DIAGNOSTIC ACCURACY OF GENEXPERT ASSAY AND COMPARISON WITH SMEAR AFB ON BRONCHIAL WASHINGS IN SPUTUM NEGATIVE SUSPECTED PULMONARY TUBERCULOSIS

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ABSTRACT

Objective: To determine diagnostic accuracy of GeneXpert Assay and compare it with smear microscopy for AFB in terms of sensitivity, specificity on bronchial washings/bronchoalveolar lavage samples in sputum smear negative suspected tuberculosis.

Study Design: A prospective cross sectional analytical study.

Place and Duration of Study: Pulmonology Department, Pak Emirates Military Hospital Rawalpindi, from Jul to Dec 2018.

Materials and Methods: Patients older than 12 years with suspected sputum negative tuberculosis were included in the study. Bronchoscopy findings were noted, bronchoalveolar lavage fluid/bronchial washings were taken. Samples were sent for AFB smear; GeneXpert Assay and Cultures. Data was analyzed in SPSS version 23.

Results: A total of 224 patients were included in the study. 151 (67.4%) were males and 73 (32.6%) females. Mean age was 48.25 ± SD 18.21. Flexible bronchoscopy was diagnostic for tuberculosis in overall 42.4% (95/224) cases. Smear microscopy for AFB was positive in 53 (23.7%) cases, GeneXpert in 82 (36.6%) cases, culture in 66 (29%) cases while 6 cases (2.7%) showed rifampicin resistance on GeneXpert RIF resistance assay. Sensitivity, specificity, PPV and NPV of smear microscopy were 69.6%, 95.5%, 86.7%, 88.3% respectively whereas for GeneXpert they were 86.36%, 84.1%, 69.5%. 93.66% respectively; when compared to culture considered as gold standard. GeneXpert assay activity had statistically significant (p-value<0.01) association with smear and culture positivity.

Conclusion: GeneXpert assay had higher sensitivity than AFB smear microscopy and it is a rapid and effective tool on bronchial washings, reducing the time constraint associated with culture based diagnosis and determining Rifampin Resistance simultaneously.

Keywords: Bronchoalveolar lavage fluid/microbiology, Bronchoscopy, Tuberculosis.

INTRODUCTION

Tuberculosis, once a death statement known as the Captain of all these men of death[1] is now treatable. However, it still remains the leading cause of death by single infectious agent resulting in estimated 1.3 million deaths in HIV-negative and 300,000 deaths in HIV positive individuals annually[2]. An estimated 10 million individuals developed tuberculosis in year 2017, among these only 6.4 million cases were notified, the rest are suspected to be either under reported or undiagnosed. Some 23% of total world population estimated 1.7 billion have latent TB and 5-10% of these individuals are at risk of developing active tuberculosis in their life time. In Pakistan, an estimated 525,000 developed TB in 2017 out of which 165,776 were either not notified or remained undiagnosed. TB treatment coverage in Pakistan remains 68% with target of achieving 90% coverage at end of 2025 as part of End TB Strategy[2]. Globally, Pakistan also has the fourth highest prevalence of MDR-TB. Late diagnosis delays treatment increases risk of transmission and results in poor health outcomes.

Some one-third of tuberculosis cases are sputum negative or scarce[3,4]. These cases are infectious[4] and early diagnosis and treatment initiation requires good quality specimen. From the introduction of flexible bronchoscopy in 1968

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by Ikeda, the use of flexible bronchoscopy has grown exponentially and revolutionized respiratory medicine[5]. Flexible bronchoscopy presents as an effective tool in diagnosis of sputum scarce and sputum negative patients. Various techniques include bronchoalveolar lavage, bronchial washings, various biopsy techniques such as biopsies of endobronchial TB cases, TBLB and TBNA in cases of lymphadenopathy[3]. The diagnosis can be done by smear AFB microscopy, NAAT and cultures. The combination of these increase diagnostic yield in tuberculosis. Xpert® MTB/RIF assay (Cepheid, USA) can be performed on the specimen yielded by various bronchoscopy techniques. It is the only rapid molecular test for diagnosis of MTB which has been recommended by WHO since 2010[6], and it is also the only fully automated cartridge-based real-time DNA-based test that can detect both TB and resistance to rifampicin. The results can be obtained as early as 2 hours which is in sharp contrast to 6-8 weeks required for culture based diagnosis which is considered "gold standard"[6].

Flexible bronchoscopy can be carried out as an outdoor procedure and without the need of general anesthesia. It is a safe procedure with an estimated complication rate of 1.1% with a mortality of 0.02%[5]. These complications range from minor complications such as cough, transient desaturations, mild bleeding to life threating complications such as respiratory failure, pneumothorax and cardiac arrest. These are more common in those with poor cardiac reserve or hemodynamic instability.

FB is available only in few centers in Pakistan mainly in the larger cities. Few studies describe the diagnostic utility of bronchial washings in Pakistan. This study was conducted with the objective of determining the diagnostic accuracy of GeneXpert in comparison with AFB microscopy and cultures on bronchial washings/ BAL fluid.

MATERIAL AND METHODS

This study was a prospective cross-sectional analytical study carried out in Bronchoscopy unit of Pulmonology Department, Pak Emirates Military Hospital, Rawalpindi, from July to December 2018. Approval was taken from ethical review committee. Informed consent was taken from patients prior to procedure. Study population comprised of outdoor patients, indoor patients and referrals from other hospitals. Consecutive non-probability sampling technique was used. Patients with suspected sputum scarce or sputum negative tuberculosis, undiagnosed X-ray/HRCT infiltrates, who had received empirical anti-tuberculosis treatment (ATT) for less than 7 days were included in the study. Patients younger than 12 years, who refused consent, who were hemodynamically unstable, having deranged coagulation profile and those having sputum positive tuberculosis were excluded. All patients had HRCT or CECT chest carried out, along with complete blood counts and PT/PTTK prior to bronchoscopy as per BTS guidelines[5].

Fiberoptic Bronchoscopy was carried out using diagnostic probe of Pentax model EPK-i5000 having 2.0 mm internal diameter, under procedural sedation and local anesthesia using 4% local xylocaine spray. Procedure was performed by pulmonologists, senior residents or under supervision of consultants by juniors residents. Oxygen saturation and other vitals were monitored throughout the procedure. Patients were kept under observation for at least 30 minutes post-procedure. Positive broncho-scopy findings were noted, bronchial washings were taken, when appropriate BAL was carried out and 30 ml normal saline sample was taken. Samples were sent for AFB smear, GeneXpert Assay and Cultures. Six passes were done for biopsies and sent for histopathology. Sample for GeneXpert were sent to TB center PEMH site for testing whereas rest of sample were sent to AFIP laboratory CMH Rawalpindi.

All the specimens after being digested and decontaminated processed along with MGIT growth supplement OADC and PANTA, were inoculated into MGIT 960 TB system (Becton

Dickinson, Sparks, USA) and incubated as per manufacturer instructions.

GeneXpert testing was also done as per manufacturer's instructions. GeneXpert reagent was added to sample in 2:1 ratio without initial decontamination or centrifugation and incubated for a total of 15 minutes on room temperature, agitated at least once during incubation. At least 2 ml of fluid mixture was then placed in GeneXpert®MTB/RIF cartridge. Cartridge was then placed in GeneXpert instrument chamber. Results were read after 2 hours, and classified as "detected" and "not detected"; activity was classified as very low, low, medium and high.

Pre-bronchoscopy suspected diagnosis was based on clinical and imaging findings; final diagnosis was made based on microbiological or histological evidence.

Data were entered and analyzed in SPSS version 23. A *p*-value ≤0.05 was considered significant. Data reviewed included demographic details, suspected diagnosis/ indication, bronchoscopy findings and final diagnosis of patients.

RESULTS

A total of 224 patients were included in the study. 151 (67.4%) were males and 73 (32.6%) were females. Mean age was 48.25 ± SD 18.21 yrs. 22% (51/224) had history of smoking. There were 5 HIV positive cases; HIV status of rest was unknown. 58 (25.9%) were on empirical anti tuberculosis treatment. 15 (6.7%) had history of TB contact and 23 (10.3%) had previous history of TB. Most commonly reported symptom was productive cough by 71.5%; as 86% (193/224) had complained of cough dry or productive, followed by fever 42.5% and weight loss reported by 39.8% as shown in table-I.

Flexible bronchoscopy was diagnostic for tuberculosis in overall 42.4% cases (95/224) with three tuberculosis diagnosis based on histopathology report. Smear microscopy for AFB was positive in 53 (23.7%) cases, GeneXpert was positive in 36.6% (82) cases and culture was positive in 29% (66) cases with one case being NTM. Three Histopathology reports revealed granulomatous inflammation, 1 case was diagnosed as adenocarcinoma, 1 ILD (hypersensitivity pneumonitis). 25 cases were GeneXpert positive, but culture negative and 9 cases were GeneXpert negative but culture positive. 6 cases (2.7%) showed rifampicin resistance on GeneXpert RIF resistance assay, 4 were detected on cultures.

Sensitivity and specificity, positive and predictive value (PPV), negative predictive value (NPV) of smear microscopy were 69.6%, 95.5%, 86.7%, 88.3% respectively whereas for GeneXpert they were 86.36%, 84.1%, 69.5%. 93.66% respectively; when compared to culture considered as gold standard as shown in table-II.

Table-I: Clinical profile of patients

Patient Profile	Frequency (n)	Percentage (%)
Gender		
Male	151	67.4
Female	73	32.6
ATT history	58	25.9
Previous TB history	23	10
Symptoms		
Dry cough	35	15.8
Productive Cough	158	71.5
Fever	94	42.5
Weight Loss	88	39.8
Dyspnea	47	21.3
Hemoptysis	36	16.3
Chest Pain	31	14
Hoarseness	1	0.5
Others Spontaneous pneumothorax	1	0.5
TB contact	23	10.3
Smoker (Current or former)	51	22.7
HIV status	5	2.2%

52% (13/25) cases of GeneXpert positive, culture negative cases had very low activity and 32% (8/25) had low activity on GeneXpert assay. All cases with high activity were detected on culture. 40% of these GeneXpert positive, culture negative cases had history of receiving ATT. GeneXpert activity had statistically significant

correlation (*p*-value <0.01) with smear and culture positivity as shown in table-III.

DISCUSSION

The male to female ratio in our study was comparable with the WHO statistics 1.7:1[2], as 58.9% of those diagnosed were males. Cough was the most common presenting symptom, consistent with findings of other studies[4,7-12].

Overall diagnostic yield of bronchoscopy for tuberculosis in our study was 42.4%. In a local study done in Pakistan, 34.6% were diagnosed with tuberculosis[1], 18.5% in a study by Pauld *et al*[10] and 34.8% (39/112) were diagnosed[13] in a study by Bernard *et al* in South Africa. Similarly, 40% (24/60) were diagnosed in a study in neighboring country India by Gowda *et al*[4]. A considerably higher, 78% yield for tuberculosis was seen in a study (85/108) by Choudhary *et al* in India[12]. Diagnosis of tuberculosis was made in 17 (42.5%) of the 40 patients[14] in a study done by Quaiser *et al*. Diagnostic yield was lower in other studies as 6.7% (5/74) and 13.5% (10/74) were diagnosed on smear microscopy and GeneXpert respectively in a study done in Rome[15].

Culture confirmed TB was seen in 29.5% (66/224) cases in our study. Variable rates were seen in other studies, 18%(27/154) cases were culture positive in study by Theron *et al*[16], 91.4% (85/93) in a study by Kanwal *et al* in Pakistan[17], 26.7% (16/52) in an Indian study[18], 14.2% (23/162) by Palud *et al*[10] and 22.4% (38/170) by Agrawal *et al*[19].

Although sensitivity and specificity of GeneXpert was comparable to other studies, some studies had higher specificity. Our sensitivity of 86.36% was comparable to local studies in Pakistan, as it was 91.86% in a study by Kanwal *et al*[17] and 80% by Ullah *et al*[20]. PTB was confirmed in 69.2% patients by GeneXpert and 4 (3.3%) patients were found to have rifampicin resistant in another local study done in Karachi region of Pakistan[21]. The sensitivity and specificity of the XpertMTB/RIF assay were 84.5% and 98.9%, respectively, and those for smear microscopy were 36.2% and 100%, respectively in a study done in China[7], MTB/RIF had a sensitivity and specificity of 81% and 73% in culture confirmed cases by Gowda *et al*[4]; considerably higher sensitivity and specificity were observed in a study by Theron *et al* 93% and 96%[16] and they were 80%and 98% respectively in a study done in Hong Kong[8].

Pooled sensitivity and specificity of GeneXpert on BAL and washings was 87% and 97% in a meta analysis done by Li *et al*[22].

Table-II: Comparison of AFB Smear and GeneXpert Assay.

	Culture				Culture		Total
	Positive	**Negative**	**Total**		**Positive**	**Negative**	
GeneXpert Positive	57	25	82	Smear Positive	46	7	53
GeneXpert Negative	9	133	142	Smear Negative	20	151	171
Total	66	158	224	Total	66	158	224
	Sensitivity	Specificity		PPV	NPV	*p*-value	
AFB Smear	69.6%	95.5%		86.7%	88.3%	0.019	
GeneXpert	86.36%	84.1%		69.5%	93.66%	0.009	

*As compared with culture using McNemar test

Table-III: GeneXpert assay activity and culture results (n=82).

GeneXpert Activity	Total	Culture Positive	Culture Negative
Very low	27 (20.7%)	42 (23.5%)	13 (76.5%)
Low	26 (31.7%)	18 (69.2%)	8 (30.8%)
Medium	34 (41.5%)	30 (88.2%)	4 (11.8%)
High	5 (6.1%)	5 (100%)	0

GeneXpert sensitivity for BAL sample was 81.4%, superior to that of 22.2% of smear microscopy on BAL in a study by Agrawal *et al*, and specificity of GeneXpert and smear microscopy were 93.1% and 100% respectively which were higher than our study[19]. Sensitivity and specificity values were 80.0% and 98.6% for the GeneXpert assay, and 25.0% and 95.8% for Smear microscopy in study by Palud *et al*[10] on BAL samples.

GeneXpert was superior to smear microscopy in terms of sensitivity in all of these studies which is consistent with the findings in our study. GeneXpert detection limit of around 131 cfu/ml(colony forming units) in contrast to 10,000 cfu/ml required for AFB smear[18] explains this finding. GeneXpert activity was significantly associated with culture and smear positivity in our study, in the study by Theron *et al* MTB/RIF-generated CT (cycle threshold) values had a strong correlation with smear grade and culture[16].

These differences in sensitivity and specificity can be explained due to different TB burden, bronchoscopic techniques, patient population and variable patient selection such as inclusion of patients on empirical ATT in our study and in various others studies; most using cut-off of one week[17,21], whereas Gowda *et al*[18] and Chaudhary *et al*[12] used 2 weeks and 1 month cut-off respectively. Different references such as cultures alone or combination of cultures with radiological and clinical findings were used for calculating sensitivity and specificity in various studies. Varying micro-biological techniques such as use of LJ medium instead of MGIT by Kanwal *et al*[17] also factor into this.

The lower specificity in our study is due to GeneXpert positive, culture negative cases. Among the 158 culture negative patients we had 15.8% (25/158) cases who were GeneXpert positive but culture negative. One study done in China had a relatively higher number with 29.6% culture negative cases[23], whereas this was 12.5% in a study by Bernard *et al* (9/73) and 18% (18/100) in the study by Ullah *et al*[20]. Theron *et al* detected 12 MTB/RIF-positive, culture-negative results[16]. Some 80 cases (14.3%) were GeneXpert positive/culture negative in a study done on sputum samples and previously treated patients were more likely to yield negative culture results in the study[24]. One reason for GeneXpert positive culture negative results could have been the inclusion of cases who had received ATT for less than 7 days. The use of broad spectrum antibiotics in patients with pulmonary infections is common and B-lactams given for infections haves hown activity against MTB in various studies[4]. Studies have also shown GeneXpert remains positive in cases with previous TB having received ATT in previous 5 years as dead bacilli can be amplified by GeneXpert, and low bacillary load can also contribute to this[23]. Majority of these cases in our study had very low or low activity, this was also seen in study by Barnard *et al*[13] in which 7/9 and by Agrawal *et al*[19] in which in all 9 similar cases, the result of GeneXpert was very low or low positive. Similarly, these cases had extremely high cycle threshold (Ct) values (>28) i.e. low bacillary load in the study done in China[7], 13 of 15 responded to ATT in the study[7].

LIMITATION OF STUDY

Limitations of our study were, a smaller sample size as this was a single-center study, a large number of culture negative GeneXpert positive cases which reduced specificity. We only used cultures as reference for calculating sensitivity and specificity, whereas various studies have used clinically response to ATT over months in combination with radiologically criteria as an additional reference.

CONCLUSION

Flexible bronchoscopy procedures, associated specimen and use of GeneXpert had higher sensitivity than AFB smear microscopy and is a rapid and effective tool, reducing the time constraint associated with culture based diagnosis and determining Rifampin Resistance simultaneously. GeneXpert positive, but culture

negative cases should be interpreted in context of clinical picture and history of treatment.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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