Journal of Clinical and Medical Images

Technical Paper

ISSN: 2640-9615 | Volume 6

Comparative Study of Truenat MTB/RIF Assay and MGIT Culture in Testing Different Types of Spinal Tuberculosis Tissue Specimens in T Ward Mumbai

Viswanathan R*, Kumar SU, Mehta SS

Department of Medicine, Qualilife diagnostics, India

***Corresponding author:**

Roopa Viswanathan, Department of Medicine, Qualilife diagnostics, India,

E-mail: ruvishy@rediffmail.com

Received: 01 Jul 2022

Accepted: 11 Jul 2022

Published: 16 Jul 2022

J Short Name: JCMI

Citation:

Roopa Viswanathan, Comparative Study of Truenat MTB/RIF Assay and MGIT Culture in Testing Different Types of Spinal Tu-berculosis Tissue Specimens in T Ward Mumbai. J Clin MedImg. 2022; V6(13): 1-4

1. Abstract

1.1. Introduction: The World Health Organization says there are nearly 1 million cases of rifampicin-resistant tuberculosis (RR-TB) worldwide in 2019. About 78% of cases are multidrug-resistant (MTB), of which India bears the heaviest burden. Early diagnosis and determination of drug resistance could promote effective treatment of spinal tuberculosis and reduce therisk of MTB.

1.2. Aims: The study aims to compare the sensitivity and specificity of BACTEC (MGIT) culture and Truenat MTB/RIF test on different types of spinal TB tissue specimens to improve the early diagnosis of spinal TB. Methods: In 198 patients diagnosed with spinal tuberculosis, different specimens were obtained through needle biopsy under CT or fluoroscopy guidance. The specimens were sent to the laboratory in normal saline. The precipitate was divided into two parts, with one for bacterial culture and pDST and the other for Truenat MTB/RIF testing.

1.3. Results: The positive rate (73.3%) from the Truenat MTB/RIF assays of spinal TB patients' tissue specimens was higher compared with bacterial culture (43.4%), and the positive rates from True MTB/RIF assays on the three types of specimens were all higher than those of bacterial culture, with statistically significant results for pus and caseous tissue specimens. The positive rates for pus using the two bacteriological tests were higher than those for caseous tissue but were not statistically significant. However, the positive rates obtained from granulation tissue were statistically significantlyhigher than those obtained from caseous necrotic tissue.

1.4. Conclusion: Early detection and treatment is the only strategy to prevent DR in spinal TB. Various types of specimens are obtained from spinal TB patients, especially pus specimens will give more accurate diagnosis of TB infection and DR using the Truenat MTB/RIF assay. Meanwhile, as the percentage of patients with DR-TBresistant to various first- and second-line drugs rises, it's vital to enhance existing detection methods, expand genomic locus coverage, as well as provide comprehensive evaluations.

2. Introduction

Tuberculosis (TB) is a bacterial infection caused by Mycobacterium tuberculosis complex (MTBC) [1]. In 2019, an estimated 10.0 million (8.9-11.0 million) people globally contracted tuberculosis. In India and across the world, drug-resistant tuberculosis (DR-TB) continues to be a significant health problem. In 2019, nearly 10% people worldwide were suffering from rifampicin-resistant tuberculosis, of which 78% were suffering from multidrug-resistant tuberculosis, of which India bears the world's largest burden (27%) [2]. Early diagnosis and determination of drug resistance could promote effective treatment of spinal tuberculosis and reduce the risk of multidrug-resistant TB [3]. When compared to pulmonary samples, the bacterial load in extra pulmonary samples is relatively verylow, which has a negative effect on the sensitivity of tests like acid-fast bacilli (AFB) microscopy, line probe assays, and other nucleic acid amplification tests (NAATs) [4]. This reduced sensitivity leads to delays in diagnosis and treatment, leading to the development of multi-drug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis. Previously, phenotypic drug sensitivity testing (PDST) and Lowenstein – Jensen (LJ) culture medium were common methods for diagnosing DR-TB. However, these tests take 1-2 months to process and diagnose, leading to delayed diag-

nosis and treatment, as well as an increased risk of acquired drug resistance (DR) [5]. The recently introduced nonradiometric Bactec Mycobacterial Growth Indicator Tube (MGIT) System (Becton Dickinson Diagnostic Systems) can constantly monitor the fluorescence produced by mycobacteria growth, providing rapid and consistent test findings as well as first- and second-line treatment sensitivity [6]. Hence, MGIT culture has been widely used in recent days. The GeneXpert MTB/RIF test is a commercial real-time polymerase chain reaction (PCR) kit that detects Mycobacterium tuberculosis complex and rifampicin resistance at the same time. In 2013 World Health Organization (WHO) recommended it for the diagnosis of extrapulmonary tuberculosis. The results show that it hashigher specificityin the MTB test but only the average sensitivity and higher sensitivity and specificity in the rifampicin DR test [7]. To establish and implementation is difficult in most of the developing countries because it is expensive and needs more infrastructure to establish. In India there is an urgent need for the development of cost-effective molecular tests, that can be used with less infrastructure backup. Bigetec laboratories in India have developed a battery-based Truenat MTB device as well as Trueprep MAG for extraction of DNA from the samples for early detection of TB [8]. The assay required minimal training to technicians. Truenat instruments were battery operated and temperature stable thereby needs less infrastructure to establish. So, it is widely used in developing countries like India. Clinical specimens of spinal TB are more difficult to obtain than sputum because it generally affects the peri-discal region, which is more difficult to reach. Pus, caseous tissue, and bone tissue are among the tissues that are impacted. Obtaining a suitable and sufficient sample sometimes necessitates the use of fluoroscopy and CT guidance. However, due to the low bacterial population, the specificity and sensitivity of these samples varied. The study aims to compare the sensitivity and specificity of BACTEC (MGIT) culture and Truenat MTB/RIF test on different types of spinal TB tissue specimens to improve the earlydiagnosis of spinal TB. Materials and methods Specimen collection and selection This is a multi-centricretrospective study carried out in two tertiary care hospitals from January 2016 to August 2021 in the T ward, Mumbai. In 198 patients diagnosed with spinal tuberculosis in clinical and imaging studies, different specimens were obtained through needle biopsy under CT or fluoroscopy guidance. These specimens include 158 pus specimens, 142 granulation tissue specimens, and 126 bone tissue specimens. The specimens were sent to the laboratory in sterile container. The precipitate was divided into two parts, with one for bacterial culture and pDST and the other for Truenat MTB/RIF testing. None of these patients were on anti-tubercular drugs at the time of biopsy. Culture Samples were inoculated in Mycobacteria Growth Indicator Tube 320 (MGIT) which isa liquid culture. MTB was identified by colony morphology and growth rate. The culture was taken as the reference standard. Truenat test Two drops of liquefaction buffer provided with the Trueprep Auto Sample Pre-treatment Pack (TSPP) were applied to 0.5 ml of homogenised sample, gently mixed, and left to stand for five minutes with intermittent shaking. The liquefied mixture was then transferred to 2.5 mL of TSPP lysis buffer, vigorously shaken, and set it aside for 3-5 minutes. Using a Pasteur pipette, the whole contents were transferred to the sample chamber of the Trueprep Auto Sample Prep Kit's cartridge. The cartridge was placed in the Trueprep Auto Sample Prep Device. The device automatically extracted DNA in 25 min which was collected in the DNA elution chamber. Six microlitres of the DNA were placed on the Truenat MTB micro-PCR chip placed on the chip tray of the Truelab Uno (V1.5) analyzer. Thetest was completed in 35 min and the displayed results were recorded [9, 10]. Statistical method With the culture report considered as the gold standards, the sensitivity and specificity were calculated for the comparison of positive rates from bacterial culture and Truenat MTB/RIF assays of the same specimen and those of different specimens in the same bacteriological test were examined using Z-values. $p < 0.05$ was considered statistically significant. Results Comparison of positive rates from bacterial culture and Truenat assay Among the specimens of the 198 patients, 86 patients had a positive culture, representing a positive rate of 43.4 %. Among the 426 specimens, there were 189 that tested positive. Specifically, the positive rate of pus specimens was 48.10%, which was moderately higher but not statistically significantly different from the positive rate of 47.89% of the granulation tissue specimens. However, the positive rate of the granulation tissue specimens was statistically significantly higher compared with bony necrotic tissue specimens (35.71%). No patient had culture-positive in bone tissue but negative pus and granulation tissue. Among the 198 cases, the specimens of 145 patients tested positive with the Truenat assay, accounting for a positive rate of 73.23%. Among the 426 specimens, there were 259 that tested positive, in which the positive rate of the pus specimens reached (72.78%) and was higher but not statistically significantly higher compared with the granulation tissue specimens (64.78%). However, the positive rate of granulation tissue specimens was statistically significantly higher compared with the bony tissue specimens (41.27%). Meanwhile, the positive rates of both the pus and granulation tissue specimens using the TrueNAT assay were statistically significantly higher than those for bacterial culture (pus specimens: $p < 0.01$; granulation tissue specimens: $p < 0.01$), but the positive rate of bony tissue specimens using the Truenat assay was higher but not statistically significantly higher than that for bacterial culture ($p > 0.05$). The data is summarised in [Table 1]. Sensitivity of Truenat assay in MTB test Among the 86 cases of bacterial culture with positive results, 82 also tested positive using the TrueNAT assay, indicating a sensitivity of 95.35%, and 63 of the remaining 112 cases with negative results from the bacterial culture tested positive with the Truenat test, producing a sensitivity of 56.25%. The data is summarised in [Table 2]. Discussion Spinal tuberculosis is one of the commonest EPTB and the leading cause of infection-related mortality worldwide. EPTB accounts for 10%–

20% of all TB cases, with skeletal TB accounting for 10-15% of EPTB cases. Vertebral involvement occurs in about half of all cases of musculoskeletal tuberculosis. It has the potential to result in long-term morbidity and impairment. Nearly half a million persons worldwide developed (MDR/RR-TB) in 2019, increasing 10% from the preceding year. India and China account for about half of the worldwide MDR-TB burden. This emphasizes the seriousness of the situation and highlights the requirement for prompt attention. The main factors for the development of drug resistance include delayed diagnosis and inadequate/incomplete treatment. Laboratory technique such as smear microscopy has very low sensitivity. Culture techniques are time-consuming and the sensitivity rate range from 10% to 60 [11]. PCR-based molecular diagnosis method is an efficient method for early detection of TB and drug resistance. Gene Xpert testing is expensive which makes it inaccessible for the patients in TB endemic countries [12]. Especially in India, there is a need for a more cost-effective rapid method for diagnosis. Truenat is a promising technique used to detect MTB in clinical specimens that is a point-of-care, battery-operated, chip-based RT-PCR microdevice [13]. It may be utilized to provide a rapid diagnostic with a very small amount of sample in low-infrastructure circumstances. The type of specimen and bacterial count of the specimen was linked to the accuracy of this PCR-based molecular diagnostic method. Compared with sputum, clinical specimens of spinal tuberculosis are difficult to collect due to the less number of bacteria count and high heterogeneity like pus, caseous tissue, and bony tissue. In addition, the use of needle biopsy for specimen collection is more difficult. In order to minimize the damage caused by puncture and obtain valuable specimens for early diagnosis and effective treatment, it is necessary to compare the positive rates of bacteria in different types of tissue specimens in the lesion. In our study, 428 spinal tuberculosis specimens were subjected to the BACTEC MGIT 320 culture test and Truenat assay, the positive rate of pus with MGIT culture reached 48.10%, followed bygranulation tissue (47.89%) and bone tissue (35.71%). This study has high concordance with other studies. Dong et al. (2014) selected 50 postoperative specimens of patients who were pathologically confirmed as having spinal and articular TB. Concerning thespecimens in the BACTEC MGIT 960 culture, thepositive rate of the sequestrum reached 41.9%, followed by pus (40.0%), granulation tissue (33.3%), and caseous tissue (33.3%), which reflected the difference in the viable count of the different types of tissue specimens. But culture test is time-consuming and leads to delaydiagnosis and acquired drug resistance [14]. The sensitivity of truenat showed that the positive rate of pus was 72.78%, followed by granulation tissue (64.79%) and bone tissue (41.27%). this studyhashigh concordance with other studies. Lin et al. (2015) carried out a study involving 43 patients who were pathologically confirmed as having late-stage articular TB. In the study, 43 postoperative specimens were collected and subjected to PCR-based fluorescence detection. It was found that the positive rate for granulation tissue was up to 74.42%, which was higher compared with caseous tissue (58.14%) and pus (37.21%) and reflected the difference in the MTB DNA content contained in different tissue specimens [15]. The Truenat MTB test detects TB in around 2 to 3 hours and can be used in near-care settings to provide a timely and effective diagnosis. In terms of each type of specimen, the positive rates for pus and granulation were also higher compared with bony tissue specimens in the Truenat MTB/RIF assay. Using bacterial cultureand pDST as the gold standards, the sensitivity of TB detection with the Truenat MTB/ RIF assaywas up to 95.34%, and the sensitivity and specificity in the DR test were relatively high. These results indicated that, in terms of spinal TB specimens, especially pus and granulation tissue, the Truenat MTB/RIF assay had higher sensitivity than bacterial culture. However, there is a paucity of research and studies on the differences in detection of bacteria between different tissue specimens. Furthermore, due to the significant differences in clinical progression, tissue composition, and tissue specimen collection between spinal and articular tuberculosis, studies on spinal and articular tuberculosis must be analysed separately. The study involved 198 patients with spinal tuberculosis. In both the Truenat assay and the combined culture method, the positive rates for pus specimens were higher than those for granulation tissue and bony tissue, which indicated that clinical puncture was useful for the collection of pus for early bacterial detection. Different types of specimens, especially pus specimens, are needed to carry out the Truenat MTB/RIF assay and bacterial culture, determine the TB infection and DR as early as possible, and commence effective treatment.

Table1: Comparison between Tryenat assayand culture in different types of samples in spinal TB

	Specimens Culture	Culture Positive	Truenat Positive	P value
PUS- 158	158	76 (48.1%)	115(72.7%)	<0.0001
Granulatio NTissue	142	68 (47.8%)	92 (64.7%)	0.004086
Bony Tissue	126	45 (35.7%)	52 (41.2%)	0.364805

Table 2: Sensitivity of Truenat assay with MGIT Culture

	Culture Positive	Culture Negative	
Total	86 (43.4%)	112	198
Truenat Positive	82	63	145 (73.3%)
Truenat negative	4	49	53
Sensitivity	95.30%	56.20%	

3. Conclusion

Early detection and treatment is the only strategy to prevent DR in spinal TB. Various types of specimens are obtained from spinal TB patients, especially pus specimens will give, fast, and accurate diagnosis of TBinfection and DR using the TrueNAT MTB/RIF assay. Meanwhile, as the percentage of patients with DR-TB resistant to various first- and second-line drugs rises, it's vital to enhance existing detection methods. The commonest target used in PCR test is IS6110 gene locus it is necessary to expand genomic locus coverage, as well as provide comprehensive evaluations.

References

1. Lawn SD, Zumla AI. Tuberculosis. Lancet. 2011; 378: 57-72.
2. World Health Organization. Global Tuberculosis Report 2020, CIP.
3. Pawar UM, Kundnani V, Agashe V, Nene A, Nene A. Multidrug resistant tuberculosis of the spine—is it the beginning of the end? A study of twenty-five culture proven multidrug-resistant tuberculosis spine patients. Spine. 2009; 34: E806-10.
4. Pandey S, Congdon J, McInnes B, Pop A, Coulter C. Evaluation of the GeneXpert MTB/RIF assay on extrapulmonary and respiratory samples other than sputum: a low burden country experience. 2017; 49: 70-4.
5. Li L, Zhang Z, Luo F, Xu J, Cheng P, Wu Z, et al., Management of drug-resistant spinal tuberculosis with a combination of surgery and individualised chemotherapy: a retrospective analysis of thirty-five patients. Int Orthop. 2012; 36: 277-83.
6. Ardito F, Posteraro B, Sanguinetti M, Zanetti S, Fadda G. Evaluation of BACTEC Mycobacteria Growth Indicator Tube (MGIT 960) automated system for drug susceptibility testing of Mycobacterium tuberculosis. J Clin Microbiol. 2001; 39: 4440-4.
7. World Health Organization. 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: Policy Update. World Health Organization, Geneva. 2013.
8. Kumar KK, Jayaraman R, Narasimha SK, Radhakrishnan RM, Viswanathan S, et al., Handheld Micro PCR Device. PCT Pub. No.WO/2009/04780. 2009.
9. Nikam C, Jagannath M, Narayanan MM, Ramanabhiraman V, Kazi M, Shetty A, et al., Rapid diagnosis of Mycobacterium tuberculosis with Truenat MTB: A nearcare approach. PLoS One. 2013; 8: e51121.
10. Vijayalakshmi J, Surekha A, Renuka devi A, Uma devi S. Truenat -A novel diagnostic tool for rapid detection of Mycobacterium tuberculosis and rifampicin resistance in pulmonary samples. Int J Curr Microbiol App Sci. 2019; 8: 1260-7.
11. Magnussen A, Dinneen A, Ramesh P. Osteoarticular tuberculosis: increasing incidence of a difficult clinical diagnosis. Br J Gen Pract. 2013; 63: 385-6.
12. Parsons LM, Somoskövi A, Gutierrez C, Lee E, Paramasivan CN, Abimiku A, et al., Laboratory diagnosis of tuberculosis in resource-poor countries: challenges and opportunities. Clin Microbiol Rev. 2011; 24: 314-50.
13. Jose RA, Gopal K, Samuel Johnson AK, Johnson Samuel JA, Abraham SS, Goswami, T, et al., Evaluation of Truenat MTB/RIF Test in Comparison with Microscopy and Culture for Diagnosis of Extrapulmonary Tuberculosis in a Tertiary Care Centre. Journal of Clinical & Diagnostic Research. 2021; 15.
14. Li Y, Jia W, Lei G, Zhao D, Wang G, Qin S. Diagnostic efficiency of Xpert MTB/RIF assay for osteoarticular tuberculosis in patients with inflammatory arthritis in China. PLoS One. 2018; 13: e0198600.
15. Lin QX, Huang XW, Li HM, et al., PCR detecting comparison in different materials from patients with active joint TB. Orthop J Cn. 2015; 23: 1212–5.