Role of CB-NAAT in Diagnosis of Mycobacterial Tuberculosis and Rifampicin Resistance among Key Population under Programmatic Condition in Gujarat, India

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Abstract :

Introduction : India is the country with the highest burden of TB infection. The World Health Organization has endorsed the Gene Xpert MTB/RIF assay for rapid detection of tuberculosis with rifampicin resistance. Testing specimens with CB-NAAT can detect pauci bacillary mycobacterial tuberculosis which is potentially contribute to microbiological confirmation of tuberculosis. Diagnosing tuberculosis (TB) in people living with HIV/AIDS (PLHIV) , paediatric age group and extrapulmonary samples is challenging as microscopy is negative due to low bacillary load .TB culture is a slow method which takes 2-6 weeks for growth of the mycobacteria. **Objective :** To assess the the role of Cartridge based nucleic acid amplification test (CBNAAT) to diagnose TB and rifampicin resistance in PLHIV , paediatric age group and extrapulmonary samples. **Method :** The study is based on the secondary analysis of data derived from testing by Xpert MTB/RIF testing among presumptive TB cases of HIV/AIDS (PLHIV), paediatric age group and extrapulmonary samples in Gujarat. Under this study, 28,304 presumptive TB cases of HIV/AIDS (PLHIV), paediatric age group and extrapulmonary samples were tested between January to September 2019. **Results:** Overall, 1,40,177 specimens were tested, of which 10,018 (7.14%) samples were PLHIV presumptive TB , 7,380 (5.26%)samples were Paediatric Presumptive TB and 10,906(7.78%) samples were extrapulmonary Presumptive TB. These 28,304 presumptive cases , in 3994(14.11%) cases TB detected. Out of these 3994 TB detected cases , 1068 were PLHIV Presumptive TB , 724 were paediatic presumptive TB and 1987 were extrapulmonary presumptive TB. Total 2220 rifampicin resistant TB cases were detected from January 2019 to September 2019 , of which 288(10.27%) were rifampicin resistant TB in key population .Out of these 288 rifampicin resistant TB cases 63 were PLHIV Presumptive TB ,47 were paediatic presumptive TB and 178 were extrapulmonary presumptive TB cases. **Conclusion :** CBNAAT has advantages of rapid case detection bacteriologically confirmed TB in less than 2 hours and simultaneously detecting rifampicin resistance in PLHIV , paediatic age group and extrapulmonary samples in which bacillary load is very low .

Key Words : CBNAAT, Key population, Paediatric Extrapulmonary, PLHIV, Presumptive tuberculosis

Introduction :

Tuberculosis is the commonest infectious disease caused by Mycobacterium tuberculosis complex worldwide. Worldwide, TB is one of the top 10 causes of death and the leading cause from a single infectious agent (above HIV/AIDS). Millions of people continue to fall sick with TB each year. According to global tuberculosis 2018 WHO reports , 100 lakhs newly detected cases and 13 lakhs death due to tuberculosis.[1] It is estimated that approximately 70 million people die from tuberculosis within 20 years and it is because of inadequate measures for TB control.[2] Standard sputum based diagnostic methods to detect pulmonary tuberculosis include sputum microscopy and culture . However in Key population like PLHIV , Paediatric patients and extrapulmonary infection due to paucibacillary

condition of mycobacterial Tb , microscopy is very less sensitive and specific diagnostic tool.

To overcome these problems, sputum culture and sensitivity for Mycobacterial Tb can be used but this technique is usually takes 4 to 8 weeks , and not cost effective for screening purpose. This delays initiation of anti-tuberculosis treatment leads to transmission of Tb in the community and increase risk of spread of pulmonary Tb to extrapulmonary site. CBNAAT is automated cartridge based nucleic acid amplification test assay, having integrated and automated amplification and detection using real time PCR, provide result within 2 hours. It is highly specific test as it uses 5 unique molecular probes to target rpoB gene of M. Tuberculosis which detect M. Tuberculosis and rifampicin resistance.

Diagnosis is often difficult because of low number of bacilli and scanty sputum production due to lack of caseous necrosis in PLHIV, Difficult to collect sputum sample in children and collection of extrapulmonry sample is the major challeng

Objective :

This study was carried out to evaluate role of CBNAAT in early diagnosis of TB and rifampicin resistance in key population like PLHIV, Paediatric and extra pulmonary samples .

Method :

Study Design : This study was a secondary analysis

Study participants : Samples from of presumptive TB cases of HIV/AIDS (PLHIV) , paediatric age group and extrapulmonary patients

Study Duration: Samples received from January to September 2019.

Study site: Data collected from 60 CBNAAT sites across the Gujarat state, India.

Sample Collection: For PLHIV patients sputum samples and in pediatric age group sputum or gastric lavage collected with complain of cough more than 2weeks / weight loss /low grade fever or X-ray suggestive of pulmonary tuberculosis/ history of contact with infectious TB cases and extrapulmonary cases organ specific samples like Pus, Lymphnode, Pleural fluid, CSF, Ascitic fluid ,synovial fluid ,bone etc. were collected. These all samples were tested upfront in CBNAAT. From collected sample 1 ml was separated in sterile container and was analyzed by CBNAAT on Xpert MTB/RIF manufactured by cephaid, endorse by WHO(2010).The sample was diluted with three times the reagent ,incubated at room temperature for 15 minutes and loaded cartridge in to the CBNAAT machine for automated analysis with result within 2 hours. CBNAAT machine will detect mycobacterial tuberculosis complex and rifampicin resistance simultaneously.

Data analysis: Data was analysed using Microsoft Excel.

Results:

Overall, New TB diagnosis(Smear+ve/-ve), Contact of MDR/RR TB patients , Follow up patients whose Smear +ve, HIV TB co-infected ,private sector and Presumptive Tuberculosis etc. total 1,40,177 specimens were tested for tuberculosis in CBNAAT.

Upfront CBNAAT testing in 28304 samples were done in presumptive TB cases in PLHIV , Peadiatic and Extrapulmonary patients .10,018(7.14%) samples were PLHIV presumptive TB , 7,380 (5.26%)samples were Paediatric Presumptive TB and 10,906(7.78%) samples were extrapulmonary Presumptive TB.

CBNAAT diagnosed tuberculosis complex in 3994(14.11%) patients of total 28304 presumptive tuberculosis samples . out of these 3994 diagnosed presumptive case 1068 were PLHIV Presumptive TB ,724 were Paediatric Presumptive TB ,2202 were EP Presumptive TB. (Table 1)

In these 3994 mycobacterium complex detected presumptive case 288(7.21%) were rifampicin resistant. Out of 288 rifampicin resistant mycobacterium tuberculosis complex 63 cases were presumptive PLHIV , 47 were presumptive paediatric, 178 were extrapulmonary cases. Which indicating that in key population 5.90% , 6.49% , 8.08 % rifampicin resistance detected in PLHIV , Pediatric and extrapulmonary cases respectively. (Table 2)

Discussion :

Upfront CBNAAT testing was offered to all presumptive TB cases in defined 60 CBNAAT laboratory in Gujarat. Participating providers were linked through rapid specimen transportation linkages and rapid result reporting mechanisms.

Table 1: Mycobacterium complex detection by upfront CBNAAT testing in key population

Presumptive Tb Cases	Samples tested for CBNAAT	Mycobacterium complex present by CBNAAT	Mycobacterium complex absent by CBNAAT	Invalid / errors in results
PLHIV Presumptive TB	10,018	1068(10.66%)	8806(87.90%)	144(1.43%)
Paediatric Presumptive TB	7,380	724(9.81%)	6569(89.01%)	87(1.17%)
Extrapulmonary Presumptive TB	10,906	2202(20.19%)	8595(78.81%)	109(0.99%)
TOTAL	28,304	3994(14.11%)	23970(84.69%)	340(1.20%)

Table 2: Rifampicin resistance detected by upfront CBNAAT key population

Presumptive Tb Cases	Mycobacterium complex detected by CBNAAT	Rifampicin resistance detected
PLHIV Presumptive TB	1068	63 (5.90%)
Paediatric Presumptive TB	724	47 (6.49%)
Extrapulmonary Presumptive TB	2202	178 (8.08 %)
TOTAL	3994	288(7.21%)

CBNAAT testing was extended to non-sputum specimen under routine programmatic conditions in India, in line with the recent WHO recommendations [3] This led to overall improvement in bacteriologically confirmed TB cases, as well as detection of significant numbers of rifampicin resistant TB cases in presumptive TB cases. All the TB cases diagnosed under the project were notified under RNTCP irrespective of type of referring provider.

Smear microscopy for AFB is simple ,economical and easy to test for diagnosis of tuberculosis. However , it needs at least 10,000 bacilli per ml to give a positive result and being highly subjective (operator dependent) test. Its sensitivity has been shown to range from 20 to 60% under different condition.[4] This sensitivity is further decrease in PLHIV due to lower rates of caseous necrosis and sputum production which leads to paucibacilli in sputum[5] For children specimens like gastric lavage and induced sputum is difficult which indicate that presumptive TB and DR Tb in childrens may be underdiagnosed. Current World Health Organization guidelines advise that all children <5 years of age who are in close contact with sputum smear positive index patient should be actively traced , screened for TB and provided preventive chemotherapy after active TB has been excluded.[6] And extrapulmoary samples due to low number of bacilli , its challenging to diagnose Tb by direct Zn smear microscopy. Utilization of upfront use of CBNAAT in these key population improve bacteriological confirm cases of tuberculosis with Rifampicin susceptibility.

CBNAAT performance on both sputum and non-sputum was found to be highly satisfactory, with overall 98.80% cases getting valid results. These findings are similar to other studies conducted on CBNAAT assay on sputum and non-sputum specimens[7-12]. Polymerase chain reaction inhibition leading to invalid test results is a major concern while testing specimens on various types of molecular assays, especially non-sputum specimen[13–15] Invalid or false negative results in various PCR based tests are mostly due to the presence of inhibitors, sub-optimal assay conditions or omission of key steps[16] However, this issue was seen to be of lesser concern on CBNAAT due to automation and self contained test which offers minimal manual manipulation of samples which is leading to low PCR inhibition rates.

NeerajRaizada[21]et al study indicating 6.3 % and 8.10 % M.Tb detection and Rifampicin resistanace in pediatric age group with presumptive tuberculosis

which is similar to our study.[17] This is indicating that by offering upfront CBNAAT to presumptive case we can diagnose M.Tb with resistance of rifampicin .Pediatric case of tuberculosis is directly related to contact of Tb patient so by diagnosing Tb in peadiatric case we can trace tuberculosis in adult also .We should focus contact tracing on pediatric tuberculosis.

Lesley Erica Scott et all study on Extra pulmonary samples , incidence of 22.13% M.tb in extra pulmonary samples of which 9.6% where Rifampicin resistant which is similar to our study.[18] Providing upfront CBNAAT to extrapulmonary samples reduce diagnostic delay and provide microbiological confirm report to clinician.

According to 2019 global report total of 4,77, 461 TB cases among HIV-positive people were reported till 2018. In 2018 , globally 937 500 cases were newly enrolled in HIV care , out of these 79 285 notified as TB case. In India 29 766 cases were noted as new TB HIV co-infected cases.[19] TB is the leading cause of death among people living with HIV. Persons co-infected with TB and HIV are more likely to develop active TB disease than persons without HIV infection[20] Neeraj Raizada et al published article in 2015 , Enhancing TB & DR-TB Detection by proving proving Upfront Xpert MTB/RIF Testing for people living with HIV in India which shows 28% detection of M.Tb and in these detected cases 9.5% were Rifampicin resistant. [21] These data shows detection of Tuberculosis and rifampicin resistant is higher compare to our study in which 10.66 % were M.Tb detected and 5.9% were rifampicin resistence in preumptive Tb in PLHIV. This may be due to geographical variation of study conducted in India. As HIV related immune-suppression increases, the clinical pattern of TB disease changes, with increasing numbers of smear-negative and extra pulmonary cases [22]

Sputum smears tend to be negative, as tubercle bacilli do not appear in sputum because of the paucity of pulmonary inflammation at early onset of disease and decreased cavitation. Further, though TB is the most common opportunistic infection among PLHIV, clinical decision-making is complicated because HIV infection broadens the scope of differential diagnosis of smear-negative pulmonary TB to include diseases such as Pneumocystis carinii pneumonia (PCP), pulmonary Kaposi's sarcoma, and Gram-negative bacteremia[23]

Furthermore, up to one third of HIV-TB co-infected cases might have completely normal chest radiographs due to less cavitation leading to increased chances of under diagnosis or missed diagnosis of TB in such cases. [24] Culture of sputum for M. tuberculosis though considered as the gold standard, is difficult to use and in resource-limited settings challenging to implement[25] Culture result provided after 2–8 weeks are not available to guide immediate treatment decision-making needs[26] Capacity of CBNAAT is to diagnose 131 cfu/ml TB bacilli and Rifampicin resistance in one cartridge so that it is promising tool for diagnosis of TB in PLHIV and starting early treatment .

Conclusion:

CBNAAT has advantages of rapid case detection bacteriologically confirmed TB in less than 2 hours and simultaneously detecting rifampicin resistance in key population like PLHIV , paediatric age group and extrapulmonary samples in which bacillary load is very low . This rapid turn around time of CBNAAT will helpful to start early treatment under field conditions. Upfront CBNAAT , leading to overall strengthening of care and support package for PLHIV, Pediatric group and Extrapulmonary presumptive Tuberculosis diagnosis under programmatic condition.

Declaration:

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Conflict of interests: Nil

References:

1. Global tuberculosis report 2018 WHO, https: // www. aidsdatahub.org/global-tuberculosis-report-2018-who-2018
2. Giri PK ,Khuller GK. Is intranasal vaccination a feasible solution for tuberculosis? Expert Rev vaccines 7,2008 ,1341-1356
3. World Health Organization. Policy update: automated real time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system for the diagnosis of pulmonary and extrapulmonary TB in adults and children 2013; Available http://apps.who.int/iris/bitstream/10665/112472/1/9789241506335_eng.pdf?ua=1. Accessed 26 August 2014.

4. Rdewan et al , Role of CBNAAT for early diagnosis of pulmonary tuberculosis in HIV ,Journal, Indian Academy of clinical Medicine . April-june ,2015; 16 ; 2 ;114-17.
5. Hopewell p, Pai M ,Maher D et al.International standards for tuberculosis care. Lancet Infect Dis 2006;6:70-25.
6. World Health Organization. Guidelines on the Management of Latent Tuberculosis Infection. Geneva, Switzerland: World Health Organization; 2015
7. Raizada N, Sachdeva KS, Sreenivas A, Vadera B, Gupta RS, Parmar M, et al. Feasibility of Decentralised Deployment of Xpert MTB/RIF Test at Lower Level of health System in India, 2014; PLOS ONE 9(2): e89301. , Available: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0089301 pmid:24586675
8. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, et al. Rapid Molecular Detection of Tuberculosis and Rifampin Resistance, 2010; New England Journal of Medicine, 363: 1005–1015 Available: http://www.nejm.org/doi/full/10.1056/NEJMoa0907847. pmid:20825313
9. Boehme CC, Nicol MP, Nabeta P, Michael JS, Gotuzzo E, Tahrili R, et al. Feasibility, diagnostic accuracy, and eff effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study, 2011; Lancet 377: 1495–1505 Available : http://www.ncbi.nlm.nih.gov/pubmed/21507477pmid:21507477
10. Steingart KR, Sohn H, Schiller I, Kloda LA, Boehme CC, Pai M DN. Xpert ® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults (Review). Chochrane Database Syst Rev, 2013; Available: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD009593.pub2/pdf
11. Scott LE, Beylis N, Nicol M, Nkuna G, Molapo S, Berrie L, et.al. The diagnostic accuracy of Xpert MTB/RIF on extra pulmonary tuberculosis specimens: 2 Establishing a laboratory testing algorithm for South Africa, 2014; Journal of Clinical Microbiology, , Available: http://jcm.asm.org/content/early/2014/03/06/JCM.03553-13.full.pdf+html
12. Hillemann D, Gerdes SR, Boehme C, Richter E. Rapid Molecular Detection of Extrapulmonary Tuberculosis by the Automated GeneXpert MTB/RIF system, 2011; Published ahead of print 26 January 2011, J. Clinical. Microbiology. April 2011 vol. 49 no. 4 1202–1205, Available: http://www.ncbi.nlm.nih.gov/pubmed/21270230
13. Antonenka U, Hofmann-Thiel S, Turaev L, Esenalieva A, Abdulloeva M, Sahalchyk E, et.al. Comparison of Xpert MTB/RIF with ProbeTec ET DTB and COBAS TaqMan MTB for direct detection of M. tuberculosis complex in respiratory specimens, 2013; BMC Infectious Diseases 2013, 13:280 doi: 10. 1186/1471-2334-13-280, Available: http://www.biomedcentral.com/1471-2334/13/280 PMID: 23786563
14. Eing BR, Becker A, Sohns A, Ringelmann R.Comparison of Roche Cobas Amplicor Mycobacterium tuberculosis Assay with In-House PCR and Culture for Detection of M. tuberculosis, 1998; J. Clinical Microbiology, July 1998 vol. 36 no. 7 2023–2029, Available: http://jcm.asm.org/content/36/7/2023.
15. GV, Voit A, Ritter C, Deggim V, Böttger EC. Evaluation of Cobas TaqMan MTB for Direct Detection of the Mycobacterium tuberculosis Complex in Comparison with Cobas Amplicor MTB, 2013;doi:10.1128/JCM.00142-13JClin.Microbiol.July2013vol. 51no.72112–2117,Available: http://jcm.asm.org/content/51/7/2112
16. World Health Organization, Policy Statement : Molecular Line Probe Assays For Rapid Screening Of Patients At Risk Of Multidrug-Resistant Tuberculosis (MDR-TB), 2008; pp6–7 Available:http://www.who.int/tb/features_archive/policy_statement.pdf.
17. Raizada N, Sachdeva KS, Swaminathan S, Kulsange S, Khaparde SD, Nair SA,et al. Piloting upfront Xpert MTB/RIF testing on various specimens underprogrammatic conditions for diagnosis of TB & DR-TB in paediatric population. PLoS ONE 2015; 10:e0140375.
18. Lesley Erica Scott,a Natalie Beylis,d,e Mark Nicol,c,d Gloria Nkuna,a Sebaka Molapo,b Leigh Berrie,b Adriano Duse,d,e Wendy Susan Stevensa,b .Diagnostic Accuracy of Xpert MTB/RIF for Extrapulmonary Tuberculosis Specimens: Establishing a Laboratory Testing Algorithm for South Africa. Journal of Clinical Microbiology. June 2014 Volume 52 Number 6. p. 1818–1823
19. Global tuberculosis report 2019, World health organization https://www.who.int/tb/publications/global_report/en/
20. TB HIV Facts 2012–2013, Available: http://www.who.int/hiv/topics/tb/tbhiv_facts_2013/en/. Accessed 2014 May 20.
21. Neeraj Raizada, Kuldeep Singh Sachdeva, Achuthan Sreenivas, Shubhangi Kulsange, Radhey Shyam Gupta, Rahul Thakur, Puneet Dewan, Catharina Boehme, Chinnambedu Nainarappan Paramsivan Catching the Missing Million: Experiences in Enhancing TB & DR-TB Detection by proving Upfront Xpert MTB/RIF Testing for people living with HIV in India. PLoS ONE 10(2): e0116721. doi:10.1371/journal. pone.0116721.
22. Agarwal S, Roy D, Chauhan LS. Book Chapter 16 TB-HIV Co-infection: A Lethal Combination, Available:http://tbcindia.nic.in/pdfs/Tuberculosis%20Control%20in%20India16.pdf. Sputum smears tend to be negative, as tubercle bacilli do not
23. Hargreaves N, Scano F (2003) Guidelines for Implementing Collaborative TB and HIV Programme Activities, World Health Organization, Geneva, 2003;7,9,11,13–15. Available: http://www.popline.org/node/241599 PMID: 25057689
24. Cain KP, McCarthy KD, Heilig CM, Monkongdee P, Tasaneeyapan T, et. al. (2010) An algorithm for tuberculosis screening and diagnosis in people with HIV. N Engl J Med 2010; 362:707–16, Available: http://www.ncbi.nlm.nih.gov/pubmed/20181972 doi: 10.1056/NEJMoa0907488 PMID: 20181972
25. Centres for Disease Control and Prevention, Morbidity and Mortality weekly report (MMWR Weekly) / Vol.61/ No.26 /, July 06, 2012. Available: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6126a3.
26. World Health Organization (2009) Pathways to better diagnostics for tuberculosis: a blueprint for the development of TB diagnostics by the new diagnostics working group of the Stop TB Partnership