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Xpert MTB/RIF – A new gold standard for extrapulmonary Tuberculosis?

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Abstract

Background: Tuberculosis infection can develop in the forms of Pulmonary as well as Extra Pulmonary form which are seen as major complications in HIV/AIDS. Tuberculosis is still a diagnostic dilemma in low resource countries. In cases of Extra Pulmonary tuberculosis, the problem is further compounded by presence of low numbers of bacilli in patient sample. We have made an effort to compare the results of microscopy with results of Xpert MTB/ RIF assay in evaluation of Extra Pulmonary samples

Materials and Methods: 139 consecutive samples from patients suspected to have any form of extra pulmonary tuberculosis were subjected for microscopy with Ziehl Nielsen staining and simultaneous testing for of Xpert MTB/ RIF assay. The results were compared.

Results: Xpert MTB/RIF positivity for MTB observed in 56.12% (78/139) while 61 (43.88%) were negative on Xpert MTB/RIF. Out of 125 ZN smear negative subjects 66 (52.8%) were found to be positive for MTB by Xpert MTB/RIF. Out of 14 ZN smear positive cases, Xpert MTB/RIF negative cases were found to be 2 (14.28%).

Keywords: Extra Pulmonary Tuberculosis, Xpert MTB/ RIF.

Introduction

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. It usually affects the lungs (pulmonary form, PTB) but can produce disease at other sites as well (extrapulmonary form, EPTB). The disease is commonly spread when persons who are affected with pulmonary TB expel bacteria by coughing. Of the 9.6 million new TB cases detected in 2014, India accounted for largest number of cases (23%). Overall, a small proportion (5–15%) of the people infected with M. tuberculosis will develop tuberculosis disease during their lifetime. However, the chances of developing the disease is much higher among people co-infected with HIV.[(1)]

Early diagnosis of tuberculosis is an important step to allow timely initiation of anti tubercular treatment (ATT). For a long time the diagnosis tuberculosis has been around sputum microscopy for the detection of acid fast bacilli (AFB) and by specific mycobacterial culture. Cultures done by conventional methods take up to 6 to 8 weeks for positivity. Smear microscopy using Ziehl-Nielsen (ZN) staining is usually the first modality of microbial analysis performed at the clinical suspicion of pulmonary tuberculosis, this modality has been around close to 100 years. Main reasons for using the ZN smear microscopy is its high specificity, low cost, not requiring sophisticated equipment or high laboratory standards. Provisional results can be obtained usually within two hours; however test is less sensitive and normally requires bacilli as high as 5000- 10,000 per ml of specimen for the smear to become positive.[(2)] For diagnosis of PTB, doctors in the developing world often rely only on chest X-rays without referring patients for sputum smears; confirmation by culture is also not done routinely.

Globally, an estimated 3.3% of new TB cases and 20% of previously treated cases have multi drug resistant tuberculosis (MDR-TB), a level that has changed little in recent years. An estimated 9.7% of people with MDR-TB have XDR-TB. An unprecedented rise in the incidence of infections caused by *Mycobacterium tuberculosis* coupled with emergence of MDR and XDR strains has prompted the need for rapid diagnostic techniques. The use of the rapid test Xpert MTB/RIF® has expanded substantially since 2010, when WHO first recommended its use.[(3)] This assay is a hemi-nested real-time PCR assay for the diagnosis of TB as well as rapid detection of rifampin (RIF) resistance in clinical specimens within two hours.

EPTB is equally found to be prevalent in all the high burden zones. According to WHO, 34,000 (15%) of newly reported TB cases in 2007 were extrapulmonary.[(4)] The diagnosis of EPTB is difficult to establish due to the low number of bacteria in clinical specimens. Rapid and accurate diagnosis of pulmonary and extrapulmonary TB is still a great challenge in developing countries due to limited resources and a lack of laboratory expertise.[(5)] Data originating from sputum microscopy studies reveals Xpert MTB/RIF assay is able to diagnose significantly high number of cases compared to microscopy alone and has been regarded as a high sensitive and specific diagnostic modality. This prompted us to evaluate Xpert MTB/RIF assay for extra pulmonary specimens.

Aim of this study was to assess the value of Xpert MTB/RIF Assay in diagnosis of extra pulmonary tuberculosis in comparison with Ziehl Nielsen smear microscopy and to observe its additional diagnostic value.

Methodology

This study was conducted between Jan 2011 to Sep 2016 in the Tuberculosis Laboratory of a tertiary care tuberculosis hospital, based in Pune, India. The hospital is a 600 bedded hospital and has facilities for in patient treatment of tuberculosis. The specimens collected were from patients with suspected extra-pulmonary tuberculosis infection on the basis of clinical criteria. Samples included were Biopsy, Bone Marrow aspirates, CSF, Gastric Aspirate, Pericardial fluid, Pus, lymph node aspirates, pleural fluid and Synovial fluid. Their details are given in Table 1. Since the samples were mostly obtained from sterile sites, no decontamination was contemplated. Samples from serous cavities were concentrated by centrifugation at 3000g for 20 minutes and the deposit was processed for ZN staining and Xpert MTB/RIF assay. Smears from lymph node aspirates were directly submitted for microscopy and approx 1 gm of aspirated material was mixed with diluents fluid and was processed for Xpert MTB/RIF assay. For tissue biopsies, impression smears and histopathological examination were undertaken to look for the presence of Acid Fast Bacilli. Approx 1 gm of tissue was homogenized with sterile normal saline and was processed for Xpert MTB/RIF assay.

Results

A total of 139 patients were included in this study, 108 (77.69%) of which were males and 31 (22.31%) were females. Male to female ratio was 2.77:1.

Varieties of extra-pulmonary specimens were included in this study. Greatest proportion was covered by lymph node aspirates consisting of 91 (65.47%), pus 21 (15.11%) and pleural fluid 11 (7.91%) (Table 1). Smear positivity was very rare and found to be 10.07% (14/139) and smear negative cases were 89.93% (125/139). Xpert MTB/RIF positivity for MTB observed in 56.12% (78/139) while 61 (43.88%) were negative on Xpert MTB/RIF. Out of 125 ZN smear negative subjects 66 (52.8%) were found to be positive for MTB by Xpert MTB/RIF. Out of 14 ZN smear positive cases, Xpert MTB/RIF negative cases were found to be 2 (14.28%) (Table 2).

Effort was made to categorize extent of positivity in Xpert MTB/RIF in both smear positive and negative cases (Fig. 1). It is observed that in smear negative cases there were lower levels of detection by Xpert MTB/RIF compared to higher levels of detection in smear positive cases. Comparison of ZN smear and Xpert MTB/RIF results for detection of MTB are shown in (Table 3).

Table 1: Details of samples obtained in suspected patients of extra-pulmonary tuberculosis

Sample	Numbers	Percentage
Biopsy	5	3.597122
Bone Marrow	2	1.438849
CSF	5	3.597122
Gastric Aspirate	2	1.438849
Pericardial fluid	1	0.719424
Pus	21	15.10791
lymph node aspirate	91	65.46763
pleural fluid	11	7.913669
Synovial fluid	1	0.719424
Total	139	

Table 2: Details of results obtained on different samples of extra-pulmonary tuberculosis

	Positive by ZN microscopy	Negative by ZN microscopy
MTB detected by GeneXpert	12	66
MTB not detected by GeneXpert	2	59

Table 3: Positive results comparing smear microscopy and Xpert MTB/RIF assay

Nature of specimen	Total	Smear positive		Xpert positive		Absolute difference
	N	N	%(95% CI)	N	%(95% CI)	
Biopsy	5	0	3.6-62.4	1	3.6-62.4	1
Bone Marrow	2	0	0-65.8	0	0-65.8	0
CSF	5	0	3.6-62.4	1	3.6-62.4	1
Gastric Asp	2	0	0-65.8	0	0-65.8	0
Pericardial fluid	1	0	0-79.3	0	0-79.3	0
Pus	21	4	7.7-40	15	50-86.2	11
lymph node aspirate	91	10	6.1-19.1	57	52.4-71.9	47
pleural fluid	11	0	0-25.9	3	9.7-56.6	3
Synovial fluid	1	0	0-79.3	0	0-79.3	0
	139	14		77		

Discussion

Extrapulmonary tuberculosis most often remains undiagnosed and, as a result gets untreated. A major hindrance to the diagnosis of Extrapulmonary tuberculosis is the atypical presentation, often simulating neoplasia and/or inflammatory disorders, also extrapulmonary specimens yield very few bacilli and as a result are associated with low sensitivity of acid-fast bacilli smear and culture.[6,7]

In case of pulmonary tuberculosis, in many of the resource poor countries, TB diagnosis depends mainly on smear microscopy which has a variable sensitivity ranging from 20% to 60% (10). The sensitivity performance still goes much below for extra pulmonary tuberculosis if we compare with culture or Xpert MTB/RIF assay. There is wide variation in smear positivity rates in studies conducted across the globe which range from 3.9% to 16.89%.[7,8,9]

In a study done at high prevalence setting Xpert MTB/RIF assay was able to pick up 22.5% cases while culture was able to demonstrate positivity in 10% cases comprising extrapulmonary specimens. In this above study smear microscopy was positive in 7.5% of cases.[4] In a study conducted by Tortoli et al. in on extrapulmonary samples in low prevalence country like Italy, the overall sensitivity and specificity of Xpert were found to be 81.3 and 99.8% respectively when compared to culture.[6]

Xpert MTB/RIF assay is also favorable if there if clinician wants to start the treatment without waiting for culture results, in a study conducted at a resource poor setting including both HIV negative and positive cases using sputum samples. The addition of Xpert testing increased bacteriologically-confirmed diagnoses by 27%.[11]

Other studies using fluorescent microscopy have documented superiority of Xpert MTB/RIF assay procedure translated by better case detection rate which ranges from 20.2% to 45.3%.[12,13]

The study has some limitations. We are aware that the role of MTB culture remains central in the microbiological diagnosis of Extrapulmonary tuberculosis, we have observed that cultures were not routinely requested from physicians, most likely reason seem to be a suspicion of either a reactive lesion or neoplasia in cases of tubercular lymphadenitis, and ordering smear microscopy as a ruling out measure. Xpert MTB/RIF have shown specificity of around 100% in previous studies,[6] so the possibility of over diagnosing bias due to false positive results is actually very small if someone does away with cultures. In addition, in our setting the outcome of culture in were found to be extremely low for whichever samples we found culture requests. During and after treatment, some patients continue to show dead bacilli in their specimens. Conditions where importance of culture lies would be such patients in whom there is presence of dead bacilli that will be detected both on microscopy and Xpert MTB/RIF but would not grow on culture.

We have not reported on sensitivity and specificity calculations in our study as the comparison with documented gold standard was not possible. However, the data presented shows a clear superiority of Xpert MTB/RIF assay over conventional smear microscopy which is the mainstay of diagnosis in many of the resource poor settings. The fact that the lower yield of bacilli the extra pulmonary specimen will pose in obtaining a positive culture has to emphasized. We now have a method which has capability of replacing time taking and technically demanding culture modality. It may be useful in obviating the need for routinely placing the cultures for extrapulmonary specimens altogether.

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