Section: Microbiology

Original Research Article

COMPARATIVE EVALUATION OF PCR WITH CONVENTIONAL METHODS IN THE DETECTION OF EXTRAPULMONARY TUBERCULOSIS

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Abstract

Background: Tuberculosis (TB) is an airborne communicable disease caused by the bacterium Mycobacterium tuberculosis. It primarily affects the lungs but can also affect other organs, leading to extrapulmonary tuberculosis (EPTB). The aim of this study is to compare the sensitivity and specificity of PCR with smear and culture in detecting Mycobacterium tuberculosis from different specimens of EPTB. **Materials and Methods:** In this cross-sectional study, samples were collected from extrapulmonary sites and processed them using various methods, including ZN staining, Fluorescent staining, culture on Lowenstein Jensen (LJ) Medium and Mycobacterial Growth Indicator Tube (MGIT), and Polymerase Chain Reaction (PCR). The results were analysed using SPSS version 23. **Result:** In this cross-sectional study, polymerase chain reaction (PCR) showed the highest sensitivity (62%) compared to other diagnostic techniques for tuberculosis in all samples tested. Among the conventional techniques, culture on mycobacterial growth indicator tube (MGIT) 320 media had the highest sensitivity (26%), followed by culture on Lowenstein Jensen (LJ) media (22%). ZN staining and fluorescent staining had the lowest sensitivity in all samples tested. **Conclusion:** The study showed that polymerase chain reaction (PCR) is a highly sensitive technique for diagnosing extrapulmonary tuberculosis (EPTB) and can be used in combination with other conventional techniques to improve diagnostic accuracy. The choice of diagnostic technique should also depend on the type of sample tested. The development of new and more accurate diagnostic tools for TB is essential to improve the management and control of this disease.

INTRODUCTION

Extra-pulmonary tuberculosis (EPTB) is a distinct form of tuberculosis (TB) that affects organs and tissues beyond the lungs. It occurs when Mycobacterium tuberculosis, the causative bacterium of TB, spreads through the bloodstream or lymphatic system from the primary lung infection site. Although pulmonary TB remains the most prevalent form, EPTB accounts for approximately 15-20% of all TB cases worldwide. This unique manifestation presents diagnostic and therapeutic challenges, requiring a comprehensive understanding of its diverse clinical presentations and management strategies.[1]

EPTB can affect a multiple organs and systems, including the lymph nodes, skeletal system, gastrointestinal tract, genitourinary system, central nervous system, and others. Lymph node TB, or peripheral lymphadenopathy, is the most common form of EPTB, accounting for about half of all cases. It is typically characterized by painless swelling in the neck, armpit, or groin, and requires careful evaluation to exclude other causes of lymphadenopathy. Skeletal tuberculosis is a prominent form of tuberculosis that affects bones and joints, particularly prevalent in countries with high TB burden. It can cause bone destruction, spinal deformities, and joint impairment.[2]

Diagnosing EPTB is challenging due to its varied organ involvement and nonspecific clinical manifestations. A definitive diagnosis depends on obtaining biological samples from the affected site. Procedures such as fine-needle aspiration, biopsy, cerebrospinal fluid analysis, or imaging techniques

assist in sample collection. Modern molecular tests, such as nucleic acid amplification assays, can rapidly and accurately detect M. tuberculosis in these samples.

As EPTB continues to pose a significant burden on global health, it is imperative to recognize it early and manage it comprehensively. Achieving timely diagnosis and treatment initiation of EPTB, requires a high index of suspicion, knowledge of diverse clinical presentations, and appropriate diagnostic approaches. In addition, optimizing TB control strategies, including improved access to healthcare, strengthening laboratory services, and public health interventions, can help reduce the global impact of EPTB.

Culture-based techniques are also commonly used to diagnose of EPTB. Culture of M. tuberculosis is considered the gold standard for the diagnosis of TB, including EPTB. However, the culture of M. tuberculosis from extrapulmonry sites can be challenging due to low bacterial burden, slow growth rates, and the presence of contaminating organisms. Therefore, culture-based methods should be used in combination with other diagnostic methods for the accurate diagnosis of EPTB.

The diagnosis of EPTB remains a challenge due to its diverse clinical presentations and the difficulty in obtaining adequate samples for microbiological confirmation. Imaging techniques, serological tests, and culture-based methods, can all be useful for diagnosing EPTB. However, no single test is sufficient to diagnose EPTB, and a combination of methods is recommended for accurate diagnosis.

The aim of this study was to compare results of conventional methods with PCR used to detect M. tuberculosis in extrapulmonary specimens.

MATERIALS AND METHODS

This is a prospective cross-sectional observational study, which was conducted from February 2013 to January 2014 in the Department of Microbiology at Sri Ram Murti Smarak Institute of Medical Sciences (SRMS-IMS), Bareilly. Samples were collected from both medical and surgical departments, including both outpatient and inpatient cases suspected of EPTB. The study was approved by the Institutional Ethics Committee.

This study compares the performance of conventional techniques, such as Ziehl-Neelsen (ZN) staining, Fluorescent staining, and culture on Lowenstein-Jensen (LJ) and Mycobacterium Growth Indicator Tube (MGIT) media, with the molecular technique (PCR) for detecting M. tuberculosis in various specimens. A total of 100 clinical specimens were collected, including pleural fluid (42), ascitic fluid (16), cerebrospinal fluid (12), fine-needle aspiration cytology (FNAC) of lymph nodes (11), endometrial biopsy (10), pus (4), synovial fluid (4), and pericardial fluid (1).

Inclusion Criteria

Samples taken from suspected cases of extrapulmonary tuberculosis.

Exclusion Criteria

This study excludes all cases of pulmonary tuberculosis, including those identified through sputum and bronchoalveolar lavages.

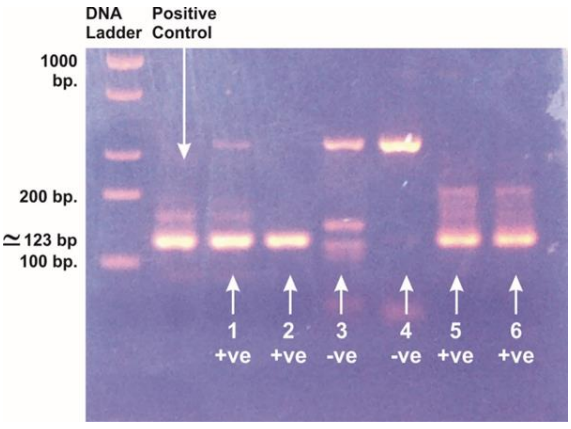
The samples received in the microbiology department were divided for conventional methods and PCR processes. Each sample underwent smear microscopy, both directly and after the required concentration. All received samples were processed according to standard guidelines.

The body fluids were centrifuged at 3000 rpm for 30 minutes, and the sediment was used for further testing. The tissue samples were placed in normal saline and then processed further. The processed samples underwent microscopy, culture on LJ medium and MGIT tubes, and PCR for the detection of M. tuberculosis.

For microscopy, smears were stained with ZN and Fluorescent stains and observed under a fluorescent microscope. For culture, samples were inoculated on LJ media and MGIT tubes. The MGIT tubes were incubated inside the BD MGIT320 instrument for six weeks, while the LJ media were incubated for eight weeks in the incubator dedicated to Mycobacterial culture. The samples were observed weekly for characteristic growth on LJ media for eight weeks and for MGIT for six weeks.

PCR was used to amplify and detect the IS6110 gene in the samples, utilizing commercial kits (Genei, Bengaluru, India). The resulting PCR products were analyzed by gel electrophoresis in 2% agarose and probed in a gel documentation system (Bio-Rad) for 123 bp using standard molecular markers. A positive result was indicated by the presence of a single band equal to 123 bp, while the negative control showed no reaction.

Statistical analysis of the results was performed using SPSS version 23



RESULTS

One hundred clinically suspected pulmonary tuberculosis specimens were analyzed by conventional and molecular methods. Of these samples, 18% of samples were positive on ZN

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staining and 20% of samples were positive on Fluorescent staining, while 22% of samples were positive on solid culture media LJ media and 26% of samples were positive on liquid culture MGIT 320 culture. Finally, 62% of the samples tested positive by PCR.

The study findings suggest that PCR is the most sensitive diagnostic technique for EPTB among the tested samples, with a sensitivity of 62%. Among conventional techniques and culture methods, culture on MGIT 320 media showed the highest sensitivity (26%), followed by culture on LJ media (22%). ZN staining and Auramine staining had the lowest sensitivity in all the samples tested, at 18% and 20%, respectively. [Table 2]

In the statistical analysis comparing the molecular technique PCR with ZN staining, PCR showed 100% positivity in ZN staining smear positive samples and 53.65% positivity in ZN smear negative samples. Similarly, when compared with fluorescent staining, PCR showed a sensitivity of 100% positivity in fluorescent staining smear-positive samples and 52.50% positivity in fluorescent smear-negative samples.

When comparing PCR to LJ culture, PCR showed 100% positivity in LJ culture positive samples and 51.2% positivity in LJ culture negative samples. Similarly, based on statistical analysis comparing PCR to MGIT, the sensitivity of PCR was found to be 84.65%. PCR showed 84.65% positivity in MGIT culture-positive samples and 54.05% positivity in MGIT culture-negative samples. PCR was positive in 40 out of 74 samples that showed no growth on MGIT culture.

Statistical analysis was conducted to compare PCR to LJ culture media as the gold standard. The results showed that PCR had a sensitivity of 100% and a specificity of 48.7%.

The study found a diagnostic accuracy of 60%, with a positive predictive value of 35.4% and a negative predictive value of 100%. The statistical analysis indicated significance with a P value of less than .001 for PCR.

Table 1: Distribution of positivity of Extra-pulmonary tuberculosis samples by different methods.

Type of specimen	ZN Staining	Fluorescent Staining	LJ Culture	MGIT Culture	PCR
Pleural fluid	5/42	5/42	8/42	11/42	24/42
Ascitic fluid	1/16	1/16	2/16	3/16	8/16
CSF	2/12	2/12	1/12	1/12	9/12
LN-FNAC	6/11	7/11	6/11	5/11	9/11
Endometrial biopsy	2/10	3/10	2/10	3/10	6/10
Pus	2/4	2/4	2/4	2/4	3/4
Synovial fluid	0/4	0/4	1/4	1/4	2/4
Pericardial fluid	0/1	0/1	0/1	0/1	1/1
Total	18/100	20/100	22/100	26/100	62/100

Table 2: Comparison of sensitivity of PCR test with other conventional tests

Test /Result Category (No.)	PCR Result		Sensitivity of PCR test (%)
	Positive	Negative	
Z-N Smear positive (18)	18	0	100%
Z-N Smear negative (82)	44	38	53.65%
FI Smear positive (20)	20	0	100%
FI Smear negative (80)	42	38	52.5%
L J positive(22)	22	0	100%
L J negative (78)	40	38	51.28%
MGIT positive (26)	22	4	84.61%
MGIT negative(74)	40	34	54.5%

Table 3: Results of conventional methods and PCR in various studies

ZN Staining % positive	Fluorescent Staining %positive	LJ Culture % positive	MGIT320 % positive	PCR % positive
S.S Negi et al 23.8%	A.Jain et al,[7] 9.9%	S.S negi (2007) et al,[9] 42.18%	S. Rishi et al,[3] study 20.28%	Shukla I et al,[8] 73%
Siddiqui Mam et al,[5] 5 .0%	Gerardo Avarez-Uria et al,[12] 9.1%	S.S negi (2005) et al,[10] 46.83%.	S.S negi (2007) et al,[9] 48.63%	S.S negi (2007) et al,[9] 77.27%
K.Desai et al,[11] 14.28%	K. Desai et al,[11] 21.42%	Siddiqui Mam et al,[5] 15%	S.S negi (2005) et al,[10] 55.6%	S.S negi (2005) et al,[10] 11 75.9%
B.Sekar et al,[4] 18%	Present study 20%	Gill Manmeet et al,[6] 13.3%	Siddiqui Mam et al,[5] 15%	Siddiqui Mam et al,[5] 70%
Present study 18%		Present study 22%	Present study 26%	B. Sekar et al,[4] 63%
				Present study 62%

DISCUSSION

Tuberculosis (TB) is a significant public health concern, with an estimated 10 million cases and 1.4 million deaths in 2019. The diagnosis of TB remains challenging due to the time-consuming and limited sensitivity of conventional techniques such as microscopy, culture, and histopathology. Molecular techniques, such as polymerase chain reaction (PCR), have emerged as promising tools for TB diagnosis.

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However, their performance varies depending on the type of clinical specimen.

The results of the present study show that 18% of samples were positive for ZN staining and 20% were positive for fluorescent staining. Additionally, 22% of samples were positive on solid culture media LJ media, 26% were positive on liquid culture MGIT 320 culture, and 62% were positive by PCR.

These findings are consistent with those of other studies, as shown in [Table 3].

The study shows that PCR is the most sensitive technique for diagnosing extrapulmonary TB (EPTB) and can be combined with other conventional techniques to improve diagnostic accuracy. PCR's high sensitivity may be due to its ability to detect low levels of TB DNA in clinical samples. These findings are consistent with previous studies that have reported PCR's high sensitivity and specificity for TB diagnosis.[1,2] However, the cost of polymerase chain reaction (PCR) is higher compared to conventional techniques, which may limit its use in resource-limited settings.

Culture-based methods remain the gold standard for the diagnosis of TB due to their ability to provide information on the drug susceptibility of the TB strain, which is crucial for selecting appropriate treatment regimens. However, these methods have several limitations, including slow turnaround time, inoculation of only 1-10 bacilli/mL in specimen, and the need for specialized laboratory facilities.

ZN staining and fluorescent staining are simple and inexpensive techniques used for diagnosing TB in resource-limited settings where more sophisticated diagnostic tools are not available. However, their sensitivity is relatively low compared to other techniques.

CONCLUSION

The study findings suggest that conventional techniques, such as ZN and fluorescent staining, have limited diagnostic performance in detecting tuberculosis. Culture methods, particularly MGIT 320, showed improved diagnostic performance, but their utility is limited due to their long turnaround time and low sensitivity. On the other hand PCR demonstrated high sensitivity and specificity, making it a valuable tool for the rapid and accurate diagnosis of tuberculosis. However, the high cost of polymerase chain reaction (PCR) limits its widespread implementation, especially in resource-limited settings.

In conclusion, our study shows that PCR is a highly sensitive technique for diagnosing EPTB and can be used in combination with other conventional techniques to improve diagnostic accuracy. The choice of diagnostic technique should also depend on the type of sample tested.

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