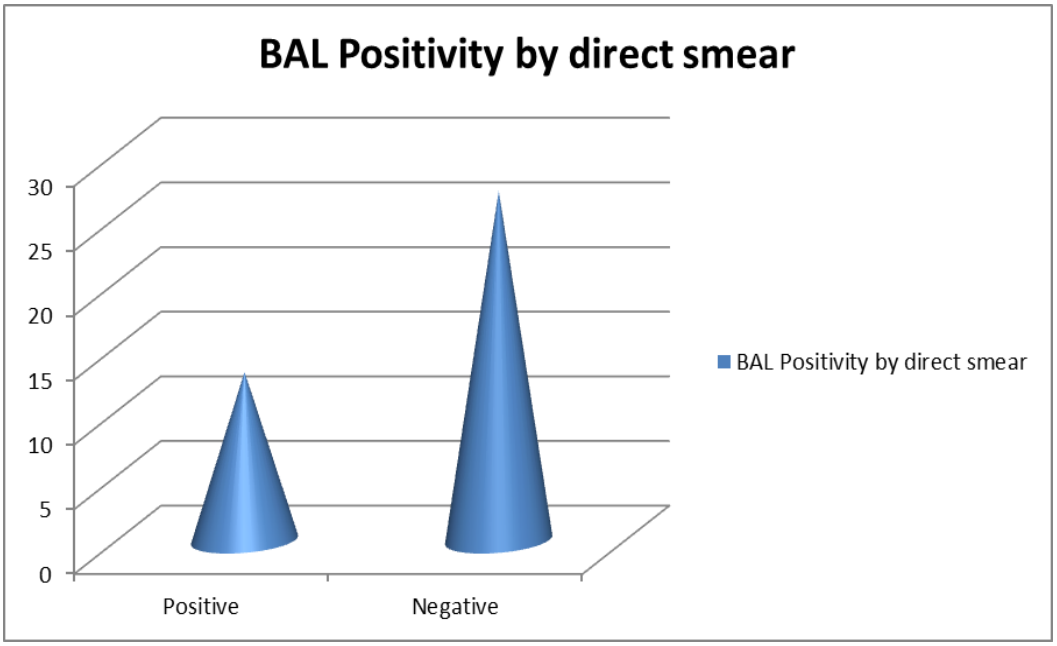
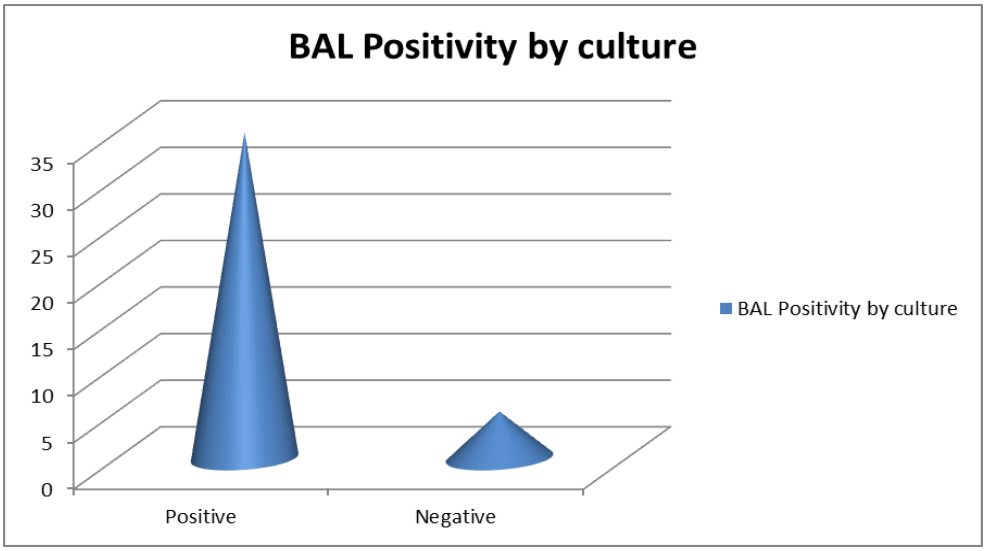


Table Xl: BAL Positivity by Culture.

BAL (Culture)	No. of People (%)
Positive	35 (88.8%)
Negative	05 (12.5%)



DISCUSSION

In this study, the diagnostic yield of BAL gene Xpert (CB NAAT) to detect MTB and rifampicin resistance in smear-negative pulmonary TB was evaluated and compared it with that of mycobacterial cultures which were taken as the gold standard.

Mycobacterial cultures for detection of Mycobacterium tuberculosis use either solid (Lowenstein Jensen media) or liquid broth system (MGIT 960). LJ medium is highly specific but is expensive, laborious, requires trained personnel, not widely available and takes 6 - 8 weeks to give the results. Though results by MGIT 960 medium come earlier as compared to LJ medium, delay in diagnosis in smear-negative especially drug resistant strains can have serious consequences for the patient as well as the community. The Xpert MTB/RIF assay is, however, a simple assay that can be performed with minimal training. The results are available within a couple of hours. At present, costs for the Gene Xpert system are similar to those required to set up an automated liquid culture system for tuberculosis but as this test is offered at WHO centres for TB control so they are available free of charge to the patient. Although it is routinely performed for identification of pulmonary tuberculosis by using frozen sputum or BAL specimens, research has shown that it may be a valuable aid in identification of mycobacteria in other body fluids like Cerebrospinal Fluid (CSF), pleural and ascetic fluid and will have wider applicability in future.

Numerous studies have demonstrated the utility of Xpert MTB/RIF assay in diagnosis of pulmonary tuberculosis. In a multicentre implementation study by Boehme and colleagues, MTB/RIF test sensitivity was 76.9% in smear-negative, culture-positive patients with 99.0%specificity while its sensitivity for rifampicin resistance was 94·4% and specificity was 98.3%. The present study showed a sensitivity of 91.86% but a specificity of 71.42% that is lower than in the international studies. However, the sensitivity and specificity of MTB/RIF assay to detect rifampicin resistance in our study was 83.33% and 100% which is higher than the international studies.

Table Xlll: Comparison of Present study with various other Studies.

Study	Sensitivity	Specificity	PPV	NPV
Present Study	**91.86**	**71.42**	97.53	41.66
Boehme et al	76.9	99	-	-
Theron G et al	93	77-98	-	-
Boehme CC et al (2010)	72.5	99.2	-	-
Lee HY et al	81.6	100	100	92.1
Kwak N et al	79.5	100	100	94
Palud P L et al	60	100	-	-
Kirwan DE et al	72.5-98.2	99.2	-	-
Steingart et al	67	99	-	-
Marlowe EM et al	72	98	-	-
Armand S et al	79	84	-	-
Moure R et al	75.3	100	-	-
Teo J et al	90.9	89	-	-
Saglam, L et al	86	57	**99**	**22**
Tortoli E et al	81.3	99.8	-	-
Zeka AN et al	68.6	100	-	-

Table XlV: Rifampicin Resistance.

Study	Sensitivity	Specificity
Present study	83.33	100
Boehme et al(2013)	94.4	98.3
Boehme CC et al(2010)	97.6	98.1
Kwak N et al	57.1	100

There were certain limitations of the study.
First, mycobacterial culture was performed by using the solid culture medium (LJ medium). Studies have shown the automated liquid culture medium (MGIT 960) to be superior to the LJ medium because of its higher sensitivity and shorter time for detection of mycobacteria. Further studies are required in this respect.

In this study, the yield of mycobacterial cultures on BAL specimens was higher than the international studies (91.4% vs. 55 - 88%), this may be due to a selection bias as patients who had a strong suspicion of active TB clinically and radiologically were enrolled in this study.

Secondly, the sensitivity and positive predictive value of Xpert MTB/RIF assay to detect MTB was very high (91.86% and 97.53% respectively), while the specificity and negative predictive value in this study was low (71.42% and 41.66%) as compared to the International studies (100 % and 92.1% respectively).

Possible explanations include false positive tests in the presence of a negative culture, use of solid culture medium as the gold standard or laboratory error. Well designed future studies eliminating these errors are required. The study was performed in smear-negative and sputum-scarce pulmonary tuberculosis that excluded many of the MDR-TB patients. This may be the reason for the low frequency of drug-resistant TB in this study (6.45%).

The key findings of our study are as follows:
MTB/RIF significantly outperformed smear microscopy on BALF, improved the time to diagnosis and detected ≥80% of TB cases that were smear negative.

Empirical treatment was higher with smear microscopy as the only available rapid test; and hence MTB/RIF did not significantly impact the overall proportion of patients initiating treatment. The present study highlights the possibility that the impact of MTB/RIF may be overestimated due to high rates of empirical treatment when smear microscopy is the only available rapid test.

Similar to what has previously been documented in sputum, this study found that MTB/RIF-generated quantitative information strongly correlated with markers of bacterial load in BALF, such as smear grade and liquid culture. This has implications for the use of MTB/RIF values to assess disease severity identify those at risk of treatment failure and stratify patients likely to be highly infectious.

One ml. of uncentrifuged BALF is sufficient for accurate performance of the MTB/RIF assay.

Only two studies have, to the best of our knowledge, examined the impact of MTB/RIF on time to TB diagnosis and TB treatment initiation; however, they were performed on sputum. We found MTB/RIF to provide a result in significantly more patients than smear microscopy, and within a similar time-frame (0–1 days), thereby significantly reducing the time to diagnosis in patients with smear-negative BALF.

Although MTB/RIF implementation facilitated a more rapid diagnosis of TB, it did not result in detectable improvements in the time to TB treatment initiation. This is because our study was underpowered to detect small differences in this outcome.(sample size calculations suggest that we had 77% power to detect a 20% difference in the proportion initiating treatment between study phases and only a 30% power to detect a difference of 3 days in the time to treatment initiation.)

This arose due to MTB/RIF becoming the standard care at the study site following endorsement of the test by WHO which resulted in us being ethically obligated use MTB/RIF for patient management. It appears that the potential impact of MTB/RIF on improving time to treatment initiation was mitigated by high rates of rapidly initiated empirical anti-TB treatment in the absence of a positive microbiological result.

A study assessing the impact of sputum-based MTB/RIF among hospitalised Ugandan inpatients has reported a similar phenomenon. These findings underscore the importance of assessing the impact of diagnostic tests in a routine environment, where clinical practice has evolved to compensate for the poor performance of smear microscopy. Large multi-centre studies are required to definitively assess this question.

This study has limitations. Although its use is increasing, bronchoscopy is not yet widely available in resource-poor settings and is usually only at a tertiary level where the burden of HIV (and smear-negative or sputum-scarce TB) is highest. Our data will also have relevance to low-burden settings where there are large immigrant populations, and where bronchoscopy is widely available and used. We did not examine the utility of MTB/RIF on induced sputum, which may reduce the need for MTB/RIF testing on BALF from patients with suspected TB. Additionally, for the secondary analysis, not all patients had test results for each of the two biological specimens examined, and this may have introduced

sampling bias. We may have used a suboptimal volume of BALF for centrifugation; however, we were limited by available volumes after specimens were sent for routine laboratory testing. The use of frozen specimens from some patients may have impacted MTB/RIF accuracy, though published data suggest that the effect, if any, is likely to be minimal.

In summary, MTB/RIF on the BALF of patients who are smear negative or sputum scarce has shown excellent performance for the detection of TB. Although in patients with smear-negative BALF the time to TB result significantly improved, this did not translate into a higher proportion of patients initiating treatment. However, it did reduce the proportion of patients initiating treatment on empirical grounds. The high baseline levels of empirical treatment in our setting may undermine the potential impact of MTB/RIF, but this requires prospective investigation.

It becomes difficult to establish bacteriological diagnosis in patients who are suspected of having pulmonary tuberculosis when they are unable to expectorate. So, we compared the yield of GeneXpert MTB/RIF (CB NAAT) on the BAL and GA samples. Our study showed difference in yield of the BAL and GL was not statically significant (48.6% vs. 38.9%) ($p > 0.05$). Combine positive results of BAL and/or GL sample were 51.4% in TB suspect cases.

Another noteworthy factor was the absence of complications due to GL during our study. Notably, it can be compared to major adverse events of bronchoscopy in a study by Dang et al. in which it was stated that this technique is safe as they came across less major complications like pneumothorax.[9] However, the aim of this study was not to evaluate the efficacy of BAL procedure, there were no major complication of bronchoscopy except desaturation, respiratory discomfort during and throat discomfort after the procedure. In the study, Tuberculosis culture was not done to compare with the outcome of Genexpert, because of resource limitations of the hospital and most patients who present to our (government) setup are poor and cannot pay the cost of the test in private labs.

And lastly, our purpose of the study was to evaluate the patient population who are out of reach of culture but have access to GeneXpert facility provided by WHO, which is free of cost.

The updated Cochrane Review on the diagnostic accuracy of Xpert MTB/RIF for TB detection and rifampicin resistance detection in adults summarizes the current literature and integrates nine new studies (33% of the included papers) identified since the original Cochrane Review (Steingart 2013). The findings in this update are consistent with those reported previously.

This study found Xpert MTB/RIF sensitivity for TB detection to be higher in fresh specimens than in frozen specimens. Although we did not find conclusive evidence, one possible explanation for this observation is that investigators may have used an increased amount of buffer volume to resuspend frozen sputum specimens causing a dilution effect. We also found that, in comparison with processed specimens, unprocessed specimens had slightly higher sensitivity in smear-negative patients. In addition, we found higher pooled accuracy of Xpert MTB/RIF in studies performed in high-income countries than in low- or middle-income countries. However, after adjustment for smear status, the strength of these associations decreased.

Therefore, there was no conclusive evidence supporting the impact of either specimen preparation or country income on Xpert MTB/RIF sensitivity for TB detection.

We acknowledge that patient outcomes are clearly important to patients, decision makers, and the wider TB community. Outcomes in addition to diagnostic accuracy, however, could not be systematically addressed, as they would have required a different methodology. Nonetheless, we looked for and summarized two 'time to event' outcomes (time to result and time to treatment initiation) when data were provided in the included studies. Xpert MTB/RIF results for TB detection were usually reported within two hours or on the same day, compared with liquid culture results reported in around 16 to 20 days. Studies reporting on time to detection of rifampicin resistance found that, compared with conventional methods, Xpert MTB/RIF greatly decreased the time to diagnosis.

However, early detection of rifampicin resistance may not lead to improved patient outcomes if the result is not linked to appropriate treatment, services, and supervision. Two studies provided information about time to treatment initiation. In Boehme 2011, for smear-negative, culture-positive TB, the median delay in beginning treatment was 56 days (IQR 39 to 81) before Xpert MTB/RIF was introduced, compared with five days (IQR 2 to 8) after Xpert MTB/RIF was introduced. In Van Rie 2013, for smear-negative, culture-positive TB patients with Xpert MTB/RIF positive results, treatment was begun on the same day compared with 13 days for patients diagnosed by other methods.

A recent analysis of cost and affordability found that, globally, the use of Xpert MTB/RIF to diagnose MDR-TB would cost less (US$70 to 90 million per year) than what it would cost to use a combination of conventional diagnostics (US$123 to 191 million per year). Conventional diagnostics may include smear microscopy, chest radiography, culture, and culture-based DST following WHO-recommended algorithms. However, for almost all countries, the deployment of Xpert MTB/RIF to diagnose TB in all individuals with signs and symptoms of TB would cost more than the use of conventional diagnostics (which may include smear

microscopy and follow-up chest radiography for those with smear-negative results.

Xpert MTB/RIF has now begun to be rolled out in over 20 countries via UNITAID, with a price drop from $16.86 to $9.98 (US) per cartridge, a price that will remain in effect until 2022 (The Gates Foundation 2012; UNITAID 2012). UNITAID is a global health initiative working to increase access for tests and medicines for HIV/AIDS, TB, and malaria. Since WHO endorsed the use of Xpert MTB/RIF, country-level policy makers have been making decisions about adoption and scale-up. The uptake has been much faster than for any other TB technology recommended by WHO over the last 10 years.

This study represents the most comprehensive review on the diagnostic accuracy of Xpert MTB/RIF and provides evidence that may help countries make decisions about scaling up Xpert MTB/RIF for programmatic management of TB and drug-resistant TB. Although the information in this review will help to inform, other factors such as level of deployment in the health system, cost, and operational considerations (including the ability to maintain an uninterrupted and stable electrical power supply, temperature control, and maintenance of the cartridge modules) will also influence those decisions, as discussed in recent publications.

There have been studies from India with large sample size that have evaluated the performance of Xpert MTB/RIF in patients with extrapulmonary TB, however, this is the first Indian study to have studied the performance of Xpert MTB/RIF assay with respect to solid medium culture as the gold standard in patients with PTB. The study results clearly show that Xpert MTB/RIF has high sensitivity of 95.7% and specificity of 99.3% for detecting MTB in pulmonary samples of patients with PTB. For detecting smear negative-culture positive cases, our study results show a sensitivity of 77.7% and specificity 99.3% respectively. This is an important finding especially in a high TB burden country like India as this test will help in rapid diagnosis of smear-negative TB cases which were earlier a challenge for the TB control programmes.

The results of our study are comparable to those a recent meta-analysis which reported the pooled sensitivity of Xpert in smear positive-culture positive PTB as 98%, and a sensitivity was 67% and specificity 99% for smear negative TB. In our study, the sensitivity of Xpert to detect RIF resistance in pulmonary samples positive for PTB was 94.5% and the specificity for excluding RIF resistance was 97.7%. These results are consistent with previously reported data for RIF resistance.

In the present study a total of 1 specimens gave ‘error’ as a result by Xpert MTB/RIF corresponding to an error percentage of 2.5% (1/40). The MTB/RIF assay for rifampicin resistance was indeterminate in 2.5% (1/40) cases. The error rate in the present study was quite less as compared to the error rates >5% which were obseved in the earlier versions of Xpert MTB/RIF cartridges. G4 cartridges were modified in terms of the modified Ct values for the probes used in the assay, modification of the probe sequence and fluidics of the assay to decrease the error rates. Further, post hoc-analysis of the study data showed that Xpert MTB/RIF was able to detect 14 out of 40 cases where smear was negative, of which three were RIF resistant by Xpert MTB/RIF. Given the high specificity of Xpert MTB/RIF (99%),these cases are less likely to be false-positive. This is a significant finding from our study, particularly in the setting of a national TB control program. In the absence of Xpert MTB/RIF such patients are likely to undergo repeat testing for sputum smear microscopy and culture resulting in an unnecessary delay in initiation of treatment.

In the present study, DST was done on solid culture which adds to its merits as previously published literature has shown that automated BACTEC MGIT 960 can miss out on strains with certain resistance conferring *rpoB* mutations that can only be detected by DST on LJ medium. Therefore, comparison of Xpert MTB/RIF with MGIT is likely to decrease specificity of Xpert MTB/RIF. Further, all pulmonary samples in this study were used directly for performing Xpert MTB/RIF without processing, and no frozen samples were included in the study.

Line probe assay (LPA), another molecular diagnostic test for TB, has shown to have sensitivity greater than 95% and a specificity of 100%. However, LPA is recommended only in smear positive cases, while Xpert MTB/RIF in our study showed a sensitivity of 77.7% for detecting TB in smear negative samples. Hence, Xpert MTB/RIF provides a significant diagnostic edge in smear negative cases, as treatment can be started immediately without waiting for culture results.

While Xpert MTB/RIF may be the foremost choice amongst all molecular diagnostic tests, it has its own limitations. Resistance to RIF is taken as a surrogate marker for MDR-TB, but certain strains may exhibit only mono-resistance to RIF that may not warrant full line MDR therapy, thus, leading to over-estimation of the MDR-TB cases.

Likewise, a study from Mumbai, India demonstrated how specimens with rifampicin results reported as sensitive by GeneXpert could be resistant to isoniazid. Other drawbacks of Xpert MTB/RIF are requirement of stable electrical power supply, temperature control and annual calibration of instrument.

Regardless of all these limitations, addition of Xpert MTB/RIF assay to the present set of diagnostic modalities for TB on account of its unambiguous, rapid results, and high sensitivity and specificity will facilitate early diagnosis.

However, this study fulfills the objective which was to evaluate the diagnostic utitlity of Xpert MTB/RIF assay in PTB samples using culture as the reference standard.

SUMMARY AND CONCLUSION

- Bronchoalveolar lavage gene Xpert had a superior diagnostic yield (with high sensitivity and positive predictive value) to detect Mycobacterium tuberculosis and rifampicin resistance (with high sensitivity and specificity), in those cases of pulmonary tuberculosis who have either smear-negative or sputum-scarce disease.
- This test has the advantages of being inexpensive, requires less manpower and gives results on the same day.
- Hence, a positive XpertMTB/RIF assay may be a useful adjunct to diagnosis Our study highlights that XpertMTB/RIF has high sensitivity and specificity for diagnosis of both smear positive and smear negative PTB cases with high rates of detection of RIF resistance and greater concordance with gene sequencing for RIF resistance when compared with culture.
- Our findings are like those reported by studies previously done in other countries. In resource-limited settings and less accessible areas where establishing a sophisticated laboratory for culture and DST conforming to the prescribed biosafety levels is difficult, XpertMTB/RIF provides a viable option.
- Widespread application of this assay can increase the case detection rates of both drug sensitive and MDR-TB, thereby facilitating early treatment decisions and curbing transmission.

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