

Comparison of GeneXpert MTB/RIF Assay in Broncho Alveolar Lavage and Gastric Lavage Samples

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Abstract

Objective: To compare the yield from Gastric lavage (GL) and Broncho alveolar lavage (BAL) samples in adult patients suspected case of Tuberculosis but not producing sputum. **Methodology:** 80 adults with suspected case of tuberculosis but not producing sputum were recruited. 72 patients were then subjected to one gastric lavage followed by Broncho-alveolar lavage in the same morning. The collected samples were subjected to GeneXpert MTB/RIF assay. **Result:** Of the 72 patients samples, the mean age was 38.6 years. 41 (56.9%) were male and 31 (43.1%) were female. History of TB contact was present in 25 (34.7%) patients. 37 (51.4%) patients had GeneXpert MTB/RIF positive on BAL and/or GL samples. The GeneXpert MTB/RIF of BAL fluid was positive on 35 (48.6%), which was not significantly greater than that for specimens from GL, which was 28 (38.9%) ($p > 0.05$). In 26 (36.1%) cases, GeneXpert MTB/RIF was positive in both BAL and GL samples. **Conclusion:** This study showed the yield of GeneXpert MTB/RIF in GL was comparable to BAL to detect *Mycobacterium tuberculosis* complex. Patients who can't produce sputum, GL can be a good alternative to BAL to detect *Mycobacterium tuberculosis* complex in resource poor areas and patients who do not tolerate Bronchoscopy.

Keywords

Mycobacterium tuberculosis Complex, Rifampicin Resistance, Bronchoscopy, TB Suspects

1. 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 2014, approximately 9.0 million people developed TB in 2013 amongst which 1.5 million died from it [1]. It ranks as the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV). Diagnosis of pulmonary TB in individuals is through clinical presentation, sputum test and bacteriological confirmation in laboratories. These diagnostic tests range from traditional smear microscopy, culture to newer rapid tests like GeneXpert MTB/RIF and line probe assay.

The GeneXpert MTB/RIF is an automated cartridge based nucleic acid amplification test that detects *Mycobacterium 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 non-tuberculous 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 [3]. 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 [1].

In December 2010, GeneXpert MTB/RIF assay was endorsed by WHO and then recommended for detection of *Mycobacterium tuberculosis* complex and rifampicin resistance. Overall, the sputum GeneXpert MTB/RIF assay has similar sensitivity, specificity and accuracy as culture on solid media for Mycobacteria [4].

In everyday clinical practice, we often encounter patients suspected of pulmonary tuberculosis but they do not produce sputum. One study showed 50% of patients with suspected active TB are either unable to produce sputum or demonstrate a negative sputum smear for acid fast bacillus (AFB) [5]. In such situations sampling through BAL, induced sputum (IS) and GL are alternatives. Where available, bronchoscopy and BAL can be performed to obtain samples. However, it requires special facilities, trained staff and is not accessible in limited-resource settings. Additionally, due to its invasive nature many patients may not prefer and tolerate it [6]. Although yield of induced sputum has favorable results, its use requires isolation rooms with negative pressure, which may not be available in many health care settings [6] [7]. The GL method is preferred in diagnosis of TB in children who swallow their sputum and cannot expectorate. Many studies have shown good result of GL for AFB smear and culture [6] [7]. In the centers where WHO has provided GeneXpert machine is available, it is possible to make bacteriological diagnosis within 2 hours. As the prevalence of MDR TB is increasing, this test also helps to detect Rifampicin resistance in suspected cases and guide further management.

Therefore, in order to confirm the clinical and radiological findings in patients with suspected active TB who are not able to produce sputum samples, GL and BAL were performed to obtain specimens for bacteriologic confirmation. In this study, comparison of the yield of GL and BAL in terms of GeneXpert MTB/RIF result was made.

2. Methods

A prospective study was conducted in the Department of Chest Medicine of Jinnah Postgraduate Medical Centre, Karachi, Pakistan. The institutional ethics committee approved the study, and written informed consent was taken from each participant. Patients were recruited from outpatient clinics of tertiary care center from March 2014 to December 2014.

The patients were not producing sputum included in the study if they met following inclusion criteria:

- TB Suspects, who had respiratory and/or constitutional symptoms for more than 2 weeks as defined by WHO.
- Chest radiography suggestive of active TB.
- Patients more than 15 years of age of both gender.
- No previous history of tuberculosis.

Patients were excluded from the study if they had:

- Previous history of tuberculosis or anti-tubercular therapy.
- History of structural lung disease like COPD, bronchiectasis, diffuse parenchymal lung disease.
- Patients who are too breathless or hypoxic to undergo the procedure.

GL: After explaining details regarding the procedure, patients were admitted one day prior to the test. After overnight fast, Gastric lavage (GL) specimens were obtained in the morning. This technique involves inserting a

appropriate size nasogastric tube into the stomach of the patient, rinsing the stomach with ~50 mL of sterile normal saline, and aspirating the stomach contents. The procedure yielded volumes of 20 - 40 ml GL specimens. The sample was collected in a sterile syringe.

BAL. Fiber-optic bronchoscopy was performed on fasting patients after gastric lavage in the same morning. Local anesthesia and sedation were administered. After the bronchial tree was inspected, 60 - 100 mL of normal saline was instilled and aspirated from lung segments involved by the disease process, as indicated by a chest radiograph. Approximately, 20 - 30 mL of BAL fluid was obtained. BAL and GL samples were transported within 20 - 30 min to the GeneXpert laboratory. Patients were discharged once they were stable enough after the procedures, and were advised to follow up in OPD with reports.

Data on age, sex, history of TB contact and report of GeneXpert MTB/RIF for both specimens was collected on a data entry form. Data analysis was performed in SPSS window version 19 software.

3. Results

Total 80 cases of suspected pulmonary tuberculosis not producing sputum were recruited for the study. 5 patients declined to undergo bronchoscopy, one refused GL later on and two patients did not tolerate bronchoscopy. Complete specimens were obtained from 72 patients. Out of these 41 (56.9%) were male, and 31 (43.1%) were female (**Figure 1**). The Mean age was 38.6 ± 18.3 (16 to 72 years) and 25 (34.7%) had TB contact history (**Table 1**).

Total 37 (51.4%) patients had GeneXpert MTB/RIF positive on either BAL and/or GL samples. In BAL fluid sample, MTB complex was detected by GeneXpert MTB/RIF in 35 (48.6%) patients and negative in 37(51.4%). In GL fluid samples MTB complex was detected in 28 patients (38.9%) and negative in 44 (62.1%) ($p > 0.05$) (**Table 2**). In 26 (36.1%) patients, both BAL and GL samples were positive (**Figure 2**). In 2 (2.7%) patients, only GL was positive and BAL was negative. Primary rifampin resistance was detected in 3 (4.1%) patients and they were subjected for further evaluation of drug resistance tuberculosis.

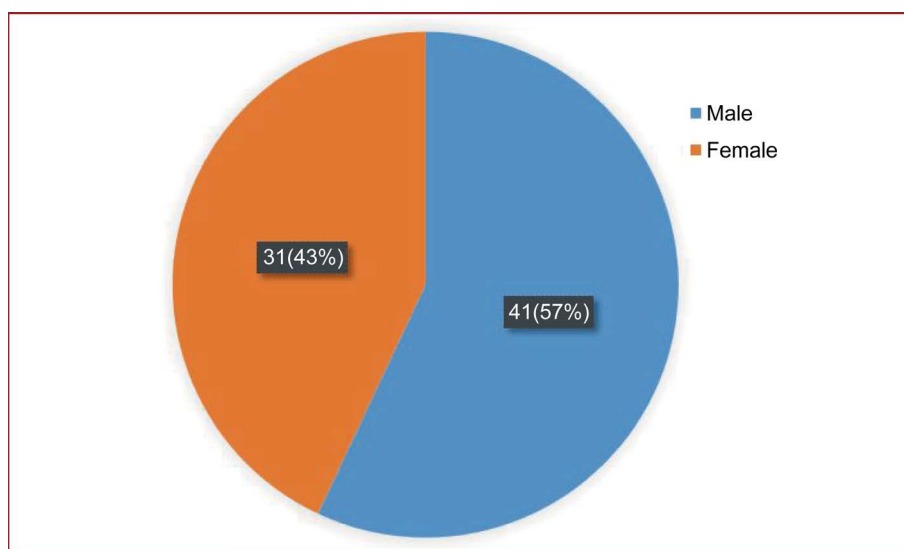


Figure 1. Gender distribution.

Table 1. Baseline characteristics of the patients.

Gender	
Male-No. (%)	41 (56.9)
Female-No. (%)	31 (43.1)
Age (mean)*	38.6 ± 18.3 years
TB contact history-No. (%)	25 (34.7)

*Plus-minus values are means \pm SD.

Table 2. Result of GeneXpert in BAL and GL samples.

	Positive n (%)	Negative n (%)
BAL	35 (48.6%)	37 (51.4%)
GL	28 (38.9%)	44 (61.1%)
Total positive cases (BAL and/or GL)	37 (51.4%)	35 (48.6%)

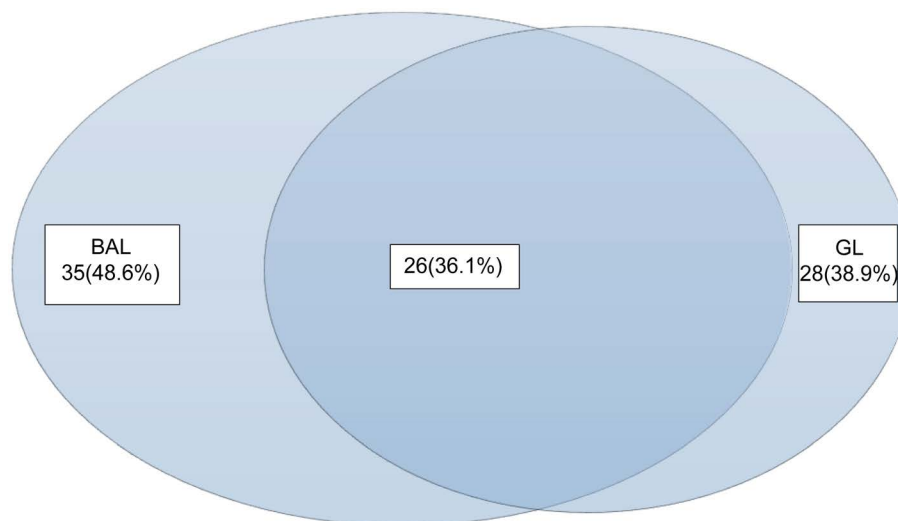


Figure 2. Number of positive GeneXpert MTB/RIF on gastric Lavage (GL) and Broncho alveolar lavage (BAL) specimens.

4. Discussion

Results in terms of AFB smear and culture were compared with several other studies on BAL, IS and GL. No direct comparative study on these sampling methods were found to be done on GeneXpert MTB/RIF on adults. 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 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. In a study by Singh *et al.* there was no difference in mycobacterial isolation rates from gastric lavage and BAL when studied in isolation [8]. However, when both GL and BAL were used, these procedures complement each other to increase the diagnostic yield [8] [9].

Most of the studies on GL were done in children. A recently published study by Pang *et al.*, showed 48.6% sensitivity of GL samples GeneXpert MTB/RIF assay in detecting TB in smear negative children [10]. Previous studies of the GeneXpert that assessed either sputum samples or concentrated decontaminated sputum pellets, have shown test sensitivity of 72% - 75% in cases of smear-negative tuberculosis and 98% - 100% in cases of smear-positive tuberculosis and specificity 99% [11]-[15]. Among patients with smear-negative, culture-positive tuberculosis, the addition of a second GeneXpert MTB/RIF test increased sensitivity by 12.6% and a third by 5.1%, to a total of 90.2% [16]. Similar study was done in our center on 2001, in which 20 patients were enrolled in the study. Yield of 2 AFB smear and a culture of bronchial washing and GL were compared. Result showed bronchial wash was superior to gastric lavage in culture, but their yield on direct smear was equal [17]. In a comparative study performed by QQ Yin *et al.* in 250 Chinese children of suspected TB showed Sensitivity of AFB microscopy, MTB culture, and GeneXpert MTB/RIF assay was 8.4%, 28.9%, and 53.0%, respectively. GeneXpert MTB/RIF assay could detect 33.9% of cases with negative MTB culture, and 48.7% of cases with negative AFB microscopy [18]. In a prospective study on children by Brown *et al.* showed culture of gastric lavage had sensitivity only 30% and induced sputum 39% [6]. Some studies show yield of 3 induced sputum is superior to 3 GL while others show equal efficacy [19].

In our study 3% patient had Rifampicin resistance. If we had relied solely on sputum smear of the samples, we would have missed those cases and putting them on new treatment regimen could have led to gene amplification to MDR. 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 [20]. 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.

5. Conclusion

In resource poor areas, Gastric lavage can have invaluable role to detect and rule out pulmonary tuberculosis in suspected cases, which are unable to produce sputum. And further, GeneXpert test can be effective to make accurate diagnosis and also detects Rifampicin resistance thereby potentially decreasing morbidity and mortality associated with diagnostic delay, dropout and mistreatment.

Conflict of Interest

The authors declare that there is no conflict of interest.

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