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Comparison Study of GENEXPERT versus TB MGIT Culture in Extra Pulmonary Tuberculosis

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Abstract Aims and objectives: To compare the diagnostic yield of Extra Pulmonary Tuberculosis, in terms of MTB isolation & detection of drug resistance via GENEXPERT & MGIT-DST culture methods. Materials and Methods: This was a prospective observational study carried out in Department of Pulmonary medicine, D.Y. Patil Hospital, Navi Mumbai. Study participants were patients above 18 years of age who had extra pulmonary tuberculosis and who were not already on Anti tuberculosis treatment in D.Y. Patil Hospital, Nerul, Navi Mumbai and willing to participate in the study and meeting all the inclusion criteria of the study. Study duration was from the date of approval by institutional ethics committee to October 2019.Sample size was 150 patients satisfying the inclusion criteria. Results: In our study, 28 % of all extra pulmonary samples were tested positive for TB MGIT culture out of which 35.2 % showed Resistance to 1st line ATT drugs on DST where as 39.33% was tested positive with Gene expert out of which 22.47% showed Rifampicin Resistance. Sensitivity of MGIT was 28.00 % and specificity was 39.50 %.Sensitivity of Genexpert was 39.33% and specificity was 26.5%. Conclusion: Our findings suggest that Gene Xpert may have a role in EPTB diagnosis in addition to PTB, particularly in low income/high-burden settings, where facilities for mycobacterial culture are limited. But Gene Xpert can detect only Rifampicin resistance where as DST by BACTEC MGIT AFB Culture detects other ATT drugs sensitivity too. Also the test results of MTB detected in GeneXpert in extra pulmonary samples was shown as low and very low based on the CT range (high, <16; medium, 16-22; low, 22-28; very low >28) as most of the extra pulmonary samples are pauci bacillary; hence the result is not totally reliable. Therefore, it should be confirmed by phenotypic DST by BACTEC MGIT Culture.

Keywords: *DST, Gene Xpert, Rifampicin Resistance, TB MGIT, CT range (Cyclic Threshold)*

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1. Introduction

Tuberculosis remains one of the deadliest communicable diseases. Tuberculosis (TB) still constitutes a major health problem worldwide, with an 8.8% incidence and 1.3% mortality rate [1]. It is worth noting that 27% of TB cases were extra-pulmonary infections. Of the 5.4 million new tuberculosis cases notified to WHO in 2013, 0.8 million were extrapulmonary disease [2]. Extrapulmonary tuberculosis (EPTB) comprises a wide spectrum of disease affecting all parts of the body excluding the lungs. Commonly affected sites include lymph nodes, pleura, urogenital tract, bones and joints, meninges, central nervous system (CNS), bowel and/or peritoneum, pericardium and skin. Although morbidity, mortality and disease sequelae are common, it is an entity that is underplayed as it does not contribute significantly to the transmission of tuberculosis (TB) [3]. Challenges faced by treating clinicians are its myriad clinical features and the difficulty in sample collection from deep-seated tissue. The laboratory diagnosis is an added hurdle, as a good number of specimens are paucibacillary or smear negative [3,4]. It is, thus, easy to misdiagnose. Patients are often started on empirical antituberculosis treatment (ATT) based on a combination of suggestive symptoms, radiology and histology. There is an urgent need for rapid and accurate laboratory diagnosis of EPTB. By detecting active Extra pulmonary TB early, an appropriate treatment can be initiated, and disease rate can be decreased. There are number of tests available for the diagnosis of tuberculosis but conventional microscopy has low sensitivity and culture although gold standard, but takes longer time for positivity. Furthermore, while mycobacterial culture remains the gold standard for laboratory diagnosis of TB, it requires 2-6 weeks to confirm a diagnosis. This results in delay in initiating appropriate treatment while waiting for this confirmation, except for cases where there is strong enough clinical suspicion to initiate a presumptive anti-TB therapy. FNAC and tissue biopsy is considered a good option for these cases that pose a

diagnostic challenge. On the other side, Nucleic acid amplification techniques due to its rapidity and sensitivity not only help in early diagnosis and management of tuberculosis especially in patients with high clinical suspicion like immunocompromised patients, history of contact with active tuberculosis patient etc., but also curtail the transmission of the disease. Several polymerase chain reaction- (PCR-) based molecular methods have recently been developed for early TB diagnosis and rapid detection of drug resistance from clinical specimens. The Xpert® MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) is one of these methods, and consists of a hemi-nested real-time PCR test that simultaneously identifies *Mycobacterium tuberculosis* and detects rifampicin resistance, as a surrogate of multidrug resistance (MDR), directly from clinical specimens. This assay requires less than 2 hours, and its key advantage over other PCR methods is that it is a fully-automated process, designed to run on the GeneXpert system. This system incorporates DNA extraction, often considered the critical step, along with real-time PCR amplification. Of the many molecular techniques that are currently available, Xpert MTB/Rif assay is unique as it has integrated sample processing and simplified testing [4]. Since December 2010, WHO has recommended the Xpert® MTB/RIF assay as a *bona fide* follow-on test due to its high-quality performance compared to microscopy. The advantages of this test include faster results, low complexity, low cost, wide availability and less manpower involved. It is widely used in diagnosis of extra pulmonary TB too [5]. As Fluid/tissue smear-negative patients form the bulk of cases and delay in diagnosis in this subset often leads to increased morbidity and mortality, gene Xpert can rapidly detect the mycobacteria and rule out rifampicin resistance on the same day, helping in the diagnosis and management of these patients [6]. In 2017, WHO formulated new guidelines, advocating the use of Xpert MTB/Rif assay for the diagnosis of EPTB. It is the only commercial nucleic acid test (NAT) recommended for the diagnosis of EPTB [7].Bacteriological confirmation plays a key role in the diagnosis of tuberculosis (TB). The most commonly used conventional Lowenstein Jensen (L.J) culture method requires at least 6-10 weeks of incubation due to the slow growth rate of the *Mycobacterium tuberculosis* complex[8].The use of the radiometric BACTEC 460 TB system considerably improves the isolation and decreases the time required to detect mycobacteria. However, this procedure is still labor-intensive and requires attention to special safety and regulatory issues regarding radioisotopes. BACTEC 460 is used in many laboratories worldwide but the increasing cost of radioactive waste disposal promoted the manufacturer to develop alternative systems. The automated BACTEC Mycobacterial Growth Indicator Tube (MGIT) 960 [TB] system is a state of the art, in-vitro diagnostic instrument designed and optimized for the rapid detection of mycobacteria from clinical specimens (except blood). This system has a 960-tube capacity for nearly 8000 specimens per year and is useful in laboratories dealing with large specimen loads [9]. It provides continuous monitoring of patient samples to identify the positive ones and refers safe, on-board incubation. MGIT utilizes a modified 7H9 Middlebrook broth base with 0.25% glycerol (7 ml) with an oxygen quenching fluorescent sensor embedded in silicon at the bottom to detect microbial growth directly from clinical specimens. Species identification of all isolated mycobacteria can be done using a para-nitro benzoic acid (PNBA) from MGIT positive vials. BACTEC MGIT 960 system and Gene Xpert MTB/RIF can detect drug resistance easier and faster. The presence of a mutation in rpoB geneleads to resistance for RIF and mutations in inhA geneand/or in katG gene lead to resistance for isoniazid (INH). BACTEC MGIT System and GeneXpert MTB/RIF assays are used for the detection of MDR-TB in selected health facilities and research centers. Although molecular assays can rapidly detect MTBC and mutation associated with drug resistance in a day(s), it should be complemented by phenotypic DST by BACTEC MGIT Culture. This study sought to evaluate the clinical value of the Xpert® MTB/RIF assay using body fluids/tissue samples for an early diagnosis of extra pulmonary TB & compare it with TB MGIT and their importance in identifying drug resistance.

1.1. Aims and Objectives

To compare the diagnostic yield of Extra Pulmonary Tuberculosis, in terms of *MTB isolation* & *detection of drug resistance* via GENEXPERT & MGIT-DST culture methods in cases above 18 years of age.

1.2. Materials and Methods

This was a prospective observational study carried out in Department of Pulmonary medicine, D.Y. Patil Hospital, Nerul, Navi Mumbai. Study participants were patients above 18 years of age who had extra pulmonary tuberculosis and who were not already on Anti tuberculosis treatment in D.Y. Patil Hospital, Nerul, Navi Mumbai and willing to participate in the study and meeting all the inclusion criteria of the study. Study duration was from the date of approval by institutional ethics committee to October 2019. Sample size was 150 patients satisfying the inclusion criteria. Ethical approval was taken from institutional ethics committee of D.Y.Patil Hospital, Nerul, Navi Mumbai prior to start of the study. Written informed consent was obtained from each patient. An information sheet was given to all participating patients. Completion of proforma which included personal data, symptoms, Tissue/Fluid Genexpert, TB MGIT investigation, procedures performed. Source of funding: Nil.

1.3. Statistical Method

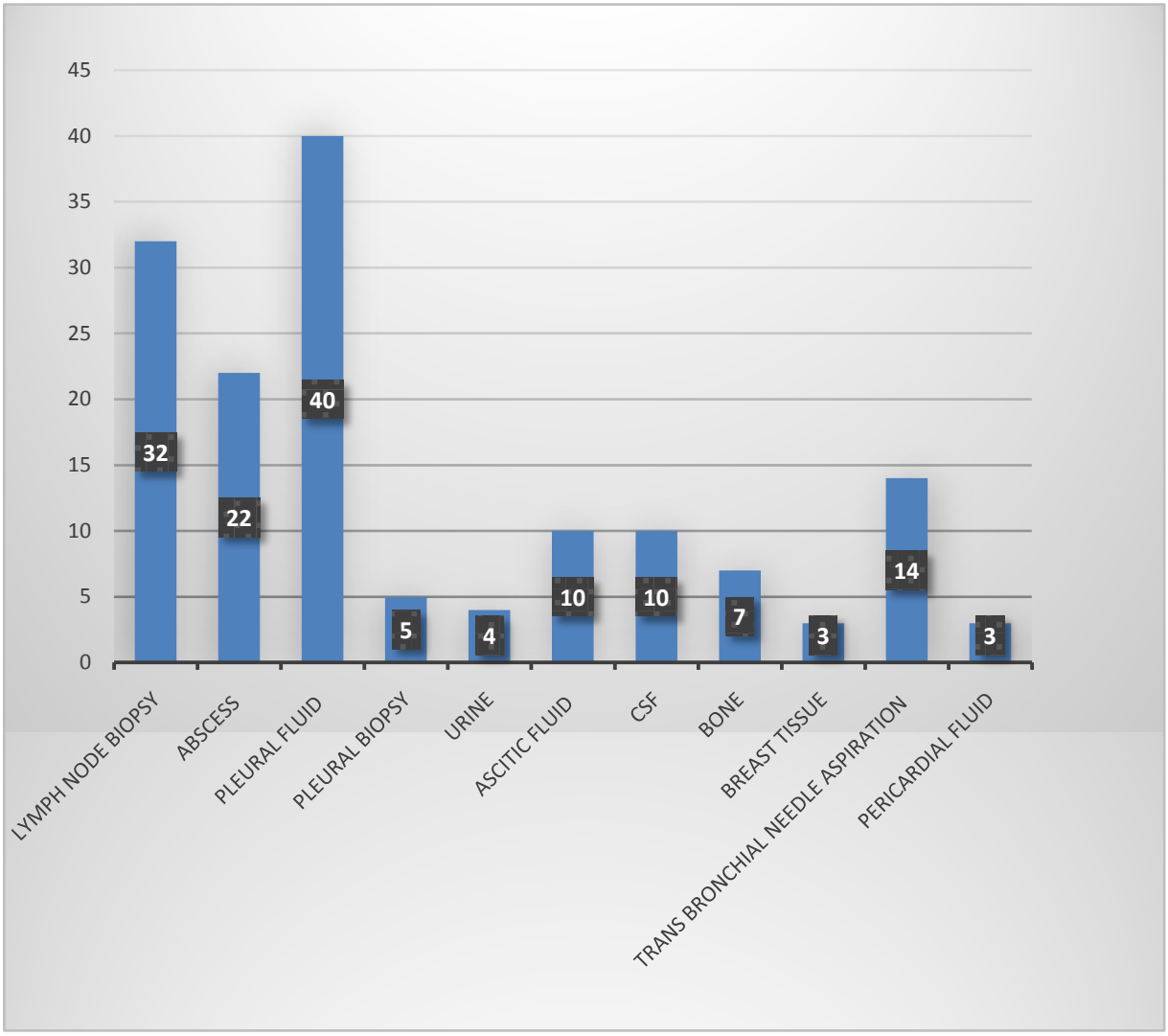
Descriptive and analytical statistics was done.The data was analyzed using statistical software (IBM SPSS V20.1, IBM Corporation, Armonk, NY, USA). The results are expressed as mean ± standard deviation and proportions. Categorical variables were compared using chi-square tests. The statistical significance was determined at $p<0.05$.

2. Observation and Results

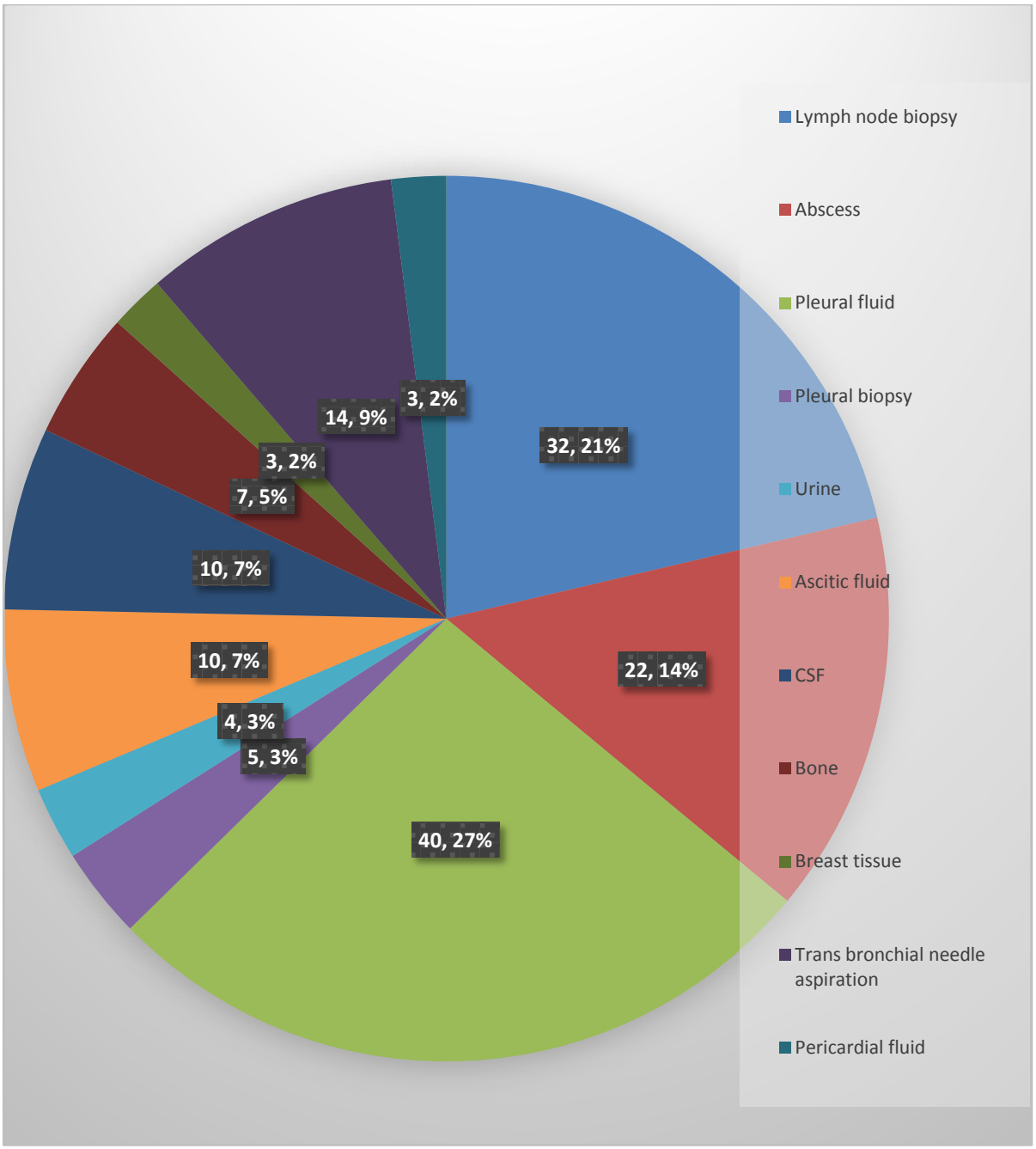
Table 1. Specimen profile

Specimen	No. of specimen	Percentage
Lymph node biopsy	32	21.34%
Abscess	22	14.67%
Pleural fluid	40	26.7%
Pleural biopsy	5	3.34%
Urine	4	2.67%
Ascitic fluid	10	6.67%
CSF	10	6.67%
Bone	7	4.67%
Breast tissue	3	2.00%
Trans bronchial needle aspiration	14	9.34%
Pericardial fluid	3	2.00%
Gastric aspirates	0	0
Total	150	100.00%

There were 32lymph node biopsy, 22 abscess samples, 40 pleural fluid sample, 5 pleural biopsies, 4 urine sample, 10 Ascitic fluid sample, 10 CSF samples, 7 bone samples, 3 breast tissue samples, 14 Trans bronchial Needle Aspiration (TBNA) samples.



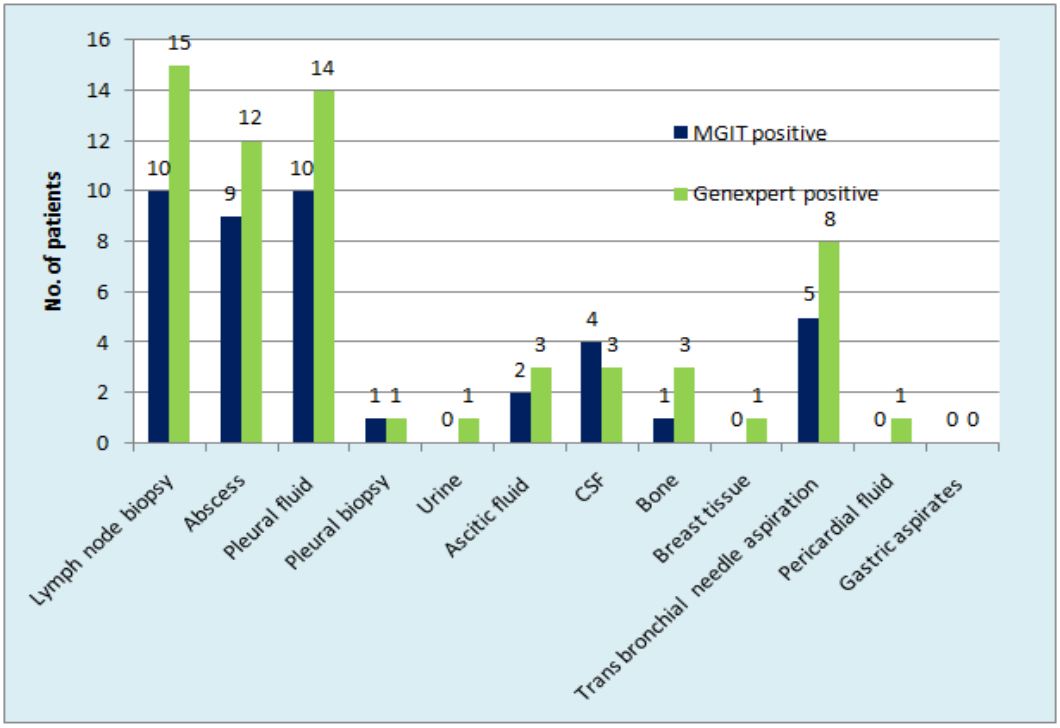
Bar Diagram 1. Specimen profile



Pie Diagram 1. Specimen profile

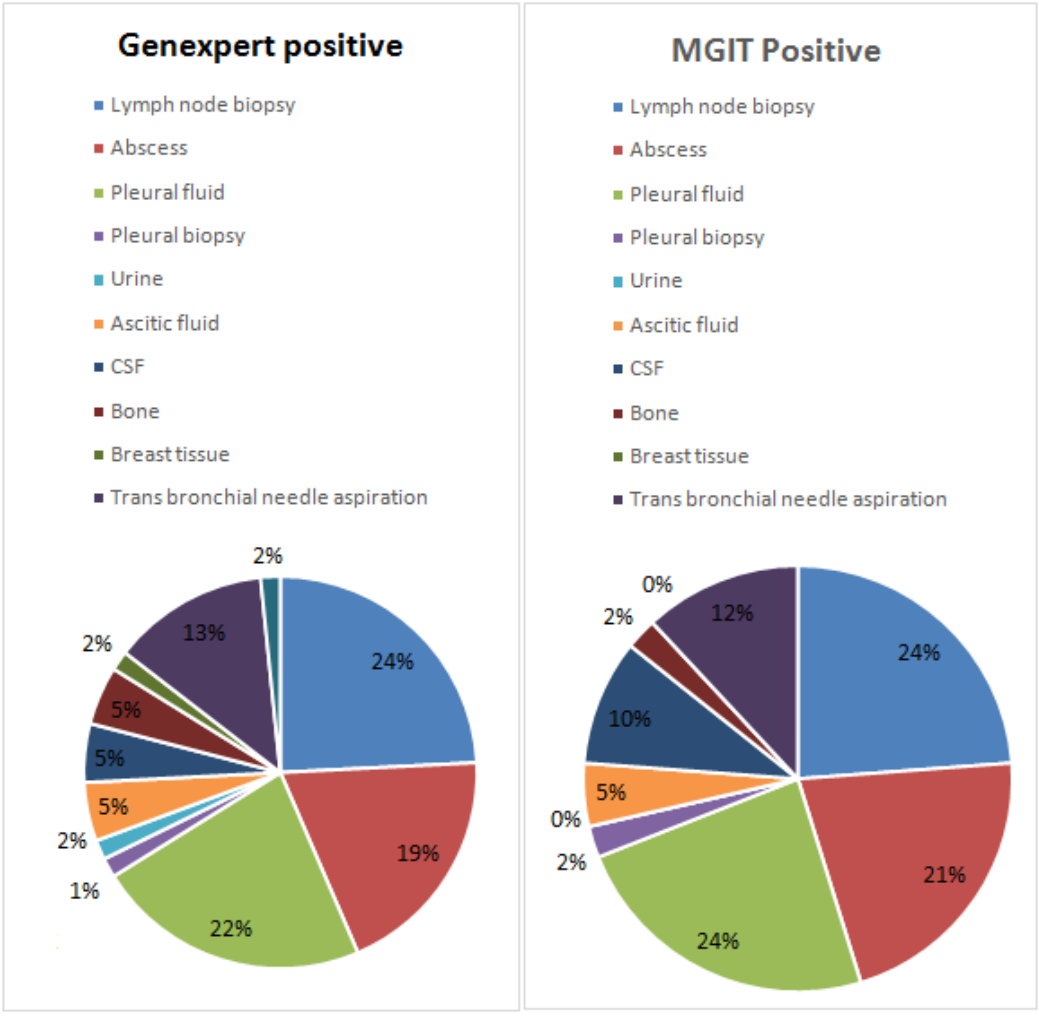
Table 2. Comparison of Genexpert versus MGIT

Specimen	No. of specimen	MGIT positive	Genexpert positive	Chi squre statistic	P value
Lymph node biopsy	32	10	15	36.3	0.021
Abscess	22	9	12	61.4	0.042
Pleural fluid	40	10	14	41.5	0.025
Pleural biopsy	5	1	1	11.8	0.41
Urine	4	0	1	0	0.00
Ascitic fluid	10	2	3	24.9	0.01
CSF	10	4	3	11.8	0.02
Bone	7	1	3	9.2	0.01
Breast tissue	3	0	1	31.6	0.06
Trans bronchial needle aspiration (Mediastinal Lymph Node)	14	5	8	5.7	1
Pericardial fluid	3	0	1	0	1
Gastric aspirates	0	0	0	100	1
Total	150	42	59	23.6	0.041



Bar Diagram 2. Comparison of Genexpert versus MGIT

Chi square test was applied. P value < 0.05 was obtained for Lymph node biopsy, abscess and pleural fluid specimen, urine, Ascitic fluid, bone implying statistically significant difference between Genexpert and MGIT cultures.



Pie Diagram 2. Comparison of Genexpert versus MGIT

Table 3. Comparison of TBMGIT DST Resistance versus Genexpert resistance

Specimen	MGIT		Genexpert	
	Sensitivity	Specificity	Sensitivity	Specificity
Lymph node biopsy	50.00%	60.00%	40.00%	48.00%
Abscess	55.00%	66.67%	41.67%	50.00%
Pleural fluid	20.00%	24.00%	28.57%	34%
Pleural biopsy	0.00%	0.00%	0.00%	0.00%
Urine	0.00%	0.00%	0.00%	0.00%
Ascitic fluid	0.00%	0.00%	0.00%	0.00%
CSF	25.00%	30.00%	33.33%	40.00%
Bone	0.00%	0.00%	0.00%	0.00%
Breast tissue	0.00%	0.00%	0.00%	0.00%
Trans bronchial needle aspiration	40.00%	48.00%	50.00%	60.00%
Pericardial fluid	0.00%	0.00%	0.00%	0.00%
Gastric aspirates	0.00%	0.00%	0.00%	0.00%

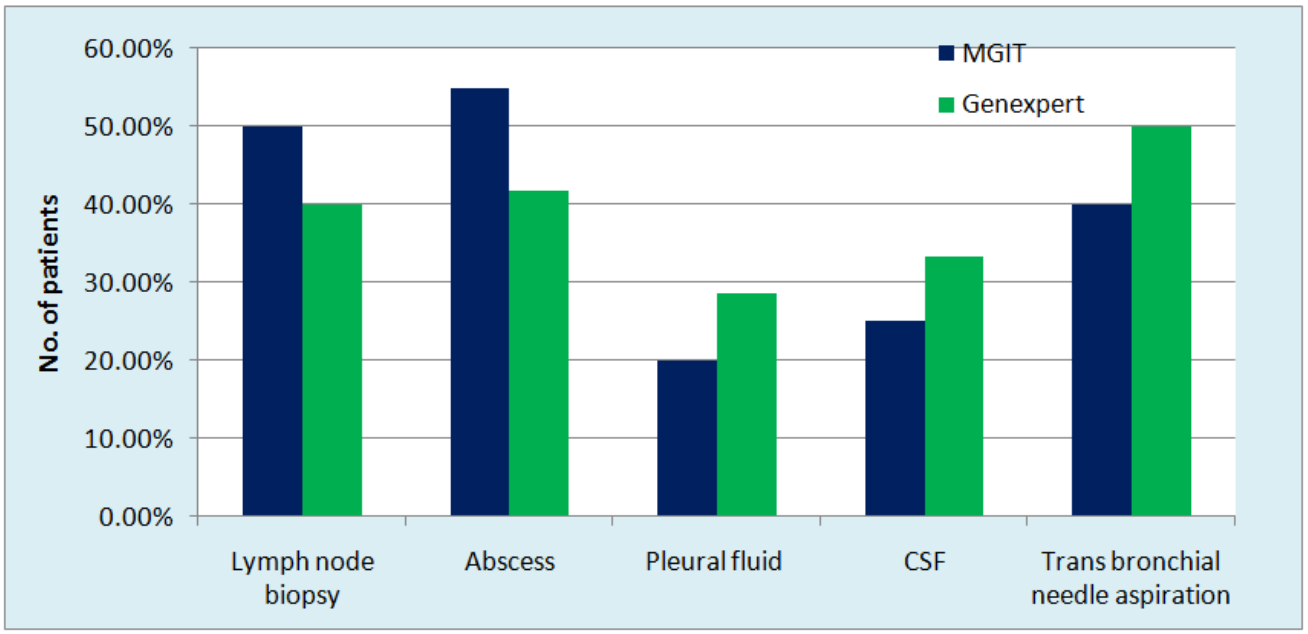


Diagram 3a. Comparison of TBMGIT DST Resistance versus Genexpert resistance: Sensitivity

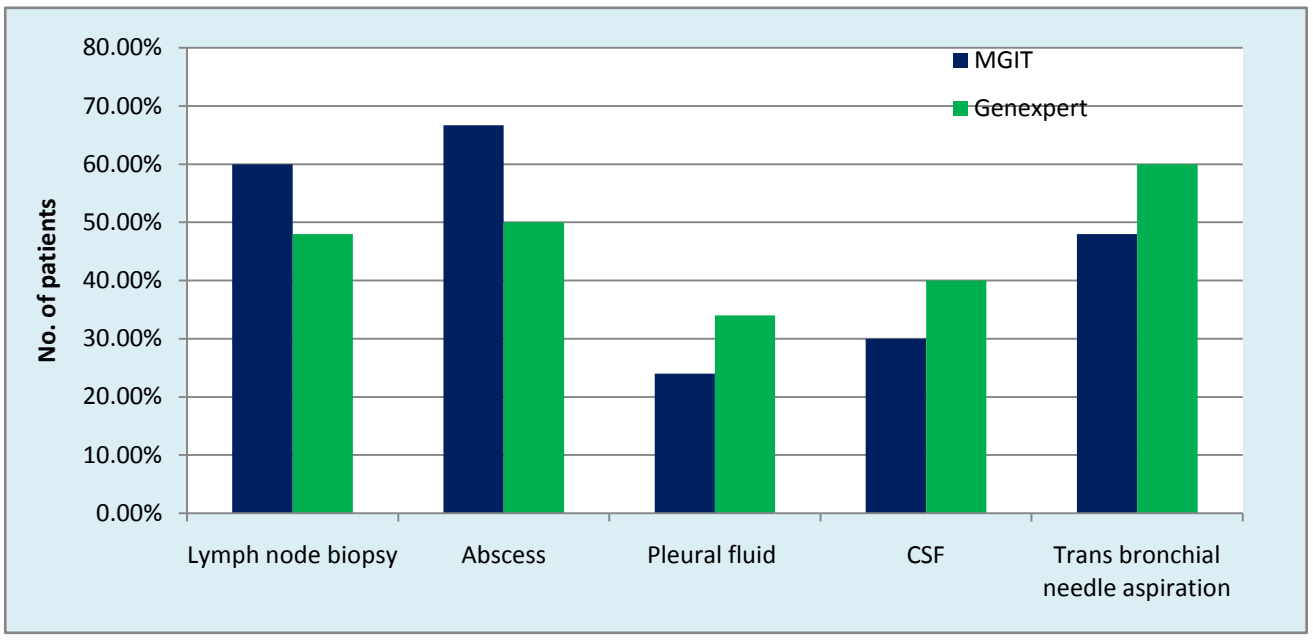
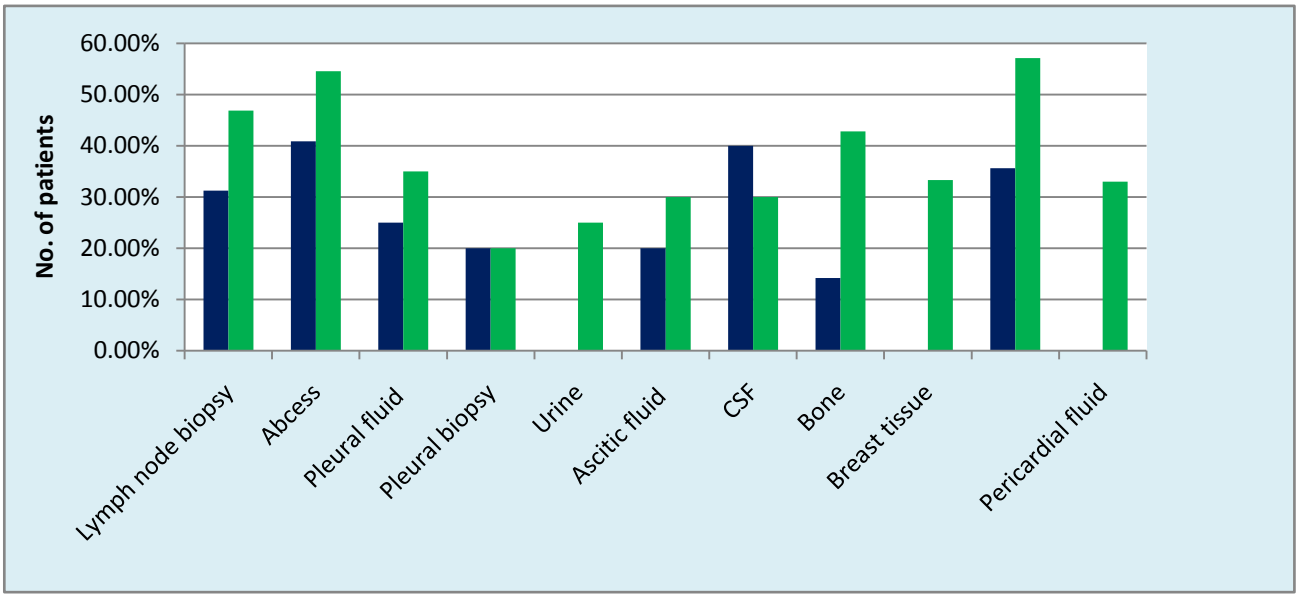


Diagram 3b. Comparison of TBMGIT DST Resistance versus Genexpert resistance: Specificity

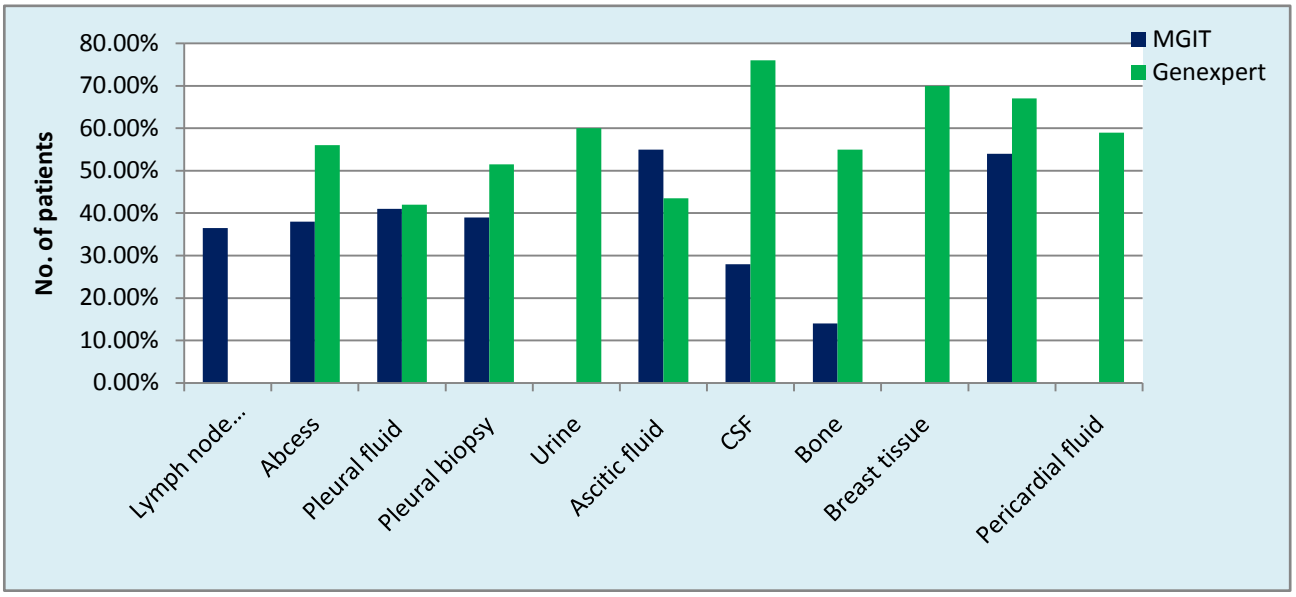
Table 4. Sensitivity & Specificity of MGIT versus Genexpert

Specimen	MGIT		Genexpert	
	Sensitivity	Specificity	Sensitivity	Specificity
Lymph node biopsy	31.25%	36.5%	46.87%	68.5.00%
Abscess	40.9%	38.00%	54.54%	56.00%
Pleural fluid	25.0%	41.00%	35.0%	42.00%
Pleural biopsy	20.0%	39.00%	20.0%	51.50%
Urine	0%	0.00%	25.0%	60.00%
Ascitic fluid	20.0%	55.00%	30.0%	43.50%
CSF	40.0%	28.00%	30.0%	76.00%
Bone	14.2%	14.00%	42.8%	55.00%
Breast tissue	0%	0.00%	33.3%	70.00%
Trans bronchial needle aspiration (Mediastinal Lymph Node)	35.6%	54.00%	57.14%	67.00%
Pericardial fluid	0%	00.00%	33.0%	59.00%
Total	28.00%	39.5.00%	39.33 %	66.50%

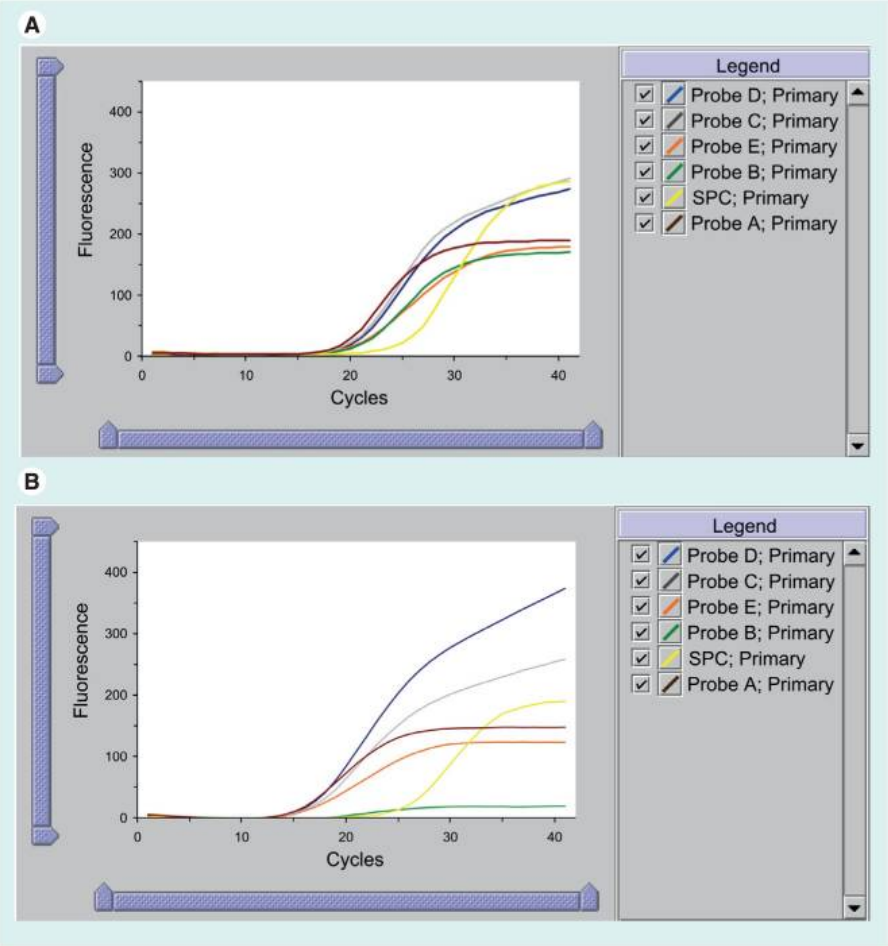
*Statistically significant
Sensitivity of MGIT is28.00 % and specificity was 39.50 %. Sensitivity of Genexpert is 39.33 % and specificity was 66.50 %.



Bar Diagram 4a. Sensitivity of Genexpert versus MGIT



Bar Diagram 4b. Specificity of Genexpert versus MGIT



Trace **(A)** shows the read-out from processing a rifampicin-sensitive strain of *Mycobacterium tuberculosis* as denoted by amplification of all five probes with a similar cycle threshold. Trace **(B)** shows the read-out of a rifampicin-resistant strain as shown by the failure of amplification of Probe B.
SPC: Sample processing control.

Figure 1. GeneXpert user view of the amplified probes A-E and the sample processing control

3. Discussion

Diagnosing EPTB remains challenging because of various reasons: symptoms varying depending on the organ involved, clinicians having low level of suspicion because of varied presentations, clinical samples obtained from relatively inaccessible sites being paucibacillary; all these factors decreasing the sensitivity of diagnostic tests. Since the conventional smear microscopy has a low sensitivity with a range of 0%–40%, negative results cannot exclude the presence of TB. The reported yields of mycobacterium culture vary from 30% to 80%, but it usually takes 2–8 weeks to receive the results, which is too slow to help treatment decisions. All these factors lead to a delay in diagnosis. Accurate and rapid laboratory investigations have therefore gained importance. Several new non radiometric technologies for growth and detection of mycobacteria have been developed to reduce the time to detect (TTD) and identify mycobacteria in clinical specimens; among these, the Mycobacterium Growth Indicator Tube (MGIT) system. There are a few published reports on the evaluation of Bactec MGIT 960 on extrapulmonary samples. The present study attempted to measure the efficacy of the Bactec MGIT 960 method for the detection of Mycobacteria in extrapulmonary specimens [10,11]. This system has been extensively evaluated, but very few studies have been carried solely on extrapulmonary specimen. The system was more useful in paucibacillary specimens like Lymph nodes, Abscess, pleural fluid and CSF. Even though AFB culture is considered the gold standard in TB diagnostics, growth on solid culture media requires four to six weeks. This delay would negatively affect patient care. To overcome this problem, we opted for automated cartridge-based molecular nucleic acid amplification (NAA) techniques, which offer a rapid diagnosis of life-threatening disease such as TB meningitis with a turn around time of 24 h. This method is said to be very sensitive as it can detect as few as 10 mycobacteria. There are number of Nucleic Acid Amplification (NAA) methods that have been developed for rapid detection and identification of Mycobacterium tuberculosis (MTB) in clinical specimens of pulmonary and extra-pulmonary tuberculosis cases. These techniques not only provide the advantage of rapidity of diagnosis but also detect even low MTB genomic copies in various specimens. More recently, the WHO endorsed the GeneXpert (Xpert® MTB/Rif assay) for the diagnosis of TB. The GeneXpert utilizes a DNA-PCR technique for simultaneous detection of Mycobacterium tuberculosis and Rifampicin resistance related mutations. Diagnostic accuracy of GeneXpert for Extra pulmonary TB has been reported high. The assay

utilizes single-use plastic cartridges with multiple chambers that are preloaded with liquid buffers and lyophilized reagent beads necessary for sample processing, DNA extraction and heminested rt-PCR. Samples are treated with a sodium hydroxide and isopropanol-containing sample reagent (SR).The test platform employs a sonic horn that inserts into the cartridge base to cause ultrasonic lysis of the bacilli and release of the genetic material. The assay then amplifies a 192 bp segment of the rpoB gene using a hemi-nested rt-PCR reaction. The assay also contains lyophilized Bacillus globigii spores which serve as an internal sample processing and PCR control. The B. globigii PCR assay is multiplexed with the M. tuberculosis assay. Mycobacterium tuberculosis is detected by the five overlapping molecular probes (probes A–E) that collectively are complementary to the entire 81 bp rpoB core region. M. tuberculosis is identified when at least two of the five probes give positive signals with a cycle threshold (C_T) of ≤38 cycles and that differ by no more than a prespecified number of cycles. The B. globigii internal control is positive when the single B. globigii-specific probe produces a C_T of ≤38 cycles. The standard user interface indicates the presence or absence of M. tuberculosis and the presence or absence of rifampicin resistance, and a semi-quantitative estimate of the concentration of bacilli as defined by the C_T range (high, <16; medium, 16–22; low, 22–28; very low, >28). Assays that are negative for M. tuberculosis and for the B. globigii internal control are reported as invalid assays. When performed on unprocessed samples, the assay can generate results within 2 h with less than 15 min of hands-on time. The basis for detection of rifampicin resistance is the difference between the first (early C_T) and the last (late C_T) M. tuberculosis-specific beacon (ΔC_T). The system was originally configured such that resistance was reported when ΔC_T was >3.5 cycles and sensitive if ≤3.5 cycles. Since the assay terminates after 38 cycles, the assay was deemed indeterminate for rifampicin resistance if the first probe C_T is >34.5 cycles and the last probe has a C_T of >38 cycles. From May 2010 the automated detection of rifampicin resistance was modified using a new ΔC_T cut-off in order to improve the specificity for rifampicin resistance detection [12,13]. Figure 1 shows traces from rifampicin-susceptible and rifampicin-resistant strains of M. tuberculosis identified in extrapulmonary samples.

Comparison of MGIT versus Genexpert

In present study, 28% of all extra pulmonary samples were tested positive for TB MGIT culture out of which 35.2% showed Resistance to 1st line ATT drugs on DST where as 39.33% was tested positive with Gene expert out of which 22.47% showed Rifampicin Resistance. In the study by Lesly scottt et al, 65.9 % of all samples were tested positive for MGIT culture where as 77.4% was tested positive with Genexpert. Results of the study by Lesly scottt et al was comparable to present study. Difference between MGIT culture and Genexpert was not only high, but statistically significant in both the studies. This provides evidence that nucleic acid amplification technique is more efficient than traditional culture methods. While Genexpert utilises the DNA/RNA for detection of TB, MGIT needs to grow the mycobacterium tuberculosis bacteria as to provide the evidence. As the bacteria is not always amenable to growth in vitro even under ideal conditions, various studies have reported less than satisfactory results with MGIT. In present study, Sensitivity of MGIT was 28.00% and specificity was 39.50%. Sensitivity of Genexpert was 39.33% and specificity was 26.5 %. In a study by Habous, of 168 non respiratory samples, 52 samples were positive by both culture and Xpert MTB/RIF, 9 samples were detected positive only by culture. Sensitivity, specificity, positive predictive value, and negative value of the Xpert MTB/RIF test were 82.69%, 100%, 100%, and 92.80%, respectively. No false positive was yielded by the Xpert MTB/RIF, and all 116 samples were true negative by Xpert MTB/RIF. The sensitivity of the Xpert MTB/RIF was 76.92% in lymph node tissue and aspirates, 66.67% in cerebrospinal fluid, 81.25% in other body fluids, 100% in pus, 85.71% in urine, and 66.67% in other tissue samples. Of 168 strains, five strains were rifampicin resistant by phenotypic and Xpert MTB/RIF and 163 were susceptible to rifampicin with culture and Xpert MTB/RIF. Study concluded that performance of Xpert MTB/RIF assay was comparable to the gold standard culture method for identification of MTB in nonrespiratory clinical specimens. It does not replace the gold standard culture method, but it helps to achieve better sensitivity and obtain rapid results within 2 h. An overall sensitivity of 83.1% and a pooled specificity of 98.7% for diagnosis of EPTB was recently published in a meta-analysis by Ghiralia et al. Raquel Moure et al in 2012 conducted a study; in which 58.3% were positive with the Xpert MTB/RIF assay for Mycobacterium tuberculosis [13]. In a similar study by Vadwai V et al16 in 2011, the sensitivity of the Xpert assay was 81% (228/283 specimens), 64% for smear-negative cases and 96% for smear-positive cases), with a specificity of 99.6% [6]. In a Cochrane review, Xpert MTB/RIF pooled sensitivity for rifampicin resistance detection was 95% (95% CI, 90% to 97%; 17 studies) which was found to be lower than in our study and pooled specificity was 98% (95% CI, 97% to 99%; 24 studies) which was again lower than our study [14]. One case found to be positive for resistance to RIF phenotypically (detected by conventional DST method), was found to be negative for resistance to RIF genotypically by Xpert. The reason for this discrepancy might be due to mutation in other sites rather than hot spots, which needed to be solved by further sequencing the rpoB gene. In Hui Jing et al study, only 77.3% (17/22) of DST proven RIF resistant cases had MDR-extra pulmonary TB disease, of which Xpert MTB/RIF assay only identified 76.5% of these MDR-Extra Pulmonary [10].

CSF

In CSF, present study observed, genexpert was positive for TB in 30 % of cases. Sensitivity and specificity of Xpert was 30 % and 76 %. Sensitivity and specificity of the MGIT culture was 40% and 28 % respectively. One of the largest studies performed to date by Patel et al found a significant improvement in the sensitivity of the Xpert MTB/RIF test in CSF when the CSF was centrifuged. However, this was only with unpaired samples from two different groups of patients; when 12 positive paired samples from the same patients were tested, no significant difference was found. Patel VB, Theron G, Lenders L et al showed Diagnostic accuracy of quantitative PCR (Xpert

MTB/RIF) for tuberculous meningitis in a high burden setting: a prospective study [12].

Lymph node biopsy

Diagnosis of EPTB, including LNTB, is very challenging, due to the characteristics of a low bacterial load. In most cases, invasive examinations are necessary. For LNTB, the most commonly used invasive procedures are FNA and biopsy. Specimens obtained by different methods have different sensitivity for the diagnosis of LNTB. Sensitivity of pathological diagnosis is the highest with the most invasive biopsy specimens. In lymph nodes biopsy, present study observed, genexpert was positive for TB in 46.87% of cases. Sensitivity and specificity of Xpert was 46.87 % and 68.50%. In MGIT culture, Sensitivity and specificity was 31.25% and 36.5% respectively. In a study by Ghariani et al, among the 174 samples of lymph nodes tested, the GeneXpert detected the DNA of MTBC in 134 samples (77%) [13]. Standard bacteriological assays, including AFB microscopy and culture, were positive, respectively, in 41 (23.6%) and 79 (45.4%) specimens. M.bovis was isolated in 76% of positive cultures. GeneXpert sensitivity and specificity results were assessed according to smear and culture results, clinical and histological findings. The sensitivity and specificity of the Xpert assay were 87.5% (126/144) and 73.3%, respectively. Sensitivity and specificity of the MGIT culture was 67.5% and 56% respectively. Study by Tadesse et al observed that Xpert detected M. tuberculosis complex (MTBC) in 60.1% (86/143) of the presumptive TBL cases. The sensitivity of Xpert compared to CRS was 87.8% [95% CI: 81.0–94.5]and specificity 91.1% [95% CI: 82.8–99.4]. The sensitivity was 27.8% for smear microscopy and 80% for cytology compared to CRS. Sensitivity and specificity of the MGIT culture was 71.5% and 69.5 % respectively [14]. In a meta-analysis by Denkinger et al., Xpert MTB/RIF pooled sensitivity and specificity were 81.2% (95% CI 72.4–87.7%) and 99.1% (95% CI 94.5–99.9%) versus CRS, and 83.1% (95% CI 71.4–90.7%) and 93.6% (95% CI 87.9–96.8%) [15]. In another meta-analysis by Yanquin Shen et al, we observed that the pooled sensitivity and specificity of Xpert MTB/RIF were 79% and 98%, respectively, when compared with a CRS, and 84% and 91%, respectively, when compared with culture. When performed on FNA samples, the pooled sensitivity and specificity were 80% and 96% versus CRS and 90% and 89% versus culture, respectively. When performed on tissue samples, the pooled sensitivity and specificity were 76% and 100% versus composite reference standard (CRS), and 76% and 92% versus culture, respectively [16]. There was no significant difference in the diagnostic efficiency for specimens obtained via different routes. Xpert MTB/RIF showed a good diagnostic efficiency on LNTB and was not related to the type of specimen. Many of the studies showed higher rates of sensitivity and specificity in both Genexpert and MGIT culture. Inter subject variation and expertise of the surgeon probably accounted for the difference. In present study, procedure was carried on by post graduate residents where as most other studies procedure was done by specialists. None the less, the significant difference between MGIT and Genexpert was maintained in present study in consonance with previous studies. Culture is the gold standard for TB diagnosis. However, It is time-consuming, requires biosafety measures and needs trained laboratory personnel. MGIT has improved lymph node TB diagnosis. Indeed, it gives a higher yield of mycobacteria and faster results than LJ medium. Although solid and liquid media were combined, culture shows a low sensitivity in case of lymph node specimens. This may be attributed to the uneven bacilli distribution and the loss of the low number of viable bacilli during NALC-NaOH processing. In developing countries, smear microscopy is the only widely implemented method for quantifying the bacterial burden at the time of the initial diagnosis. Xpert provides a semi-quantitative measurement of the number of MTBC bacilli present in a clinical sample. In various studies, more than 90% of Xpert-positive samples were scored as ‘low’ and ‘very low’ suggesting a limited number of bacilli in FNA sample. That’s where Xpert’s utility comes in to picture in extra pulmonary TB.

Tuberculous pleural effusion (TPE)

Tuberculous pleural effusion is the second most common site of extrapulmonary tuberculosis (EPTB) Currently, the best tool for diagnosing TPE is a thoracoscopic pleural biopsy, an invasive procedure, with sensitivity ranging from 93 to 100%. The most widely used diagnostic test for TPE is the pleural fluid adenosine deaminase (ADA) level, which has variable sensitivity ranging from 47% to 100%. The sensitivities of pleural fluid microscope and culture in TPE are about 10% and 20%, respectively. The absence of a simple reference standard makes the treatment of TPE empirical (nonmicrobiological) in most circumstances [28]. In pleural fluid, present study observed, genexpert was positive for TB in 35.00%. Sensitivity and specificity of Xpert was35.00% and 42.00%. A systematic review by Sehgal et al investigating the role of Xpert MTB/RIF in the diagnosis of tuberculous pleural effusion (TPE) was conducted. The pooled sensitivities and specificities of Xpert MTB/RIF were 51.4% and 98.6%, respectively, with culture used as a reference standard and 22.7% and 69.8%, respectively for MGIT, with a composite reference standard (CRS) used as the benchmark [17]. Xpert MTB/RIF has low sensitivity but excellent specificity in the diagnosis of TPE. Friedrich et al investigated the diagnostic utility of the Xpert MTB/RIF (Mycobacterium tuberculosis/rifampin [RIF] resistance) assay in 20 cases with confirmed tuberculous pleural effusion. The sensitivity and specificity of the Xpert assay in pleural fluid were 25% and 100%, respectively. All cases positive by the Xpert assay were also positive by pleural fluid culture. Sensitivity and specificity of the MGIT culture was 22% and 43 % respectively [18]. However, study by Nasir Javed et al, observed contradictory report. Among 25 patients Gene Xpert was only positive in 2(8%) and Pleural Biopsy showed definitive caseating granuloma in 14(56%), Malignancy in 6(24%), Acute Inflammation in 1(4%), Pleuritis in 1(4%) and inconclusive report in 3(12%) patients. Studyconcluded that pleural Biopsy is a diagnostic tool in diagnosing tuberculosis. Gene Expert has a limited role in diagnosing tuberculous pleural effusion [19].

Ascitic fluid

In ascitic fluid, present study observed, genexpert was positive for TB in 30 % of cases. Sensitivity and specificity of Xpert was 30 % and 43.5 %.Sensitivity and specificity of the MGIT culture was 20 % and 55 % respectively. Sensitivity and specificity of Xpert was 64% and 51% respectively. Rufai et al observed that Seventeen (25.4%) were MGIT-960 culture positive while 12 (17.9%) were detected positive by the Xpert MTB/RIF assay and 9 (13.4%) by in-house multiplex PCR. Sensitivity and specificity of the Xpert MTB/RIF assay compared with the MGIT-960 culture were 70.6% (95%, confidence interval [CI]: 44.1–89.7) and 100% (95%, CI: 92.8–100) and that of in-house multiplex PCR were 52.9% (95%, CI: 30.9–73.8) and 100% (95%, CI: 92.8–100), respectively [20]. In a study by Ahmad et al, of the 21 samples analyzed, all were positive for tuberculosis (TB) by histopathology. GeneXpert was positive in six and negative in 15 patients. The sensitivity of GeneXpert was 28.57% and specificity was 0%. The positive predictive value was 100%. The diagnostic accuracy was found to be 28.57%. Study observed that GeneXpert has shown poor sensitivity and specificity for the detection of abdominal TB from ascitic fluid samples [21].

Pus (Abcess)

In pus samples, present study observed, genexpert was positive for TB in 54.54% of cases. Sensitivity and specificity of Xpert was 54.54% and 56.00%. Sensitivity and specificity of the MGIT culture was 40.9% and 38 % respectively. In comparison, study by Sunil Narute et al, genexpert was positive for TB in 52% of cases and MGIT in 41% of cases. Sensitivity and specificity of Xpert was 91% and 76% respectively. In study done by Shakeel K et al in which out of 212 pus samples, 77 (36.3%) were positive on Gene Xpert. Rifampicin resistance was detected in 5(6.4%) pus samples by Gene Xpert.

Urine

In urine samples, present study observed, genexpert was positive for TB in 25 % of cases. Sensitivity was 25.00 % and specificity of Xpert was 60 % each. Sensitivity and specificity of the MGIT culture was 0% each. In a study by Habous et al, they observed that when compared with L-J culture,the sensitivity of acid-fast bacilli (AFB) microscopy and GeneXpert were 40.5% (15/37, 95% confidence interval [CI]: 24.7–56.4%) and 94.6% (35/37, 95% CI: 87.3–100.0%), respectively. In addition, GeneXpert identified 109 of 126 culture negative UTB cases, yielding a specificity of 86.5% (95% CI: 80.5–92.5%). Statistical analysis revealed that the sensitivity of GeneXpert was significantly higher than that of AFB microscopy (P [22]0.001)

Bone

In bone specimen, present study observed, genexpert was positive for TB in 42.8 % of cases. Sensitivity and specificity of Xpert was 42.8 % and 55%.Sensitivity and specificity of the MGIT culture was 14.2% and 14 % respectively. In a metanalysis by Wen et al, 12 studies were identified with a pooled sensitivity and specificity of respectively 0.81 (95% confidence interval [CI] 0.78-0.83) and 0.83 (95% CI 0.80–0.86) of Xpert for the diagnosis of musculoskeletal TB. Xpert was highly sensitive (0.89, 95% CI 0.79-0.95) and highly specific (0.96, 95% CI 0.92-0.98) in detecting RIF resistance.AUC (over 0.9) suggested a relatively high level of overall diagnostic accuracy of Xpert for detecting musculoskeletal TB and RIF resistance [23].

Breast tissue

In present study, sensitivity and specificity of TB genexpert was 33.33 % and 70 % respectively, sensitivity and specificity of TB MGIT was 0% and 0% respectively. In a study by poleploe et al, Gene Xpert MTB/RIF was positive for TB in 25% of cases. In lymph tissue of breast, the accuracy of the Xpert MTB/RIF assay was 41% (95%CI 27-57), not significantly better than ZN or the in-house PCR assay. In breast tissue the sensitivity of the in-house PCR assay was 82% (95%CI: 56%-95%). Study concluded that the Xpert MTB/RIF assay was potentially a useful tool for the diagnosis of TB [24]. Study by Jayashree Pandya et al stated that Gene Xpert is an important tool with moderate sensitivity (83.3%) and high specificity (99%) for detection of TB mastitis. It should be considered in all suspicious cases of TB breast, where patients fail to respond to conventional treatment of breast abscess. However, it cannot be used alone and other clinical and complementary laboratory parameters (ADA, ESR) should be used in the diagnostic pathway.

Pericardial fluid

In present study, in pericardial fluid, sensitivity of Genexpert was 33.00 % and specificity was 59.00 %. Sensitivity and specificity of TB MGIT was 0% and 0% respectively.In a study by Saeed et al, Xpert-MTB/RIF had a sensitivity and specificity (95% confidence interval (CI)) of 63.8% (52.4% to 75.1%) and 100% (85.6% to 100%), respectively. Concentration of pericardial fluid by centrifugation and using standard sample processing did not improve Xpert MTB/RIF accuracy. In a study by Guocanyu et al, sensitivity and specificity of Xpert MTB/RIF assay were 78.6% (49.2–95.3%) and 70.6%. It concluded that Xpert MTB/RIF test is a valid diagnostic technique for TBP with higher sensitivity and specificity than TB MGIT Culture.

Pleural biopsy

In present study, sensitivity and specificity of genexpert in pleural fluid was 33.00 % and 42.00% respectively. Sensitivity and specificity of TB MGIT was 20 % and 39% respectively. In a study by Saeed et al, GeneXpert showed high sensitivity (84.3%), specificity (100%), with positive predictive value (100%), and negative predictive value (96.7%) [25]. Saeed M, Ahmad M, Iram S, Riaz S, Akhtar M, Aslam M.GeneXpert technology. A breakthrough for the diagnosis of tuberculous pericarditis and pleuritis in less than 2 hours. In review of M.Kohli et al for TB pleural biopsy, Xpert pooled sensitivity and specificity (95% CrI) were 50.9% (39.7% to 62.8%) and 99.2% (98.2% to 99.7%), respectively and MGIT were 35.6% and 46.3% respectively.

TBNA

In present study, in Trans bronchial needle aspiration, sensitivity of MGIT was 35.60% and specificity was 54.00%. Sensitivity of Genexpert was 57.14 % and specificity was 67.00 %. In a study by Dhasmana et al with transbronchial needle aspiration, the positive predictive value was 88.9% (69.7–97.1%),negative predictive value was 86.5% (76.9–92.1%), and odds ratio was 51.3 (24.0-98.0) for correctly identifying culture-positive disease [26]. In a study by Hanif SN et al

on transbronchial needle aspiration, Xpert and culture were positive in 70% and 41% cases respectively. Study concluded that increased yield was associated with pneumonia, moderate and severe airway obstruction and lymph node ulcerating into the airways [27].

4. Conclusion

Gene Xpert is useful for rapid detection of TB and identification of RIF resistance especially in a high prevelance country like India. The results are superior to smear microscopy and comparable to culture with shorter turn-around time. GeneXpert can be a useful tool for early diagnosis of patients with high clinical suspicion of extra pulmonary tuberculosis. Our findings suggest that Gene Xpert may have a role in EPTB diagnosis in addition to PTB, particularly in low income/high-burden settings, where facilities for mycobacterial culture are limited. But Gene Xpert can detect only Rifampicin resistance where as DST by BACTEC MGIT AFB Culture detects other ATT drugs sensitivity too. Also in our study, the test results of MTB detected in GeneXpert in extrapulmonary samples was shown as low and very low based on the C_T range (high, <16; medium, 16-22; low, 22-28; very low, >28) as most of the extra pulmonary samples are pauci bacillary; hence the result is not totally reliable. Therefore, it should be confirmed by phenotypic DST by BACTEC MGIT Culture.

5. Summary

A total of 150 patients of age 18 years and above and both sexes with clinical, microbiological and radiological diagnosis of extra pulmonary tuberculosis, who are not already on AKT were selected for the study. The conclusion drawn from our study were. Among the samples, 28 % of all extra pulmonary samples were tested positive for TB MGIT culture and 39.33% was tested positive with Gene expert. Among the samples that were tested positive for TB MGIT culture, 35.2 % showed Resistance to 1st line ATT drugs on DST and among the samples that were tested positive for Gene expert, 22.47% showed Rifampicin Resistance. Majority of the study participants were of Pleural fluid (26.6%) followed by Lymph nodes biopsy (21.34%) followed by Abscess (Pus) (14.67%).MTB detected in GeneXpert in extra pulmonary samples in majority of the study participants was shown as low and very low based on the C_T range.

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