ORIGINAL ARTICLE

ค่าการตรวจวินิจฉัยของพันธุกรรมวัณโรคแบบเรียลไทม์เพื่อวินิจฉัยวัณโรคปอด

Diagnosis Test of Real–time Polymerase Chain Reaction (RT–PCR) for Pulmonary Tuberculosis

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บทคัดย่อ

ที่มาของปัญหา: วัณโรคปอดยังคงเป็นโรคที่เป็นสาเหตุสำคัญของการเจ็บป่วยและเสียชีวิตของประชากรไทย การตรวจหาเชื้อวัณโรคทางจุลชีวิทยามีความสำคัญเนื่องจากการวินิจฉัยที่รวดเร็วและถูกต้องรวมถึงการทดสอบการดื้อยาจะช่วยให้การรักษามีสิทธิภาพและทันท่วงที ในปัจจุบันการวินิจฉัยที่เป็นมาตรฐานคือ การเพาะเชื้อมัยโคแบคทีเรียและวิธีที่กำลังเป็นที่นิยมคือ การตรวจหาเชื้อด้วยเทคนิคเรียลไทม์พีซีอาร์ ได้แก่ Allplex™ MTB/MDRe Detection

วัตถุประสงค์: เพื่อประเมินค่าการตรวจวินิจฉัยเชื้อวัณโรคปอดด้วยเทคนิคเรียลไทม์พีซีอาร์โดยชุดตรวจสอบ Allplex™ MTB/MDRe Detection จากตัวอย่างเสมหะโดยเปรียบเทียบกับวิธีมาตรฐานการเพาะเชื้อมัยโคแบคทีเรีย

วิธีการศึกษา: การศึกษาแบบย้อนหลังในผู้ป่วยอายุมากกว่า 15 ปีและสงสัยวัณโรคปอด ดำเนินการตั้งแต่มกราคมถึงตุลาคม พ.ศ. 2566 ที่โรงพยาบาลขอนแก่น คำนวณขนาดกลุ่มตัวอย่างได้ 101 คน มีการวิเคราะห์ชุดตรวจสอบ Allplex™ MTB/MDRe Detection เพื่อทดสอบค่าความไว ความจำเพาะ ความถูกต้อง ค่าทำนายเมื่อผลเป็นลบ (NPV) และค่าทำนายเมื่อผลเป็นบวก (PPV) ที่ระดับความเชื่อมั่น 95% (95%CI) ตามลำดับ

ผลการศึกษา: จำนวนผู้ป่วยสงสัยวัณโรคปอด 120 คน การวินิจฉัยวัณโรคปอดด้วยชุดตรวจ Allplex™ MTB/MDRe และการเพาะเชื้อพบ 86 ราย (ร้อยละ 71.6) และ 73 ราย (ร้อยละ 60.8) ตามลำดับ เมื่อเปรียบเทียบผลการทดสอบของชุดการทดสอบ Allplex™ MTB/MDRe Detection พบว่าค่าความไว ความจำเพาะและความถูกต้องคือ ร้อยละ 97.3 (95%CI, 90.5-99.6), ร้อยละ 68.1 (95%CI, 52.8-80.9) และ ร้อยละ 85.8 (95%CI, 78.3-91.5) ตามลำดับ ค่าทำนายเมื่อผลเป็นบวก (PPV) และค่าทำนายเมื่อผลเป็นลบ (NPV) คือ ร้อยละ 82.5 (95%CI, 75.7-87.8) และร้อยละ 94.1 (95%CI, 80.1-98.4) อัตราของผลการตรวจเป็นบวกในผู้ที่เป็นโรคเปรียบเทียบกับผู้ที่ไม่เป็นโรค (LR+) คือ 3.1 เท่า (95%CI, 2.0-4.6) และอัตราของผลการตรวจเป็นลบในผู้ที่เป็นโรคเปรียบเทียบกับผู้ที่ไม่เป็นโรค (LR-) คือ 0.04 เท่า (95%CI, 0.01-0.16)

สรุป: ชุดตรวจสอบ Allplex™ MTB/MDRe Detection มีประสิทธิภาพในการตรวจหาเชื้อวัณโรคปอดเพื่อให้ผู้ติดเชื้อสามารถเข้าสู่กระบวนการรักษาตามมาตรฐานได้อย่างรวดเร็ว

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ClinicalTrials.gov Identifier, NCT06284187

ABSTRAC

BACKGROUND: Pulmonary tuberculosis remains the leading cause of morbidity and mortality in Thailand. The microbiological detection of TB is important because of early and correct diagnosis, drug resistance testing and it ensures that the effective treatment can be achieved and in a timely manner. Mycobacterial culture is the gold standard diagnostic test. Currently, a real-time polymerase chain reaction (RT-PCR) assay, such as Allplex™ MTB/MDRe Detection, Seegene is commonly used.

OBJECTIVE: To evaluate the diagnosis value of the real-time multiplex PCR by using Allplex™ MTB/MDRe Detection kit to detect MTB from sputum specimens with a gold standard TB culture.

METHODS: A retrospective study design of adult patients (>15 years) with suspected pulmonary M. tuberculosis infection was conducted from January 2023 until October 2023, at Khon Kaen Hospital. The sample size was 101 cases. Sensitivity, specificity, accuracy, negative predictive value (NPV), and positive predictive value (PPV) of Allplex™ MTB/MDRe Detection, each with its respective 95% confidence interval (95% CI) were analysed and compared to MTB culture as the gold standard.

RESULTS: A total of 120 cases was included, and of these, 86 (71.6%) and 73 (60.8%) cases were diagnosed with pulmonary TB by Allplex™ MTB/MDRe and MTB culture, respectively. Sensitivity, specificity, and accuracy of Allplex™ MTB/MDRe for MTB detection were 97.3% (95%CI, 90.5-99.6), 68.1% (95%CI, 52.8-80.9) and 85.8% (95%CI, 78.3-91.5), respectively. The PPV and NPV were 82.5 % (95%CI, 75.7-87.8) and 94.1% (95%CI, 80.1-98.4). Positive (LR+) and Negative Likelihood Ratio (LR-) were 3.1 (95%CI, 2.0-4.6) and 0.04 (95%CI, 0.01-0.16).

CONCLUSIONS: The Allplex™ MTB/MDRe Detection kit is effective in detecting Mycobacterium tuberculosis to achieve standard treatment.

KEYWORDS: pulmonary tuberculosis infection, real-time PCR, diagnosis value

ClinicalTrials.gov Identifier, NCT06284187

INTRODUCTION

Tuberculosis (TB) is an infectious bacterial disease caused by mycobacterium tuberculosis (MTB). Tuberculosis disease remains the leading cause of morbidity and mortality worldwide, especially in low to-middle income countries[1,2]. Approximately 5.8 million in 2020 had tuberculosis, and of these, 4.8 million people were diagnosed with pulmonary TB[1]. Thailand remains on the list of the world's 14th most burdensome country for tuberculosis[1,3]. For Health Region 7, problem solving of TB is still below the target criteria (<88%)[4]. The microbiological detection of TB is important because a correct diagnosis and drug resistance testing ensures that effective treatment can be implemented appropriately.

A variety of methods for the diagnosis of tuberculosis include the testing of 3 consecutive sputum acid fast bacilli (AFB), molecular diagnostic methods based on real-time polymerase chain reaction (RT-PCR) and mycobacterial culture as a gold standard[5]. AFB staining for detecting MTB is still a widely used initial screening method in resource-limited settings. However, 1.3-20% of positive AFB smears accounts for the proportion of non-tuberculous mycobacterium infection (NTM)[6-7]. The sensitivity of sputum AFB smear is 25.3-94%, and the specificity is 91-99%[3,8]. Mycobacterial culture remains the gold standard diagnostic test. However, culture is not recommended for use as a first-line test in clinical practice, including Thailand, due to a slow turnaround time of 2-8 weeks, biosafety requirements, and high cost[9].

A real-time polymerase chain reaction (RT-PCR) assay is commonly used to determine whether it is DNA or a sequence of MTB that presents in a sample. RT-PCR is higher sensitive, specific, and reproducible; moreover, automation of the procedure reduces hands-on time and decreases cross-contamination[10]. Previous studies among patients older than 15 years who were suspected of mycobacterial infection have reported the sensitivity and specificity of the GeneXpert MTB/RIF (Xpert) 72.7% and 100%, Xpert Ultra 87.8% and 98.1% and Anyplex™ MTB/NTM 79.7% and 94.5%[11,12]. Currently there is no evidence of the diagnostic efficacy of RT-PCR at Khon Kaen Hospital. This study will evaluate the diagnosis test of the real-time multiplex PCR by using Allplex™ MTB/MDRe Detection kit to detect MTB from sputum specimens with a gold standard TB culture.

METHODS

This retrospective study was conducted in patients older than 15 years with suspected pulmonary M. tuberculosis infection at Khon Kaen Hospital from January 2023 to October 2023 with a request to identify MTB with a routine direct examination (acid-fast bacilli; AFB) staining, real-time multiplex PCR by using Allplex™ MTB/MDRe and mycobacterial culture. A sample was collected from the same person and sent for testing on the same day and processed following the standard operating procedures of Khon Kaen Hospital. Electronic medical records were extracted after ethical approval. Children under 15 years old, non-tuberculous mycobacteria (NTM) detection and data that was missing from the results of Allplex™ MTB/MDRe Detection and culture were excluded. The IRB number of the protocol for this study was KEXP66063.

MTB culture and Allplex™ MTB/MDRe Detection

Mycobacterial culture was prepared on liquid (mycobacterial growth indicator tube; MGIT) or solid (MiddleBrook 7H10; M7H10) media according to the manufacturer's instructions at the Office of Disease Prevention and Control 7, Khon Kaen.

Allplex™ MTB/MDRe Detection (Seegene) was used for the PCR assays by using an automated NIMBUS IVD nucleic acid extraction and PCR setup system (CFX96TM Detection System). Extraction and the PCR setup was controlled with Seegene Launcher IVD (Seegene) and result analysis with Seegene Viewer IVD software (Seegene). The interpretation of the results was based on Ct value, Detected (+) for ≤45 MTB and Not detected for >45 or N/A MTB. In addition, the Allplex™ MTB/MDRe Detection was used for detection of first-line anti-tuberculosis drugs (Isoniazid and Rifampicin) resistance.

Sample size determination

Sample size estimation for test performance was calculated based on data from a previous study. The sensitivity of the diagnostic test was 68.1%[13], the prevalence of pulmonary MTB determined by PCR was 83.3%[14], and marginal error was 0.1 with a 95% confidence interval (CI). The number of participants was 101 cases.

Statistical analysis

The performance of MTB against RT-PCR was calculated according to sensitivity, specificity, accuracy, negative predictive value (NPV), positive predictive value (PPV), positive likelihood ratio (LR+) and negative likelihood ratio (LR-) each with its respective 95% confidence interval (95% CI). All statistical analyses were carried out using Stata version 11.0. Categorical and continuous variables were presented as percentage and mean±SD, respectively.

RESULTS

A total of sputum from patients with suspected pulmonary TB 120 cases were included in this study. The number of males was 82 (68.3%) with a mean±SD age 51.6± 16.7 years and females accounted for 38 (31.6%) cases with a mean±SD age 51.1±18.8 years. Forty percent of the total participants had underlying diseases such as HIV, diabetes mellitus (DM) and hypertension (Table 1).

Table 1 Characteristics of participants

	N (%)
Pulmonary TB	
Male	82 (68.3)
Female	38 (31.6)
Underlying disease	
No underlying disease	71 (59.2)
HIV	17 (14.2)
Diabetes mellitus	12 (10.0)
Diabetes mellitus and hypertension	8 (6.6)
Hypertension	4 (3.3)
Diabetes mellitus and chronic kidney disease	2 (1.6)
Hepatitis C	2 (1.6)
Myocardial infarction, SLE, stomach cancer, colon cancer	4 (3.3)

MTB was detected by real-time PCR system (Allplex™ MTB/MDRe Detection, Seegene) of 86 (71.6%) cases and by mycobacterial culture of 73 (60.8%) cases. The diagnosis value of Allplex™ MTB/MDRe for detection of pulmonary TB (MTB) was presented in Table 2.

Table 2 The diagnostic value of the Allplex™ MTB/MDRe for MTB detection

		MTB culture		
		Growth (%)	**No growth (%)**	**Total (%)**
Allplex™ MTB/MDRe	Detected (%)	71 (59.2)	15 (12.5)	86 (71.6)
	Not detected (%)	2 (1.6)	32 (26.6)	34 (28.3)
Total		73 (60.8)	47 (39.2)	120 (100.0)
Sensitivity 97.3 % (95% CI 90.5-99.6)				
Specificity 68.1 % (95% CI 52.8-80.9)				
Positive predictive value (PPV) 82.5 % (95% CI 75.7-87.8)				
Negative predictive value (NPV) 94.1 % (95% CI 80.1-98.4)				
Accuracy 85.8% (95% CI 78.3-91.5)				
Positive Likelihood Ratio (LR+) 3.1 (95% CI 2.0-4.6)				
Negative Likelihood Ratio (LR-) 0.04 (95% CI 0.01-0.16)				
Prevalence 60.8% (95% CI 51.5-69.6)				

The overall diagnosis values of Allplex™ MTB/MDRe were 97.3 % (95% CI 90.5-99.6) sensitivity, 68.1% (95% CI 52.8-80.9) specificity, 82.5% (95% CI 75.7-87.8) PPV, 94.1% (95% CI 80.1-98.4) NPV, 85.8% (95% CI 78.3-91.5) accuracy, 3.1 (95% CI 2.0-4.6) positive likelihood ratio (LR+) and 0.04 (95% CI 0.01-0.16) negative likelihood ratio (LR-), respectively.

From MTB culture and drug susceptibility testing (DST) for first line anti-TB drugs (isoniazid (INH) and rifampicin (RIF)), there were ten drug-resistance MTB positive patients in this study. Seven patients were resistant to INH and the remaining three patients were resistant to INH and RIF (multi-drug-resistance MTB (MDR-TB)). However, there was an inconsistent report which found that three out of seven patients reported INH susceptible by the Allplex™ MTB/MDRe. No false-positive drug-resistance results were reported. The Allplex™ MTB/MDRe performance for INH susceptibility testing was 57.1% (95% CI 20.5-93.8) sensitivity, 100.0 % (95% CI 94.4-100.0) specificity, 100.0 % (95% CI 39.7-100.0) PPV and 95.2 % (95% CI 90.6-98.1) NPV, respectively (Table 3).

Table 3 The diagnostic value of INH susceptibility testing

		Drug susceptibility testing (DST)		
		INH resistance	**INH susceptible**	**Total**
Allplex™ MTB/MDRe	INH resistance	4	0	4
	INH susceptible	3	64	67
Total		7	64	71
Sensitivity 57.1 % (95% CI 20.5-93.8)				
Specificity 100.0 % (95% CI 94.4-100.0)				
Positive predictive value (PPV) 100.0 % (95% CI 39.7-100.0)				
Negative predictive value (NPV) 95.2 % (95% CI 90.6-98.1)				
Accuracy 95.7% (95% CI 88.1-99.1)				

DISCUSSION

In this study, the Allplex™ MTB/MDRe demonstrated a detection rate of MTB of 86 (71.6%) when compared to MTB culture as a gold standard of 73 (60.8%). This finding was consistent with a previous study reporting that the prevalence of pulmonary TB among patients who were suspected of mycobacterial infection from Thailand, accounted

for 74.3%[12]. The diagnostic value of this study showed that the Allplex™ MTB/MDRe had a high sensitivity of 97.3% (95% CI 90.5-99.6), but slightly lower specificity of 68.1% (95% CI 52.8-80.9) for MTB detection in patients with suspected pulmonary TB. This indicated that the Allplex™ MTB/MDRe is useful in Thailand with a high prevalence of TB because it will be able to detect MTB in patients whom had suspected TB while also increase the screening rate of TB. Our findings show that nearly half of patients had an underlying disease. This was because of a low immune system, including HIV, and poorly controlled DM associated with failing to control TB infection. The Allplex™ MTB/MDRe had high sensitivity and slightly low specificity for TB, which were consistent with the previous studies that presented the sensitivity and specificity of the Allplex™ MTB/MDRe as 68.1-88.5% and 66.4-97.7%, respectively[13,15]. High sensitivity from this study may especially result from adequate preparation of specimens and a good processing procedure. Our findings of PPV 82.5% (95% CI 75.7-87.8) and NPV 94.1% (95% CI 80.1-98.4) from this study were comparable with a previous study of 88.9% (95% CI 74.1-96.2) and 91.9.9% (95% CI 87.0-95.1), respectively[(15)]. The Allplex™ MTB/MDRe can detect MTB of 15 (12.5%) cases in MTB culture negative patients. It could be explained by the fact that the Allplex™ MTB/MDRe can detect mpb64 and IS6110 genes in MTB strains up to 10 copies/reaction. Alternatively, another reason may have caused the remaining MTB DNA to be extracted from dead bacteria in the treated patients. Yet, providing TB treatment in patients with MTB detected by the Allplex™ MTB/MDRe and negative for MTB culture would be based on the clinical manifestations and imaging evaluation. Drug resistance testing by the Allplex™ MTB/MDRe provided reliable results, however, more resistance MTB would have been evaluated by MTB culture with DST.

A limitation of the study is that we did not compare the diagnostic value of the Allplex™ MTB/MDRe to microscopic smear (AFB). Nevertheless, the high sensitivity may be adequate for replacing the Allplex™ MTB/MDRe as the initial screening of TB, which was superior to a microscopic smear on the known gene target that can separate MTB from NTM and known first line anti-TB drugs susceptibility. A good quality study of cost-effectiveness analysis of the Allplex™ MTB/MDRe compared to microscopic smear (AFB) will be needed.

In conclusion, from this study findings showed that the Allplex™ MTB/MDRe is useful for MTB detection in patients suspected of pulmonary TB in high prevalence settings.

Conflicts of interest: none

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