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Research Article

Feasibility and Effectiveness of Xpert®MTB/RIF Assay in Reducing the Median Time to Diagnosis of Tuberculosis in HIV Infected Patients

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Abstract

Introduction: Multi-drug resistant tuberculosis is twice more prevalent in HIV/TB co-infected patients. Diagnosis of pulmonary TB among HIV-infected individuals remains a challenge and the need for easy to perform and accurate tests is imperative. The objective of our study was to assess feasibility and effectiveness of the assay in reducing the median time to diagnosis of TB in HIV-infected individuals.

Methods: After approval from the institutional ethics committee, a prospective study was conducted in a tertiary care hospital in Mumbai. Microscopy, culture and Xpert®MTB/RIF assay were performed on specimens collected from 224 HIV positive adults suspected of TB. Overall diagnostic yield and sensitivity were calculated with culture as reference standard. The results were statistically analysed using the Chi-square test.

Results: Microscopy, culture and Xpert®MTB/RIF assay were positive in 16(7.1%), 36(16.07%) and 34(15.17%) cases respectively. Xpert®MTB/RIF assay was effective in giving an additional yield of 8.04% (18/34) over microscopy. Overall sensitivity of the assay was 77.78% (28/36) and specificity was 96.81%(182/188). Rifampicin resistance was detected in 11 out of 34 cases (32.35%) by Xpert®MTB/RIF assay. Median time to detection of TB by Xpert®MTB/RIF assay was 0days, compared to 1day for microscopy and 30days for solid culture.

Conclusions: Xpert®MTB/RIF assay was effective in diagnosis of TB in a significantly high number of HIV-infected individuals who were sputum smear-negative and significantly reduced the median time to diagnosis of TB. The minimal expertise required for performing the assay makes it feasible to implement Xpert®MTB/RIF assay as a routine diagnostic test in a high throughput microbiology laboratory.

Keywords: Tuberculosis; Xpert®MTB/RIF Assay; Median Time; HIV; Effectiveness

Introduction

Tuberculosis (TB) is the most common opportunistic infection among Human Immunodeficiency Virus (HIV)-infected individuals. Globally in 2016, an estimated 10% of the 10.4 million incident Tuberculosis (TB) cases were among people living with HIV. India ranks 2nd and accounts for about 10% of the global burden of HIV-associated TB [1].

Multi-drug resistant tuberculosis (MDR-TB) has been almost twice as common in TB patients living with HIV than those without HIV. TB-HIV co-infected patients are categorized as Presumptive Drug Resistant Tuberculosis (DRTB) cases, as per Revised National Tuberculosis Control Programme (RNTCP) [2].

Diagnosis of TB difficult in HIV-infected individuals and emphasizes the need for more sensitive and rapid diagnostic tests both for

detection of Mycobacterium tuberculosis (MTB) and for identifying MDR-TB [3].

Xpert®MTB/RIF assay, developed by Cepheid diagnostics, is an unique, fully automated, molecular assay for detecting MTB and rifampicin resistance within two hours. It has a sensitivity of 79% and 86% for specimens from HIV infected and HIV non-infected individuals respectively) [4].

WHO policy guidance (2013) provided recommendations for use of Xpert®MTB/RIF assay in diagnosis of pulmonary and extrapulmonary TB and rifampicin resistance [5]. The RNTCP Technical and Operational Guidelines, 2016 revised diagnostic algorithm for presumptive TB providing Cartridge Based Nucleic Acid Amplification Test (CBNAAT) for these patients [6]. Universal drug susceptibility testing (DST) for at least rifampicin for all diagnosed TB patients by rapid molecular tests was rolled out phase wise in India in 2017 [7].

There are limited published studies on Xpert®MTB/RIF assay in HIV co-infected patients from India [8,9]. Hence this study was undertaken to evaluate the sensitivity and specificity of Xpert®MTB/RIF assay and to assess its effectiveness in reducing the median time to diagnosis for HIV-TB co-infected patients.

Methods

After approval from the institutional review board, a cross sectional study was conducted for 18 months at a tertiary care teaching hospital's microbiology department.

HIV positive adults (>15 years) with any symptom (cough of any duration, fever, night sweats, loss of appetite and weight loss) suggestive of TB referred from Integrated Counselling and Testing Centre (ICTC) to Designated Microscopy Centre (DMC) who were able to produce adequate sputum specimen were enrolled in the study with their informed consent. Detailed history was noted along with the latest CD4 count and patients on anti-tubercular treatment (ATT) were excluded. They were appropriately instructed for collection of sputum- two specimens (spot(A) and early morning(B)) in a 50 ml wide mouthed, graduated and screw-capped sterile plastic container as per RNTCP guidelines [10].

The sample processing was carried out in Class II Biosafety Cabinet and following biosafety level 2 practices and quality control. Macroscopic appearance of the specimen with respect to its colour, volume, consistency, presence of blood, mucus and food particles was recorded. All samples were processed for microscopy with acid fast staining and reported according to RNTCP guidelines. Patient was considered to be positive even if one of the two smear results were positive.

The early morning and spot specimen were then pooled. Xpert®MTB/RIF assay was performed on 1.5-2ml of the pooled specimen following manufacturer's instructions [11]. The negative results were generated as "MTB NOT DETECTED". The positive results were generated as "MTB DETECTED" with the bacterial load (high, medium, low and very low) and "RIF RESISTANCE DETECTED / NOT DETECTED". If results obtained were invalid, indeterminate or error, test was repeated using second specimen.

The remaining pooled specimen was digested and decontaminated using N-acetyl-L-cystine-sodium hydroxide (NALC-NaOH) method and then centrifuged at 3000 rpm for 15 minutes. Two loopfuls of the centrifuged sputum deposits were inoculated on Lowenstein Jensen (LJ) medium and incubated aerobically at 37°C. All cultures were read daily during first week for contamination and rapidly growing mycobacterial species and then weekly till growth was detected or eight weeks whichever was earlier. Any growth observed on LJ medium was confirmed for its acid-fast nature and identified as Mycobacterium tuberculosis or Mycobacterium other than tuberculosis (MOTT) using phenotypic characteristics like rate of growth, pigment production and MPT64 antigen test (SD Bioline TB Ag MPT64 Rapid) [12]. For the purpose of this study, an isolate was considered as Mycobacterium TB complex (MTBC) if, it was slow growing (>7 days), acid-fast, colonies were rough and buff, and gave positive result for MPT64 antigen.

MTB isolates recovered on LJ medium were tested for rifampicin and isoniazid susceptibility by proportion method that was performed as per the Canetti., *et al.* protocol. Incubation, reading and interpretation of test was done as per RNTCP guidelines [10].

Statistical analysis

The difference in the results between the three tests, sputum smear microscopy, culture and Xpert®MTB/RIF assay were analysed. Culture on LJ medium was considered as the gold standard in this study. The significance of the difference in the detection by the three tests was analysed using Chi-square test. P-value <0.05

was considered as significant. The significance of smear grading with time to positivity for culture was analysed by the one way ANOVA and Tukey's honest significant difference test. Correlation of Xpert®MTB/RIF assay positivity with CD4 counts was evaluated by logistic regression analysis.

Definitions

For the purpose of the study,

- Effectiveness was defined as the increase in number of TB cases diagnosed by Xpert®MTB/RIF assay over sputum smear microscopy in HIV infected patients
- Feasibilty was defined as the ease of implementing Xpert®MTB/RIF assay as a routine diagnostic test in a high throughput microbiology laboratory in addition to sputum smear microscopy and solid culture
- Confirmed case was defined as a case which was either sputum smear positive or Xpert®MTB/RIF assay positive or culture positive.

Results

Between March 2014 - October 2015, 224 HIV-TB co-infected patients were included in the study. An additional 34 patients were enrolled in the study but excluded from analysis as they were- not able to produce sputum (8), relief of symptoms (5) required hospitalization (3), were lost to follow up (5) or had a contaminated culture (13).

The mean age of study subjects was 39.57 years (range 15-67 and SD =10.38). Majority of the patients 79/224 (35.27%) were adults in 41 - 50 years age group (SD = 2.39%).

The male: female ratio was 1.9:1. Xpert®MTB/RIF assay positivity was 15.65% in males, slightly higher than that in females (14.29%) (p = 0.94). Rifampicin resistance was higher in women (36.36%) compared to men (30.43%). (p = 0.73). Both these findings were statistically not significant.

Almost 38.84% (87/224) of HIV positive cases referred from ICTC, had a history of TB. 18.39% of the patients with a history of TB were positive by the Xpert ® MTB/RIF assay. The positivity was higher than that among patients with no history of TB (13.14%) but statistically not significant. In patients with history of previous ATT, 56.25% (9/16) were detected as rifampicin resistant while only 11.11% (2/18) were rifampicin resistant in those without history of ATT and this difference was statistically significant (p = 0.014).

All smear positive cases (n = 16) were also positive by Xpert®MTB/RIF assay. Xpert®MTB/RIF assay diagnosed an additional 18 cases, giving an additional yield of 8.04% over microscopy (p = 0.0001). Culture detected 93.75% (15/16) among smear positive cases. Culture gave an additional yield of 9.37% over microscopy by diagnosing 21 cases additionally (p = 0.0001) (Table 1).

	Xpert® MTB/ RIF Positive	Xpert® MTB/ RIF Negative	Total
Smear positive-Culture positive	15	0	**15**
Smear negative-Culture positive	13	8	**21**
Smear positive-Culture negative	1	0	**1**
Smear negative-Culture negative	5	182	**187**
Total	34	190	224

Table 1: Comparison between smear microscopy, Xpert®MTB/RIF assay and culture.

Prevalence of TB in HIV infected individuals with symptoms suggestive of TB was 16.07% considering culture as gold standard. Overall, sensitivity of Xpert®MTB/RIF was 77.78% when compared to culture. Sensitivity in smear-positive, culture-positive cases was 100%. Sensitivity in smear-negative, culture-positive cases was 61.90% (13/21). Overall specificity of Xpert®MTB/RIF was 96.81%. Specificity in smear-positive, culture-positive cases was 100% while that in smear-negative, culture-positive cases was 97.33% (182/187). The higher yield by culture in comparison to Xpert®MTB/RIF assay was statistically significant (p = 0.0001) (Table 2).

55.56% (20/36) isolates were sensitive to both rifampicin and isoniazid. 30.56% (11/36) isolates were MDR. 2.78% isolates showed mono-resistance to rifampicin while 11.11% isolates showed mono-resistance to isoniazid.

	Culture positive	Culture negative	Total	Sensitivity	Specificity
Xpert® MTB/RIF Positive	28	6	34	77.78%	96.81 %
Xpert® MTB/RIF Negative	8	182	190		
Total	36	188	224		

Table 2: Performance characteristics of Xpert®MTB/RIF assay- single sputum.

Sensitivity of Xpert®MTB/RIF assay for detection of rifampicin resistance was 90% when compared to the proportion method and the specificity was 94.73%. Concordant results were obtained in 27/28 isolates. One strain reported as resistant by proportion method was reported as sensitive by Xpert®MTB/RIF assay giving an error rate of 10% and Cohens coefficient was 0.92 (Table 3).

	Rifampicin sensitive	Rifampicin resistant	Total
1% proportion method	18	10	28
Xpert® MTB/RIF assay	19	9	

Table 3: Comparison of rifampicin resistant status – Proportion method vs Xpert®MTB/RIF assay.

The earliest growth detected by culture was at the end of 3rd week in 13.89% of the samples (Table 3). All the isolates grew by 7th week. Median TAT for growth on LJ medium was 30 days. (Range= 18 to 48, SD=7.8)

The mean TAT varies inversely with the smear positivity grade. A p value < 0.05 was obtained by applying the one way ANOVA. This signified that time to positivity for culture in one or more of the smear grade categories were significantly different from that in the others. Tukey's honest significant difference test was performed on the same data set to pinpoint which smear category/categories had significantly different time to positivity for culture. It was found that there was significant difference in time to positivity for culture between negative and 1+, 2+ and 3+ smears. It was also evident that there was no significant difference in time to positivity for culture between positive smears of any grade (Table 4).

Smear grading	Number of cultures positive at different weeks (n=36)						
	Mean(in days)	3rd	4th	5th	6th	7th	Total
Negative	35.71	0	3	7	8	3	21
Scanty	30.5	0	0	2	0	0	2
1+	26.28	1	4	1	0	0	6
2+	22.4	3	2	0	0	0	5
3+	20.5	1	1	0	0	0	2
Total	NA	5	7	3	8	3	36

Table 4: Comparison of smear grading with time to positivity for culture.

CD4 counts for 113 patients were available; of which 29.20% of the patients had a CD4 count of < 200. 27.27% of the patients with CD4 cell count <200, were Xpert® MTB/RIF assay positive. By performing a logistic regression on the available data p value= 0.0024 was obtained with an odds ratio of 0.9953. This indicates that a low CD4 count significantly contributed to the outcome of Xpert®MTB/RIF assay positivity. (Table 5).

CD4 cell count	Total	% Total	Xpert® MTB/RIF assay positive	% Positivity	Rifampicin resistance by Xpert® MTB/RIF assay
<200	33	29.2%	9	27.27	2
201-350	35	30.97%	7	20	3
351-500	24	21.23%	0	0	0
>500	21	18.58%	1	4.76	1
Total	113		17		6

Table 5: Correlation of Xpert MTB/RIF assay positivity with CD4 counts.

Discussion

The present study of HIV infected patients provides evidence of additional TB and DRTB case detection using Xpert® MTB/RIF assay in comparison to sputum smear microscopy and culture and prevalence of rifampicin resistance.

Xpert®MTB/RIF assay gave 8.04% additional yield over microscopy which is in concordance with other studies [13-15]. Another study from India demonstrated a higher additional yield of 14.74%

[8]. The increase in yield can be explained based on the differential sensitivity of the two methods. Sputum smear microscopy is known to detect MTB only when the bacterial load is 10^4 or more per ml of specimen. On the other hand Xpert®MTB/RIF assay has a limit of detection of 131cfu/ml [13].

Smear examination though rapid and simple has a low sensitivity compared to culture and molecular platforms and is even lower in HIV infected patients as compared to the HIV uninfected patients [3]. Further, Xpert®MTB/RIF assay detected 100% more cases in comparison to sputum smear microscopy among HIV infected patients.

Considering the above benefits, WHO policy update 2013 recommends its usage rather than conventional microscopy, culture and DST as the initial diagnostic test in children and adults suspected of having MDR-TB or HIV- associated TB [5]. The International Standards for Tuberculosis Care 2014 also states that patients at risk for drug resistance, who have HIV risks, or who are seriously ill, should have Xpert®MTB/RIF performed as the initial diagnostic test and in patients suspected of having pulmonary tuberculosis whose sputum smears are negative, Xpert®MTB/RIF and/or sputum cultures should be performed [16].

The overall sensitivity in the present study using a single, early morning, sputum sample was 77.78%. A comparison with other studies shows that some had a better sensitivity [4,9,15,17]. (Table 6) The difference in inclusion criteria of the study subjects may be one of the reasons for the variable sensitivity. Carriquiry G., *et al*. [15] in their study have included HIV-positive individuals with cough of ten or more days with abnormal chest radiograph and at least one additional symptom suggestive of TB making their study population more selective. In our study HIV positive adult patients with any symptom of TB or abnormal chest radiograph were enrolled in the study.

	Overall sensitivity	Sensitivity in smear-positive, culture-positive	Sensitivity in smear-negative, culture positive	Overall Specificity	Gold standard
Theron G., *et al*. [14] 2011 (n=130)	69.6%	91.3%	47.3%	91.7%	Liquid culture
Boehme C., *et al*. [9] 2011 (n=1255)	82·4%	97·7%	71·8%	99·2%	Solid or liquid culture
Lawn SD., *et al*. [13] 2011 (n=445)	73.3%	100%	63%	99.2%	Liquid culture
Rachow A., *et al*. [17] 2011 (n=172)	88%	84%	68%	98%	Liquid and solid culture
Carriquiry G., *et al*. [15] 2012 (n=131)	97.8%	100%	92.2%	97.7%	Liquid and solid culture
Steingart KR., *et al*. [4] 2014	79%	97%	61%	99%	Liquid/ solid culture
Balcha TT., *et al*. [18] 2014 (n=812)	66.39%	96.4%	57.4%	98.11%	Liquid culture
Present study, 2015 (n=224)	**77.78%**	**100%**	**61.90%**	**96.81%**	**Solid culture**

Table 6: Performance characteristics of Xpert®MTB/RIF assay in HIV infected patients.

Fourteen discordant results were observed between Xpert®MTB/RIF assay and culture. Eight cases were culture positive but Xpert®MTB/RIF assay negative in the present study. These could be either false-positive cultures or false-negative Xpert®MTB/RIF assay. The common reasons for false-positive cultures include cross contamination due to inadequately functioning biosafety cab-

inets (BSC), aerosols generated during manipulation and processing of sputum specimens and use of contaminated reagents, buffer or pipettes [19]. Adequate measures were taken to avoid contamination. Periodic tests to assess efficiency of BSC were undertaken and work surfaces in BSC were routinely disinfected. The tubes were opened only 5-10mins after mixing and vortexing to prevent aerosol formation. Daily aliquots of processing reagents and buffers were used and any leftover reagents were discarded. Thus the possibility of a false-positive culture is unlikely.

Culture of MTB is highly sensitive and can detect as few as 10 viable bacilli per ml, which maybe the reason for the discrepant results. The use of concentrated specimen for culture as against direct specimen for Xpert®MTB/RIF assay may have also led to higher positivity by culture. Presence of very low copy numbers or absence of the target (for amplification) being detected in the infecting MTB strains can lead to false negativity of Xpert®MTB/RIF assay. Use of other molecular methods should have been considered to determine if the false negative Xpert® MTB/RIF assay results were some unique isolates. These culture positive, Xpert®MTB/RIF assay negative cases were lost to follow up as the results were available only after a period of three weeks to seven weeks since specimen collection.

Six patients had culture negative but Xpert®MTB/RIF assay positive result. These could be either false-negative culture or false-positve Xpert®MTB/RIF assay. All these patients had clinical symptoms and chest radiograph findings suggestive of TB. Two of them had a history of TB. Five of these patients had improved clinical condition on initiation of ATT and one patient was lost to follow up. These are unlikely to be false-positive on Xpert® MTB/RIF assay. Other studies with Xpert®MTB/RIF assay positive but culture negative results have also shown that the patients responded well to ATT [9,13,17]. The use of solid medium rather than liquid culture and possible culture contamination could lead to a false-negative culture. These five patients showed a negative culture result on follow up culture, post-treatment completion.

In the present study, one of the patients had a positive sputum smear microscopy and Xpert®MTB/RIF assay result but was negative on culture. Over decontamination of the sputum specimen, could be one of the reasons for culture negativity in this case. Contamination rates of < 2% indicate over decontamination during specimen processing [10]. However, the contamination rate in the present study was 5.48%. Use of solid culture medium with its lower sensitivity compared to liquid culture, could be a cause of false negativity in the present study. Patients on current ATT may also give false-negative results on culture but patients on treatment were not enrolled in the present study.

Using both culture and Xpert®MTB/RIF assay increased the detection to 42 cases. Xpert®MTB/RIF assay and culture should be used in conjunction wherever facility for culture is available. A positive Xpert®MTB/RIF assay result, should be interpreted in light of history, clinical symptoms and radiological findings. A negative Xpert®MTB/RIF assay in a symptomatic patient should be confirmed by culture even though this means incurring additional cost for diagnosis.

Six cases of rifampicin resistant TB were detected by proportion method. One strain was reported as rifampicin sensitive by Xpert®MTB/RIF assay and rifampicin resistant by proportion method. When this isolate was tested by line probe assay (LPA), a rifampicin sensitive report was obtained in concordance with Xpert®MTB/RIF assay. Both LPA and Xpert®MTB/RIF assay detect resistance in the 81bp RIF resistance-determining core region of the wild-type rpoB gene. More than 95% of the rifampicin resistant strains contain mutations localised within this region [4]. Other mechanisms of resistance may be present which are not detectable by the Xpert®MTB/RIF assay but are expressed phenotypically. These include mutations in the amino-terminal region of *rpo B* in the codon 176 and expression of efflux pump [20].

It is difficult to comment on the sensitivity and specificity of Xpert®MTB/RIF assay for detection of rifampicin resistance due to limited numbers in this category. Xpert®MTB/RIF assay detected 90% of the rifampicin resistant cases on day 0 in comparison to around 72 days needed by conventional LJ culture and DST. DST by conventional method should be performed to confirm results of Xpert®MTB/RIF rifampicin susceptible status when the suspicion for MDR-TB is high.

The median time to diagnosis by Xpert®MTB/RIF assay was zero days for the present study confirming the findings of other studies [9,13]. The median time to positivity for culture on LJ medium was 30 days, in the present study, similar to findings in a large multicentre study [9]. An additional 28 - 42 days were needed for phenotypic drug susceptibility testing.

A short TAT for detection of TB and rifampicin resistance by Xpert®MTB/RIF assay ensures earlier treatment initiation, better outcomes, more effective choice of ATT, lower rates of transmission and also reduces loss to follow up. Whenever available, Xpert®MTB/RIF assay should be done as an initial test to diagnose TB in HIV-infected patients.

Being a tertiary care public hospital with most patients being from out-station locations, we were unable to trace back all the patients that were tested negative by Xpert®MTB/RIF assay and culture but were initiated on ATT based on other investigations and therefore failed to evaluate the true sensitivity of Xpert®MTB/RIF assay in diagnosis of TB. Due to resource limitations we tested only one sputum specimen for Xpert®MTB/RIF assay and culture. Also we were unable to confirm bacteriologically, the Xpert®MTB/RIF assay positive but solid culture negative results using more sensitive liquid cultures. The small sample size limited the assessment of rifampicin resistance.

Conclusions

In HIV infected patients, Xpert®MTB/RIF assay established a diagnosis of TB in a significantly high number of patients who are sputum smear negative and significantly reduced the median time to diagnosis of TB. The minimal expertise and processing time required for performing the assay with minimal bio-hazard component makes it feasible to implement Xpert®MTB/RIF assay as a routine diagnostic test in a high throughput microbiology laboratory setting like ours, in addition to sputum smear microscopy and culture. Although Xpert®MTB/RIF assay has the ability to detect TB early, its lower sensitivity using one specimen compared to culture is its drawback. Culture is therefore recommended in TB suspects who are Xpert®MTB/RIF assay negative to provide confirmation.

Key Messages

Xpert®MTB/RIF assay should be used in conjunction with culture to reduce the median time to diagnosis. In settings with high MDR-TB prevalence, Xpert®MTB/RIF helps optimise the initial regimen. The minimal expertise and processing time required makes it feasible to implement Xpert®MTB/RIF assay as a routine diagnostic test in a high throughput microbiology laboratory.

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