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Comparative diagnostic value of GeneXpert MTB/RIF, PCR targeting IS6110 and culture for the diagnosis of spinal tuberculosis

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Abstract

Introduction Spinal tuberculosis is an extra-pulmonary tuberculosis infection caused by *Mycobacterium tuberculosis* (MTB) which affects the vertebrae. Culture test is the «gold standard» diagnostic method, but it takes a long time. PCR is a method of a shorter time than the culture test, so it can be an alternative diagnostic method for MTB. **Materials and Methods** This study is a cross-sectional study. The data were analyzed with diagnostic tests on patients with suspected spinal tuberculosis who performed surgery in Hasan Sadikin Hospital in Bandung. Clinical examinations and diagnostic examinations were done in 40 patients and surgery was performed to obtain samples from the spinal cord and the infected tissue. GeneXpert, PCR targeting IS6110 and culture tests were performed. The research was conducted at the Department of Orthopaedics and Traumatology and the Clinical Pathology Laboratory of FK UNPAD/ RSHS from September 2019 to September 2020. **Results** GeneXpert assay compared with culture tests as the standard diagnostic method showed sensitivity of 96.67 %; specificity of 90.00 %; positive predictive value 96.67 %; and a negative predictive value of 90.00 %, with an accuracy of 95.00 %. The PCR targeting IS6110 against culture showed that the sensitivity of MTB bacterial infection was 93.33 %, the specificity was 80.00 %, the positive predictive value was 93.33 %, the negative predictive value was 80.00 %, and the accuracy was 90.00 %. **Conclusion** This study concluded that the GeneXpert MTB/RIF RT-PCR assay has a high sensitivity, specificity, and accuracy compared to PCR targeting IS6110 in examining tissue samples in patients with spinal TB.

Keywords: spinal TB, GeneXpert, PCR target IS 6110, culture, diagnosis

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INTRODUCTION

Tuberculosis (TB) is a disease caused by the bacteria *Mycobacterium Tuberculosis*. The World Health Organization (WHO) estimates that 10.4 million people have TB, and as many as 1.3 million people died due to TB in 2016. In 2013, the number of TB sufferers in Indonesia was in fifth place after India, China, Nigeria, and Pakistan [1]. In general population, the estimated prevalence of TB was 647 per 100,000 population, and the incidence of TB in Indonesia in 2013 was 410,000 – 520,000 cases [2].

Approximately 75 % cases of tuberculosis are cases that occur in the pulmonary organs and the other 25 % affect organs outside the lungs. About 10 % of extrapulmonary tuberculosis occurs as musculoskeletal tuberculosis. In 50 % of cases, musculoskeletal tuberculosis attacks the spine, in 30 % the hip joint, and 20 % occurs in other joints [3–5].

To ensure a definite diagnosis of spinal tuberculosis, it is necessary to collect a specimen for culture examination to determine the infection bacteria. Culturing technique is still considered the gold standard method. However, a slow growth of M. tuberculosis bacteria is a major obstacle in rapid diagnosis of the disease, which is 3 to 12 weeks [6].

Culturing takes a long time to determine a definite spinal TB diagnosis. A diagnostic tool that is fast and has high sensitivity and specificity is needed to improve the conventional diagnostic method, namely Polymerase Chain Reaction (PCR). PCR is a process of forming DNA prints repeatedly using a certain procedure and time. The DNA amplification process carried out by PCR can detect tuberculosis bacilli which are insufficient to be examined microscopically [7, 8].

Various PCR modalities are being developed as diagnostic tools. The PCR examination with IS 6110 and rpoB gene targets obtained high sensitivity and specificity for detecting tuberculosis [9]. In the diagnostic evaluation of target IS 6110 PCR for pulmonary tuberculosis showed a sensitivity of 97.2 % and specificity of 99.7 %. For spinal TB, the sensitivity was 85.7 % and the specificity was 100 %. GeneXpert is a semi-quantitative Real-Time Polymerase Chain Reaction Assay (RT-PCR) method that targets the rpoB gene's hotspot in M. Tuberculosis [10, 11].

Also, GeneXpert is able to detect resistance from rifampin, but the examination cost is relatively high. Among patients with positive cultures, the smear-positive MTB/RIF test for pulmonary tuberculosis showed sensitivity (98.2 %) and specificity (99.2 %). The positive predictive value was 97.7 %, and the negative predictive value was 92.6 % [12]. The aim of this study was to determine the sensitivity and specificity of the GeneXpert assay compared to the PCR targeting IS 6110 in diagnosing spinal tuberculosis.

MATERIALS AND METHODS

This study is a retrospective analysis of 40 patients with spinal tuberculosis treated in Hasan Sadikin General Hospital from September 2019 to September 2020. Medical history, physical examination, laboratory tests, X-ray and spine MRI were studied in all patients. Posterior stabilization followed and tissue samples were obtained. Samples were taken if the patients' criteria were: 1) age 15–65 years old; 2) diagnosed with paravertebral abscess; 3) patient underwent spine surgery. The exclusion criteria were 1) patients with spinal tuberculosis who underwent surgery, took anti-tuberculosis drugs and relapse; 2) pregnancy.

Research methods

This study is a cross-sectional observational study. All patients who met the inclusion and exclusion criteria were included as study subjects. This study analyzed the diagnostic test to obtain sensitivity, specificity, positive predictive values, negative predictive values, positive likelihood ratios, negative likelihood ratios, and accuracy values. The tissue samples were stored in the prepared culture tube and each was examined using the PCR targeting IS 6110, GenExpert tool, and culturing.

The sample was taken by consecutive sampling. The results of GeneXpert test and PCR targeting IS 6110 was positive if bacteria of MTB were found and stated negative if no MTB bacteria were detected. The culture tests results were positive if there is bacterial colonization on Loweinstein Jensen media, whereas it is stated negative if there is no growth of bacterial colonization in 7 weeks.

RESULTS

This study found that the average age was 36.07 years with the youngest age being 18 years and the oldest age being 61 years (Table 1). The largest age group was aged 20–29 years, with 23 males (57.5 %), and 17 females (42.5 %) (Table 2).

Table 1

Distribution of Spinal Tuberculosis according to Age

Age (years old)	N (%)
< 20	3 (7,5)
20-29	12 (30)
30-39	10 (25)
40-49	8 (20)
50-59	6 (15)
≥ 60	1 (2,5)
Total	40 (100)

Table 2

Distribution of Spinal Tuberculosis according to Sex

Sex	N (%)
Male	23 (57.5)
Female	17 (42.5)
Total	40 (100)

A clinical pathologist interpreted the results of GeneXpert MTB/RIF tool, PCR target IS 6110 and tissue culturing. The result was stated positive if the MTB was found and negative if not found. The diagnostic test results are presented in the form of a 2×2 table with the Chi-square test. After being given positive and negative limits from patient's data who had been tested using the GeneXpert tool, PCR target IS 6110 and culture, the results are shown in Table 3.

From the Chi-square test between GeneXpert tool and diagnostics tools using culture methods, the p-value was = 0.000 < 0.05, indicating a significant relationship between GeneXpert diagnostics and tissue culture diagnostics. Also, significant relationship was found between diagnostic tools using PCR target IS 6110 and tissue culture, with the p-value < 0.05 (Table 4).

Table 3

Diagnostic testing for GeneXpert and Culture

GeneXpert	Culture		Total	p Value*
	Positive (+)	Negative (-)		
Positive (+)	29 (96.7 %)	1 (3.3 %) 9	30 (100.0 %)	0.000
Negative (-)	1 (10.0 %)	(90.0 %)	10 (100.0 %)	
Total	30 (75.0 %)	10 (25.0 %)	40 (100.0 %)	

Note: * – according to Chi-Square test.

Tabel 4

Diagnostic testing for PCR target IS 6110 and Tissue Culture

PCR target IS 6110	**Culture**		**Total**	**p value***
	Positive (+)	**Negative (-)**		
Positive (+)	28 (93.3 %)	2 (6.7 %)	30 (100.0 %)	0.000
Negative (-)	2 (20.0 %)	8 (80.0 %)	10 (100.0 %)	
Total	30 (75.0 %)	10 (25.0 %)	40 (100.0 %)	

Note: * – according to Chi-Square test.

Tabel 5

Diagnostic results of GeneXpert and PCR target IS 6110

Diagnostic Testing	GeneXpert	PCR target IS 6110
Sensitivity	96.67 %	93.33 %
Specificity	90.00 %	80.00 %
Positive Predictive Value	96.67 %	93.33 %
Negatif Predictive Value	90.00 %	80.00 %
Accuracy	95.00 %	90.00 %

In Table 5, the chi-square test using the GeneXpert tool, PCR target IS 6110 and culture media, the p-value

was $p = 0.000 < 0.05$ indicating a significant relationship compared with the gold standard diagnostic tools. The GeneXpert MTB/RIF test has a higher sensitivity, specificity, positive predictive value, negative predictive value, and accuracy than the PCR target IS 6110 for the diagnosis of spinal tuberculosis.

DISCUSSION

In this study, the specificity value, positive predictive value and accuracy of GeneXpert were higher than the PCR target IS 6110 so that the detection of *Mycobacterium Tuberculosis* bacterial infection was more accurate in GeneXpert diagnostics. This study supports the research conducted by Sharma et al. that concluded that the GeneXpert assay had a sensitivity of 93.4 % for detecting MTB bacteria [13]. In the study by Held et al., GeneXpert sensitivity results for spinal TB also had similar sensitivity (95.6 %) and specificity (96.2 %) with the positive predictive value of 97.7 %, and the negative predictive value was 92.6 % [14]. Similar results were obtained by Boehme CC et al., which obtained results from MTB/RIF test among culture-positive patients. The sensitivity of patients with smear-positive (98.2 %) and the specificity of patients without TB (99.2 %) [11].

The GeneXpert MTB/RIF system is a fully automated, Real-Time PCR system that detects DNA complex MTB in sputum and other sample types (i.e. pleura, aspirated lymph nodes or tissue, CSF, gastric fluid and non-lymph node tissue) [11, 15]. Mechal et al. in their study stated that GeneXpert is a breakthrough discovery for TB diagnosis based on molecular examinations using the semi-quantitative real-time polymerase chain reaction assay (RT-PCR) method targeting the rpoB gene hotspot in M. tuberculosis, which is integrated and automatically processes preparations by extracting deoxyribonucleic acid (DNA) in a single-use cartridge [16]. The time to get results is less than 2 hours and only requires simple training to use this tool.

The examination with GeneXpert RT-PCR method is based on repeated amplification of the target DNA, and then it is detected fluorimetrically. This technique can identify the M. tuberculosis rpoB gene and its sequence more easily, quickly and accurately. This gene is closely related to cell resistance and targets the bactericidal rifampin drug in M. tuberculosis and M. leprae. Preliminary research states that the sensitivity and specificity are high enough in respiratory tract samples to detect M. tuberculosis and simultaneously detect the resistance of M. tuberculosis to rifampin. The GeneXpert test showed better results compared to smear microscopy and performed equally well with fluid culture [17].

Examination by PCR target IS 6110 was to detect the DNA sequence complex of *Mycobacterium Tuberculosis* (MTB). Identification of M. tuberculosis was performed using a specific pair of primers designed to amplify the insertion sequence of IS6110 in the M. tuberculosis complex [18]. These primary sequences, T4 and T5, were: 5'-CCT GCG AGC GTA GGC GTC GG 3 'and 5' CTC GTC CAG CGC CGC TTC GG 3', respectively. Due to the repetitive nature of the insertion sequence IS6110, this primer is commonly used as a target in the detection of MTB in pulmonary or extra lung specimens [19]. However, this DNA sequence has limitations despite its high sensitivity, as several strains of *M. tuberculosis* were found to have no IS6110 sequence.

A prospective analysis by Leung KS et al. showed a diagnostic evaluation test for pulmonary tuberculosis with a target PCR of IS 6110 had a sensitivity and specificity of 97.2 % and 99.7 % respectively [9]. Wang et al. found that IS 6110 can be used as a diagnostic tool in differentiating MTB species, with the results obtained that clinical sensitivity and specificity for pulmonary tuberculosis were 92.00 % (23/25) and 76.92 % (30/39), and extrapulmonary 92.31 % (12/13) and 86.79 % (46/53), respectively [11]. Based on the study of Sharma K et al. in 2017 on specimens from spinal tissue examined by PCR target IS 6110, the results obtained a sensitivity of 85.7 % and specificity of 100 % [10].

CONCLUSION

This study strength is that it is the only study in Indonesia that assessed the diagnostic accuracy of GeneXpert and PCR target IS 6110 in patients with extrapulmonary TB, specifically patients with spinal TB. This is important because the main advantage of GeneXpert is that it can provide fast diagnostic results without sacrificing diagnostic accuracy for patients with TB. Faster testing can help with an earlier diagnosis, especially in the regions with high incidence of TB such as Indonesia. In addition, this study is expected to be used as a recommendation for mandatory examination and become the gold standard for all spine MTB patients. This study concludes that the GeneXpert RT-PCR assay has a higher sensitivity and specificity value for diagnosing spinal tuberculosis compared to the PCR target IS 6110 method at Hasan Sadikin General Hospital.

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