Xpert MTB/Rif assay in the diagnosis of smear-negative pulmonary tuberculosis: a prospective study from Nepal

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Research article

Keywords: Xpert MTB/Rif assay, Mycobacterium tuberculosis, Line Probe Assay, MDR-TB

Posted Date: September 4th, 2019

DOI: https://doi.org/10.21203/rs.2.13972/v1

Version of Record: A version of this preprint was published at BMC Infectious Diseases on December 1st, 2019. See the published version at https://doi.org/10.1186/s12879-019-4728-2.

Abstract

Background For improving patient care and abbreviating the disease transmission chain, speedy detection of tuberculosis and its drug-resistance with precision is crucial. Methods We analyzed, pulmonary tuberculosis (PTB) suspected, 360 smear-negative sputum from the patients attending Tribhuvan University Teaching Hospital (TUTH). The patients were selected as per the algorithm of National Tuberculosis Programme(NTP) for Xpert MTB/RIF testing. Participants' demographic and clinical information were collected using a pre-tested questionnaire. The specimens were collected, processed directly for Xpert MTB/RIF test according to the manufacturer's protocol. The same samples were stained using Ziehl-Neelsen technique then observed microscopically. Both findings were interpreted; rifampicin-resistant, if obtained, on Xpert testing was confirmed with Line Probe Assay. Result Of 360 smear-negative sputum samples analyzed, 85(23.61%) found positive while 3 of them were rifampicin resistance. The infection was higher in male, i.e. 60(25.3%) compared to female 25(20.3%). The age group, >45(nearly 33%) with median age 42± 21.5, were prone to the infection. During the study period, 4.6% (515/11048) sputum samples were reported as smear-positive in TUTH; consequently, with Xpert MTB/RIF assay, additional case 16.5% (n=85/515) missed on the smear microscopy, were detected—surging overall confirmed cases. Among the most occurring clinical presentations, cough and chest pain were more evident in PTB with relative-risk at 95% confident-levels i.e. 3.03(1.01-9.11) and 3.47(2.29-5.27) respectively. A higher number of new suspects (n=63) were found positive compared to previously treated suspects. The upper lobe infiltrates (36.4%) and pleural effusion (40.4%) were peculiar radiological impression noted in PTB patient. 94 MDR suspected cases were enrolled; of total suspected cases, 29 samples were found rifampicin sensitive, 1 indeterminant while 2 of them were rifampicin-resistant. However, a single rifampicin-resistant; case was detected in patient which was not MDR suspected. Conclusion Additional cases of PTB which are neglected as smear-negative on microscopy and other conventional tests can be detected with gene Xpert test. Hence, recommended to every suspect as a presumptive test could be a wise investment in diagnosis to restrict the global burden to some extent.

Keywords: Xpert MTB/Rif assay, Mycobacterium tuberculosis, Line Probe Assay, MDR-TB

Background

Tuberculosis (TB) is a treatable and curable disease (if early diagnosis of etiology and its drug resistance status could be made) but has existed for epochs and remains a major global health problem(1). The reasons behind this obstinacy around the globe are due to inaccessible or lacking diagnostic tool which carries higher precision and over-relying on clinical presentations, chest radiography and/or sputum smear microscopy—in most health centers of developing countries(2)(3)(4). Billions of dollars are being spent in developed nations; however, is worthless in its control and preventions. It could be fruitful only if interrupting transmission chains from developing nations is possible or by helping them financially in disease diagnosis and treatment.

To address this issue, world health organization (WHO) endorsed Xpert MTB/RIF assay as accurate, feasible, rapid, affordable, and near-point-care TB diagnostic test for resource-limited settings of

developing countries in December 2010. As collaborative efforts of global fund and National Tuberculosis Programme, Nepal (NTP) the test was made available, for all patients acquiring the test, in absolutely free of cost. However, only limited health centers provide this facility since fund circulation in every public hospital for a minimum laboratory set-up is yet to be done.

The study was aimed to characterize the patient for Xpert MTB/RIF testing, intended for diagnosis of smear-negative PTB, in Tribhuvan University Teaching Hospital, Nepal.

Materials And Methods

Study design and settings

A cross-sectional study was conducted among smear-negative but presumed pulmonary tuberculosis suspected patient. The study was conducted at Tribhuvan University Teaching Hospital, the largest public tertiary care hospital, in Kathmandu, Nepal between13th April 2016 -14th April 2017. Before the introduction of Gene Xpert, the laboratory setting of this hospital was limited with AFB smear microscopy and *Mycobacterium tuberculosis* (MTB) culture but no drug susceptibility test (DST) facility. After then, with an aid of Global Fund and NTP, Nepal the diagnostic test was made accessible for all suspected PTB patient—those meeting algorithm endorsed by National Tuberculosis Center (NTC)—as cost-free service, from our study hospital (Additional file 1).

For an estimation of additional yield in diagnosis from Gene Xpert test over smear microscopy; the smear microscopy result over the study period was extracted from the laboratory record file where 4.66% (515/11048) positive cases were recorded. About the data, the sample size was determined and result of Gene Xpert MTB/RIF assay was compared.

Participants' demographic and clinical information were collected using a pre-tested questionnaire. The clinical diagnosis and MDR suspecting were done by an expert chest physician; chest X-ray features were classified as per radiologist reports. To optimize the applicability of Gene Xpert MTB/RIF assay, as per algorithm provided by the National Tuberculosis Center, chest X-Ray was chosen as triaging test. The patient with abnormal chest X-ray (upper lobe infiltrates, pleural effusion, diffuse infiltrates, cavitary lesions, other infiltrates, consolidation, other abnormalities) and having clinical presentations: persistent cough (≥2 weeks), fever, drenching night sweats, weight loss (>1.5 kg in a month), loss of appetite, malaise, and shortness of breath or chest pain; were presumed as PTB. However, as MDR-TB, based on the smear results, previous treatment history and clinical improvement in response to the ATT (anti-tubercular therapy) after two months.

Xpert MTB/RIF assay and MDR confirmation

All the procedures for gene Xpert testing were followed as per the manufacturers' specifications and guidelines(5). In brief, 0.5ml of expectorated sputum sample and Xpert sample reagent (SR) was added in the ratio 1:2 and was vortexed twice with 15 minutes incubation at room temperature until the sample

gets emulsified completely. After then, 2 ml of the mixture was transferred to Xpert test cartridge; the cartridge was then loaded into Xpert machine. Gene Xpert DX system, interprets the results, from measured fluorescent signals and display automatically either MTB complex detected, not detected, or rifampicin-resistant(if present) only after 90mins.

Cases of rifampicin-resistance detected by Xpert were confirmed in the NTC Laboratory using MTBDR plus line probe assay (Hain Life science Germany) as per the manufacturers' instructions. In brief, bacterial DNA presumed as Rif resistant from sputum sample was extracted using cetyl-trimethyl ammonium bromide (CTAB) method which was then amplified, purified and hybridized with strips of MDRTB plus. The evaluation and interpretation of the results were done as per the interpretation chart provided with the kit.

Results

Patients' demographics

A total of 360 smear-negative sputum samples (including 237 male and 123 female clinically suspected pulmonary tuberculosis patient) were tested with Xpert MTB/RIF assay. Among smear-negative cases enrolled 85(23.61%) of them were positive for PTB where 3 rifampicin resistance cases observed (Fig.1). The infection was higher in male, i.e. 60(25.3%) compared to female 25(20.3%). The age group, >45(nearly 33%) and below 15(20%) years, with median age 42± 21.5, found prone to the infection (Table1).

During the study period, 4.6% (515/11048) sputum samples were reported as smear-positive in TUTH. Our study sum-up the additional case of pulmonary tuberculosis i.e. 16.5% (n=85/515), which were missed on the smear microscopy.

Based upon the clinical history obtained from the pre-tested questionnaire, 268 new suspects, 89 previously treated and 2 Loss to follow-up cases were registered. The higher number of new suspects were found positive on Gene Xpert testing i.e. new suspects (n=63), previously treated cases (n=20), and loss to follow up (n=2).

Clinical presentation

Among most occurring clinical presentations, cough and chest pain were more evident to pulmonary tuberculosis with relative-risk at 95% confident-levels i.e. 3.03(1.01-9.11) and 3.47(2.29-5.27) respectively. However, other clinical presentations: fever, weight loss, and night sweat found as non-specific clinical presentations in PTB patient, as shown in (Table2)

Radiological impression on chest X-ray

Of total enrolled patient, 345 were presented with an abnormal radiological impression on chest X-ray while 15 of them had normal findings. Excluding patients with normal radiological findings, 23.38%

(n=83/355) with abnormal chest X-ray had acquired PTB. The upper lobe infiltrates (36.4%) and pleural effusion (40.4%) were evident in PTB patient; nevertheless, other impressions, like hilar/mediastinal lymphadenopathy (19.2%), cavitary lesion (15.6%), diffuse infiltration (12.2%), segmental/lobar consolidation (3.2%), were also noted.(Table 3)

MDR case findings with Gene Xpert and its' confirmation

In our study, 94 MDR suspected cases were enrolled. Among them, 29 samples were found rifampicin sensitive, 1 indeterminant while 3 of them were rifampicin-resistant. Of total MDR cases, 2 of them had a previously treated history with ATT.

MDR confirmation was done with Line Probe Assay where distinct bands revealing the genomic sequences i.e. TUB(+), rpoBWT(-), rpoBMUT(+), kat GWT(-), kat GMUT(+) ,inhAWT(+) , inhAMUT(-);RMP(resistant), INH(resistant) was observed(Fig.2)

Discussion

Our findings revealed an application of Xpert MTB/RIF assay can substantially increase the yield of confirmed PTB cases. During our research period, 4.6% smear-positive PTB cases were reported at TUTH while upon execution of gene Xpert as diagnostic tool, therefore resulted in 16.5% (n=85/515) surge of confirmed TB cases. Our study is consistent with other studies, conducted in developing countries, which have suggested the benefit of Xpert in smear-negative patients(6)(7)(8)(9).

We aimed to include all possible positive cases, the infective forms i.e. PTB and tries to break the transmission chain with speedy diagnosis as far as possible. Hence, we opted the Xpert MTB/RIF assay. In most, research studies and meta-analysis performed to this date, higher specificity of the test up to 99% has been observed on sputum samples; although, lower on other body fluids(10)(11)(12)(7). Hence we include the test as an alternative to culture—plus of its extra benefits of speedy detection along with its inherent drug-resistance status of the pathogen.

In our study, we compare, likely occurring clinical features present in PTB patients with that gene Xpert results. The only clinical feature to show a statistically significant difference between the groups was cough and chest pain. As portrayed with the latest meta-analysis and perspectives study the sensitivity of cough as a positive predictive value from 79.9% to 82% (13)(14). Hence, these clinical features could be one of the triaging features in optimizing the efficacy of the test.

The cost expenses is another tethering truth with which the developing countries are being suffering. NTP, Nepal with a policy to economize cost per cartridge on par to patient's number (due to constricted resources) and also to optimize the efficacy of test on targeted population, had endorsed a guideline for the test. As per the guideline, the chest X-ray was taken as an exclusive triaging test. Relying upon the guideline, we included the chest X-ray as a triaging test. Referring to our study, the common radiological findings evident in positive cases were upper lobe infiltrates (36.4%) and pleural effusion (40.4%). A

similar study was conducted in our nearby hospital and neighboring country(India) where upper-lobe infiltrates and cavitary lesions were the common features(4)(15). Further, as to extrapolate the role of X-ray in PTB diagnosis, we compared the relationship between abnormal impressions vs. positive cases. We found, 23.38% of patients with abnormal chest X-ray had acquired PTB while Dutta et al. reported 34.4% in patients with similar features (15)

Although, a higher number of new suspects were found positive on Gene Xpert testing i.e. new suspects (n=63), previously treated cases (n=20), and loss to follow up (n=2). 2 MDR cases were recovered from the patient with a previous history of ATT. However, a small population of these categories was included in our study, the findings portrayed a picture similar to a drug resistance survey conducted in 1992–1993 in the Western Cape Province where 8.6% acquired and 3.2% initial drug resistance noted in a previously treated patient (16). In another study, conducted in high burden region, the highest i.e. >15%, MDR-TB was attributed in previously treated patient(17). Besides, the previously treated patients may be on high risk to extensively drug-resistant(XDR)TB (16).

Of total positive cases, 3.5% (n=3) were valid rifampicin-resistant (confirmed with Line probe assay); no false-positive rifampicin resistance was noted as reported in different literature with gene Xpert testing(12)(10)(18)(19). Round the globe, about one-third of the countries had surveyed on the incidence of MDR-TB which was in between 2-14%(20)(21)(22); our findings coincide within this range.

For improving patient care and abbreviating the disease transmission chain, speedy detection of tuberculosis and its drug-resistance with precision is crucial. In this perspective, endorsing gene Xpert as a diagnostic tool by WHO is the commendable action; further making it more accessible, affordable, and even upgraded version covering disseminated TB also could be a wise investment to restrict the global burden.

Limitations

Inability to include samples other than sputum was the major limitation of our study since a larger number of disseminated TB could be missing from our study frame. Some positive cases might be skipped as negative even with gene Xpert since is not of absolute accuracy. Also, we could not run the phenotypic DST and Line Probe assay for all Xpert MTB/RIF positive specimen. If it was possible, clear MDR status could be traced which might be missed even from Xpert MTB/RIF assay.

Conclusion

Xpert/MTB RIF assay yields an additional case detection of PTB along with their drug-resistance status which is neglected as negative with conventional diagnostic tests. Hence, its' recommendation for every suspect as a presumptive test could be a wise investment in diagnosis, clinical management, prevention, and control.

Declarations

Authors' contributions

PK conceived the study, design the manuscript, review of the literature. RBB, JT, GPR, and JA, reviewed the manuscript and gave the concept of the research paper. BPPR critically reviewed the manuscript. All authors read and approved the manuscript.

Acknowledgments

We would like to thanks Dr. Kedar Narshing KC (Medical Director of NTC) for his tremendous technical support.

Competing interest

The authors declare that they have no competing interests.

Availability of data and materials

Data generated or analyzed during this study are included in this manuscript and remaining are available from the corresponding author on reasonable request.

Consent to publish

Not applicable.

Ethics approval and consent to participate

This research was approved by the Institutional Review Committee of National Tuberculosis Center and Trichandra Multiple Campus, Nepal.Written informed consent was taken from all patients or their parents before participating in the study. Data regarding personal information and infectious disease were coded and kept confidential.

Funding

Not applicable (Nil)

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Tables

Table 1- Patients' demographics

ents ographics	smear-negative PTB not detected with Xpert MTB/RIF assay	smear-negative PTB detected with MTB/RIF assay	Total
ler			
	177	60	237
ale	98	25	123
group			
ear	36	9	45
29 years	65	8	73
44 years	62	14	76
59 years	53	25	78
ears	59	29	88
al history			
suspects	205	63	268
ously treated	69	20	89
o-follow up	1	2	3

Table 2- Comparison of clinical features of patients with Xpert MTB/RIF assay result.

Clinical features		Result of Gene Xpert MTB/Rif		Total (%)	P value	Relative risk (95%CI)
		Not detected (%)	Detected (%)			
Fever	Y	207(74.7)	70(25.3)	277(100)	0.18	1.39(0.84-2.30)
	N	68(81.9)	15(18.1)	83(100)		
Cough	Y	242(74.7)	82(25.3)	324(100)	0.02	3.03(1.01-9.11)
	N	33(91.7)	3(8.3)	36(100)		
Weight loss	Y	229(76.1)	72(23.9)	301(100)	0.86	1.08(0.64-1.82)
	N	46(78.0)	13(22)	59(100)		
Chest pain	Y	87(59.1)	60(40.9)	147(100)	0.01	
	N	188(88.2)	25(11.8)	213(100)		3.47(2.29-5.27)
Night sweat	Y	146(76.0)	46(24)	192(100)	0.99	1.03(0.71-1.49)
	N	129(76.8)	39(23.2)	168(100)		

Table 3- Comparison of chest X-ray features of patient enrolled with Xpert MTB/RIF assay result.

Features on chest Xray	Result of Xpert MTB/Rif assay		Total(n)
	Not detected (%)	Detected (%)	
Upper lobe infiltrate (%)	70a(63.6)	40b(36.4)	110
Pleural effusion (%)	34a(59.6)	23b(40.4)	57
Hilar /mediastinal lymphadenopathy (%)	21a(80.8)	5a(19.2)	26
Cavitary leision (%)	27a(84.4)	5a(15.6)	32
Normal (%)	13a(86.7)	2a(13.3)	15
Diffuse infilteration (%)	29a(87.9)	4a(12.1)	33
Abnormal (%)	51a(91.1)	5b(8.9)	56
Segmental/lobar consolidation(%)	30a(96.8)	1b(3.2)	31
Total Count (%)	275(76.4)	85(23.6)	360

*Each subscript letter denotes a subset of result of Xpert MTB /RIF test categories whose column proportions do not differ significantly from each other at the .05 level

Additional File Legend

Additional file-1: Algorithm of gene Xpert testing

Figures

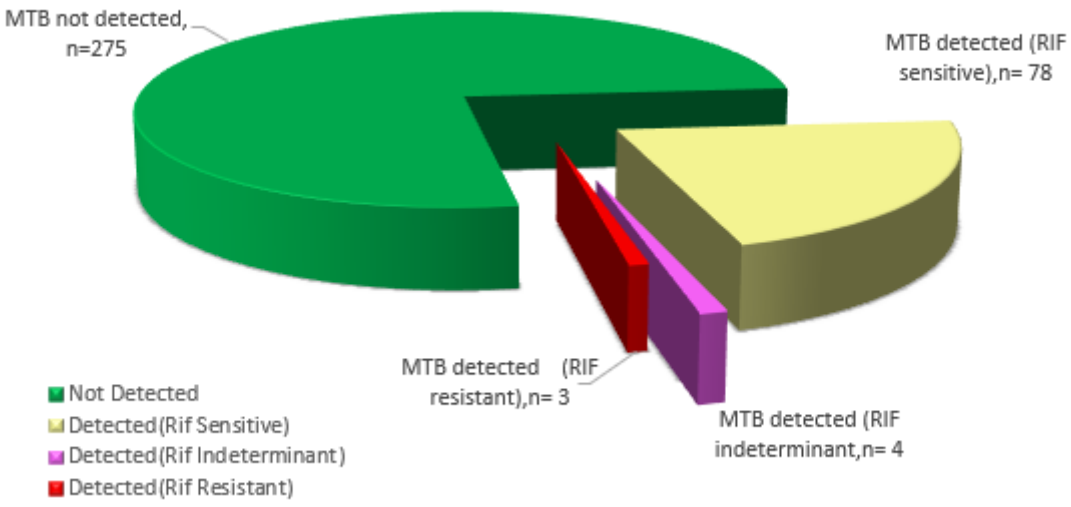

Figure 1

The total case detection of smear-negative pulmonary tuberculosis with Xpert MTB/RIF Assay is shown in the pie chart. Each portion of pie chart indicates the number of enrolled cases and their result.

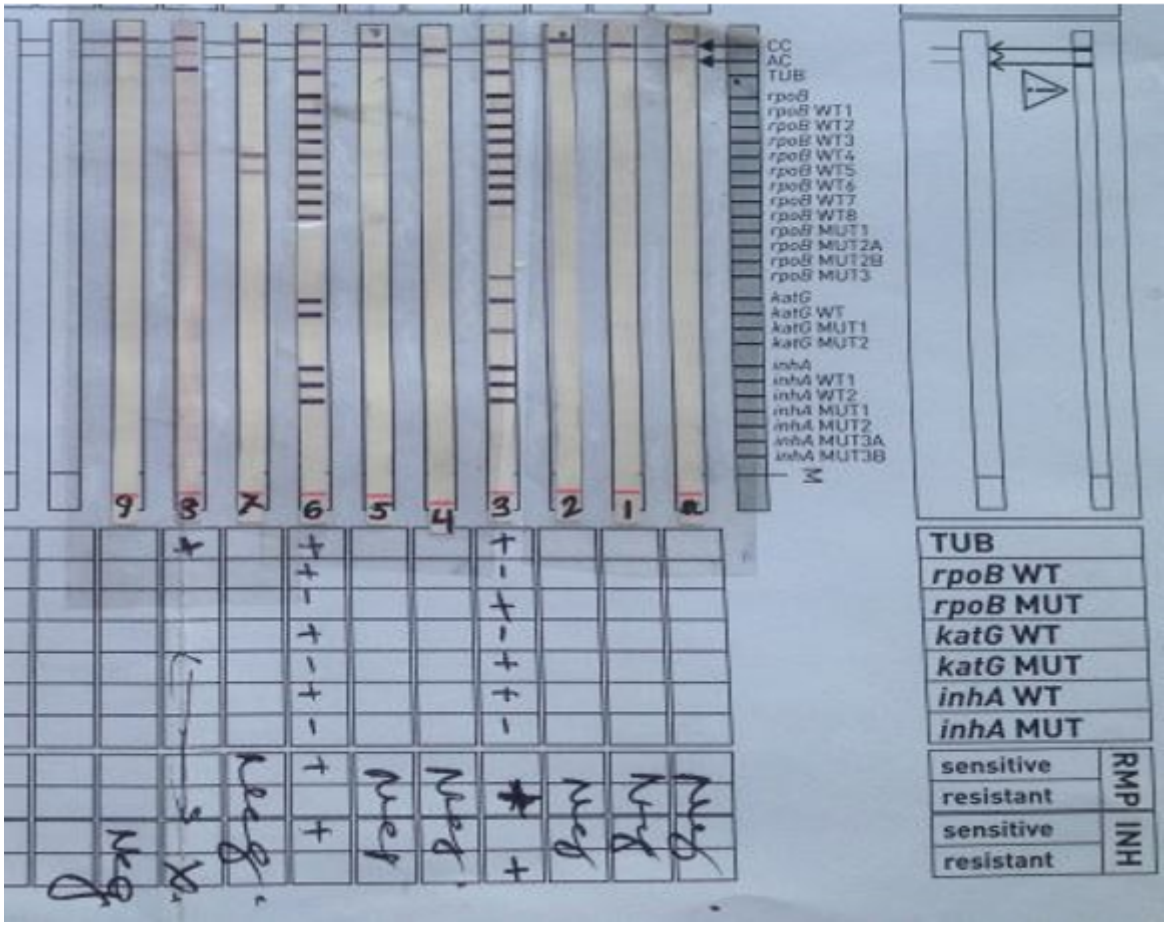

Figure 2

Line probe assay showing mutated genomic sequences.

Supplementary Files

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- supplement1.pdf