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Original Article

Comparison of GeneXpert MTB to Mycobacterium tuberculosis culture in children with tuberculosis

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Abstract

Background Diagnosing tuberculosis (TB) in children is difficult. Typical methods take a long time to achieve results, or have a low sensitivity. GeneXpert is a nucleic acid amplification test used to identify Mycobacterium tuberculosis bacteria (MTB) in only 2 hours.

Objective To compare the sensitivity and specificity of GeneXpert MTB to MTB culture in children with TB, and to assess factors associated with GeneXpert MTB test in predicting which children were likely to have positive results.

Methods This descriptive, analytical study was done in children with suspected TB, aged 1 month to 18 years in Hasan Sadikin Hospital, Bandung, West Java, from January 2016 to December 2017. The data were taken from the medical records and included age, gender, nutritional status, symptoms of TB, chest x-ray, and tuberculin test results. The GeneXpert MTB test was compared to cultures from the same patient, with regards to sensitivity, specificity, and agreement using Kappa index. We analyzed factors associated to GeneXpert MTB test using logistic regression analysis.

Results From 454 inpatients and 1,750 outpatients with suspected TB, there were 251 children who were tested by MTB culture and 722 children tested by GeneXpert MTB. Of the 70 cases who met the inclusion criteria and underwent both tests, factors associated with positive GeneXpert MTB results were age 10 to 18 years, female gender, and positive tuberculin skin test (TST). The GeneXpert MTB test showed sensitivity 78.9% (95%CI 56.7 to 91.5) and specificity 86.3% (95%CI 74.3 to 93.2), with accuracy of 84.3% (95%CI 74 to 91), and agreement value of κ=0.62 (95%CI 41.6 to 82.7).

Conclusion Specificity of GeneXpert MTB is higher than its sensitivity compared to TB cultures in children. The tests were in good agreement. Age 10 to 18 years had the strongest association with positive GeneXpert MTB results. **[Paediatr Indones. 2019;59:113-8; doi: http://dx.doi.org/10.14238/pi59.3.2019.113-8]**.

Keywords: *agreement; GeneXpert; sensitivity; specificity; tuberculosis*

Tuberculosis remains a major problem worldwide. In 2013, 9 million people developed TB and 1.5 million people died from the disease.[1] Every year, TB in children accounts for at least 6% of the global burden of disease. These numbers (530,000-999,792 cases) underestimate the burden of childhood TB, which is higher due to difficulty in diagnosing childhood tuberculosis, emphasizing the need for improved diagnostics.[2]

Tuberculosis in children manifests with severe dissemination and clinical presentations. In this age group, hematogenous and lymphatic spread of the primary infection cause extrapulmonary symptoms such as miliary and meningitic disease. Young children with severe and complicated disease have a much higher mortality rate than adults.[2] Tuberculosis is among the 10 major causes of mortality among children, with a global estimate of 130,000 deaths per year.[3]

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In children, culture methods have a greater, yet highly variable, sensitivity than other typical diagnostic methods. For this reason, microbiological confirmation of childhood tuberculosis is rarely performed, and clinical diagnosis relies on a combination of signs, symptoms, radiological findings, and identification of a tuberculosis contact.[4] Newer tools such as nucleic acid amplification tests have a slightly higher sensitivity of 40-60%. Other diagnostic tests, including the tuberculin skin test and the interferon gamma release assay, are limited in differentiating between latent infection and active disease.[5] There is an urgent need for a rapid, sensitive, and specific test for tuberculosis and for identification of drug-resistant disease in children.[6] The recent innovation of the Xpert MTB/RIF test by *Cephedi* (Sunnyvale, CA, USA) has greatly transformed the field of TB diagnostics. The Xpert MTB/RIF test simultaneously detects Mycobacterium tuberculosis (MTB) and resistance to rifampicin using real-time polymerase chain reaction (PCR) analysis, and produces results within 2 hours. The other main advantage of the Xpert MTB/RIF compared to traditional PCR methods is that it is fully automated.[7]

In the 2011 policy statement on the Xpert MTB/RIF test, the World Health Organisation (WHO) recommended the test as an initial diagnostic tool among children with suspected HIV-associated TB or multi-drug resistant (MDR) TB, based on successful data in adults.[8] There has been limited published data on the utility of the Xpert MTB/RIF test in the pediatric population with tuberculosis. Nicol *et al.* found an overall sensitivity of 100% for smear-positive/culture-positive cases, 61.1% for smear-negative/culture-positive, and specificity of 98.8% when two induced sputum samples were assessed in children aged less than 15 years with suspected pulmonary tuberculosis in Cape Town, South Africa.[6] Statistical guidance on reporting results from studies evaluating diagnostic tests was used to calculate sensitivity and specificity of the assay and value of agreement with Kappa scores.[9] Sekadde *et al.* in Uganda described clinical characteristics associated with a positive Xpert MTB test. The clinical characteristics which were independently associated with a positive Xpert MTB test included age > 5 years, a positive history of TB contact, and a positive tuberculin test.[7]

The aim of this study was to assess the sensitivity, specificity, and agreement of the GeneXpert MTB test to MTB culture as the gold standard for the diagnosis of childhood tuberculosis in Hasan Sadikin Hospital and describe factors associated with positive GeneXpert MTB test results.

Methods

This descriptive, analytical study with cross-sectional design, was done with data collected from medical records of children with TB from January 2016 until December 2017 at Hasan Sadikin Hospital, Bandung, West Java, Indonesia. The study inclusion criteria were inpatients and outpatients in Hasan Sadikin Hospital aged 1 month until 18 years, diagnosed with suspected TB based on the WHO case definition for a TB suspect,[10,11] who underwent MTB culture and GeneXpert testing, and had complete medical records. We excluded patients with incomplete medical records. The study forms were filled in based on secondary data from medical records.

All statistical analyses were performed using the *SPSS software (version 15.0)*. Chi-square test was done to analyze for associations with positive GeneXpert test results. Results with P value less than 0.25 were analyzed by multivariate logistic regression; and P values less than 0.05 were considered to be statistically significant. Sensitivity and specificity were calculated for the diagnostic test. Kappa analysis was used to determine agreement between GeneXpert MTB and solid MTB culture as the gold standard. Following conversion, four levels of agreement for Kappa were reported: <0.40 (poor), 0.40-0.59 (fair), 0.60-0.80 (good), and >0.80 (excellent).[12,13] The study was approved by the Research Ethics Committee of Hasan Sadikin General Hospital, Bandung.

Results

During the two-year study period, there were 454 inpatients and 1,750 outpatients with the diagnosis of TB. The laboratory data showed 251 MTB cultures and 722 GeneXpert MTB results were positive. Of these, only 70 children underwent both GeneXpert MTB and MTB culture tests. The general characteristics of

subjects are shown in **Table 1**. The age group with the most TB cases was 10 to 18 years, and most of them were female (61.4%). Of the 70 subjects, most had well-nourished nutritional status (64.3%).

The factors that were associated with positive GeneXpert MTB results are listed in **Table 2**. Factors with P value less than 0.25 were entered into the logistic regression model shown in **Table 3**. From three factors associated with GeneXpert test, age 10 to 18 years had the strongest association.

There were 22 positive GeneXpert results and 19 positive culture results (**Table 4**). Gene Xpert MTB test identified MTB in 15 of 22 case-confirmed cultures, with higher specificity 86.3% (95%CI 74.3 to 93.2) than sensitivity 78.9% (95%CI 56.7 to 91.5%). The value of agreement was measured by Kappa index κ=0.62 (95%CI 41.6 to 82.7), with good agreement.

Discussion

Microbiological confirmation of childhood TB with culture is rarely performed and clinical diagnosis

Table 1. Characteristics of subjects

Characteristics	Total (N=70)
Age, n (%)	
1 month - 1 year	7 (10)
1-4 years	20 (28.6)
5-9 years	19 (27.1)
10-18 years	24 (34.3)
Gender, n (%)	
Male	27 (38.6)
Female	43 (61.4)
Nutritional status, n (%)	
Low	25 (35.7)
Well	45 (64.3)
Signs and symptoms, n (%)	
Cough ≥ 2 weeks	17 (24.3)
Fever ≥ 2 weeks	38 (54.3)
Lymph node enlargement	37 (52.8)
History of contact, n (%)	17 (24.3)
Type of TB, n (%)	
Pulmonary	46 (65.7)
Extrapulmonary	17 (24.3)
Tuberculin skin test, n (%)	
Positive	36 (51.4)
Negative	33 (47.1)
Chest x-ray, n (%)	51 (72.8)

Table 2. Factors associated with positive GeneXpert MTB results

Variables	OR adjusted*	95% CI**	P values
Age (10-18 years old)	13.00	2.9 to 57.92	0.001
Gender (female)	6.42	1.35 to 30.59	0.02
Nutritional status	0.61	0.15 to 2.50	0.49
History of contact	0.43	0.09 to 1.88	0.26
TST (positive)	4.26	1.06 to 16.98	0.04
Lymph node enlargement	3.44	0.72 to 16.48	0.12

*OR=odds ratio;** CI=confidence interval

Table 3. Multivariate logistic regression analysis of factors associated with positive GeneXpert MTB test results

Variables	OR adjusted*	95% CI**	P values
Age (10-18 years old)	8.39	2.32 to 30.39	0.001
Gender (female)	5.15	1.24 to 21.37	0.024
TST (positive)	3.64	1.02 to 12.92	0.05

*OR=odds ratio;** CI=confidence interval

Table 4. Sensitivity, specificity, accuracy, and Kappa index of Gene Xpert MTB compared to culture tests

Gene Xpert	Culture		Sensitivity (95%CI)	Specificity (95%CI)	Accuracy (95%CI)	Kappa index (95%CI)	P value
	Positive	Negative					
Positive	15	7	78.9 (56.7 to 91.5)	86.3 (74.3 to 93.2)	84.3 (74 to 91)	62.1 (41.6 to 82.7)	0.549
Negative	4	44					

depends on a combination of signs, symptoms, radiological results, and identification of TB contact.[4] Culture is the gold standard for microbiological confirmation, but it takes 2 to 8 weeks to yield results.[14] In our study, we compared GeneXpert MTB test to cultures as the gold standard to detect MTB from respiratory specimens. There were 251 children who underwent MTB culture and 722 children who underwent GeneXpert MTB testing according to Clinical Pathology Laboratory and MDR TB outpatient data. However, only 70 children underwent both tests. The proportion of positive GeneXpert test (31.4%) was higher than in a previous South African study.[6] A negative GeneXpert test result did not necessarily exclude a TB diagnosis, given the fact that the test was unable to identify 27% of children with culture-confirmed tuberculosis. A clinical decision in the context of the patient, therefore, is important in initiating anti-tuberculosis therapy even for children with negative GeneXpert MTB test results.[7]

The GeneXpert MTB assay performance has been previously evaluated mostly on sputum samples collected from adult TB patients, showing a high sensitivity in smear and culture-positive specimens (98-100%), but a much lower sensitivity in smear-negative sputum specimens (43-70%).[15,16] Few such studies have been conducted in children. Nicol et al. showed an incremental increase in sensitivity of 27.8% for GeneXpert MTB, with specificity of 98.8%.6 Bunyasi et al. showed low sensitivity (26.7% for induced sputum samples and 22.6% for gastric lavage samples) and high specificity (100% for induced sputum samples and 99.6% for gastric lavage samples).[17] Our study showed that the GeneXpert MTB assay had 78.9% sensitivity (95%CI 56.7 to 91.5) and 86.3% specificity (95%CI 74.3 to 93.2). The Kappa value was $\kappa=0.62$, indicating a good level of agreement.[12,13]

Other studies also reported good agreement between GeneXpert MTB and cultures. Tang et al. showed a Kappa value of 0.73, in their study of both adults and children.[18] Hasan *et al.* showed very good agreement (Kappa values >0.8), but with stool specimens,[19] and Li *et al.* showed a Kappa value of 0.6 for extrapulmonary TB.[20]

We assessed what factors were associated with GeneXpert MTB test in predicting which children were likely to have positive results. Age (10 to 18 years), female sex, and positive tuberculin skin test (TST) were associated with positive GeneXpert MTB results. Primary infection during adolescence was associated with a high risk of developing adult-type disease. Adult-type disease results from primary infection, endogenous reactivation, or exogenous reinfection. Adult-type disease was most common after recent primary infection in children over 10 years of age.[21] Sekadde *et al.* reported that age >5 years, positive TST, and positive history of TB contact were independently associated with positive Xpert MTB/RIF test results.[7]

Teenagers, especially girls in menarche period, have the highest risk of having tuberculosis after the primary infection during adolescence.[21] Marais et al. showed that adolescent girs were at higher risk of developing tuberculosis after recent primary infection than were boys.[22] Also, unlike younger children who get paucibacillary primary disease, older children, especially those above 10 years of age, are more likely to get reactivation/cavitatory disease, thereby increasing the likelihood of a positive GeneXpert MTB test. Moreover, a positive TST response is a marker of TB exposure and increases the likelihood of TB in a child with suspected pulmonary TB.[7]

This study had some limitations. The data were taken from medical records, but some medical records were incomplete, so they were excluded. Also, this study was conducted only at a 3rd level referral hospital, so we could not generalize to lower service level conditions.

In conclusion, the specificity (86.3%) of GeneXpert MTB is higher than the sensitivity (78.9%), compared to TB culture results in children. GeneXpert MTB and cultures had good accuracy and agreement. The factor most strongly correlated to positive GeneXpert MTB results is age 10 to 18 years.

Conflict of Interest

None declared.

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References

1. Sharma SK, Kohli M, Yadav RN, Chaubey J, Bhasin D, Sreenivas V, *et al.* Evaluating the diagnostic accuracy of Xpert MTB/RIF assay in pulmonary tuberculosis. PLos One. 2015;10:e0141011.
2. Elhassan MM, Elmekki MA, Osman AL, Hamid ME. Challenges in diagnosing tuberculosis in children: a comparative study from Sudan. Int J Infect Dis. 2016; 43:25-9.
3. Swaminathan S, Rekha B. Pediatric tuberculosis: global overview and challenges. Clin Infect Dis. 2010;50: S184-94.
4. Detjen AK, DiNardo AR, Leyden J, Steingart KR, Menzies D, Schiller I, *et al.* Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: a systematic review and meta-analysis. Lancet Respir Med. 2015;3:451-61.
5. Lighter J, Riguaud M. Diagnosis childhood tuberculosis: traditional and innovative modalities. Curr Probl Pediatr Adolesc Health Care. 2009;39:61-88.
6. Nicol MP, Workman L, Isaacs W, Munro J, Black F, Eley B, *et al.* Accuracy of the Xpert MTB/RIF test for the diagnosis of pulmonary tuberculosis in children admitted to hospital in Cape Town, South Africa: a descriptive study. Lancet Infect Dis. 2011;11:819-24.
7. Sekadde MP, Wobudeya E, Joloba ML, Ssengooba W, Kisembo H, Bakeera-Kitaka S, *et al.* Evaluation of the Xpert MTB/RIF test for the diagnosis of childhood pulmonary tuberculosis in Uganda: a cross-sectional diagnostic study. BMC Infect Dis. 2013;13:133.
8. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, *et al.* Rapid molecular detection of tuberculosis and rifamipicin resistance. N Engl J Med. 2010;363:1005-15.
9. Hasan Z, Shakoor S, Arif F, Mehnaz A, Akber A, Haider M, *et al*. Evaluation of Xpert MTB/RIF testing for rapid diagnosis of childhood pulmonary tuberculosis in children by Xpert MTB/RIF testing of stool samples in a low resource setting. BMC Res Notes. 2017;10:473.
10. World Health Organization. Guidance for national tuberculosis programmes on the management of tuberculosis in children [Internet]. Geneva: World Health Organization; 2006 [cited 2018 March 1]. Available from http://whqlibdoc.who.int/hq/2006/WHO_HTM_TB_2006.371_eng.pdf
11. World Health Organization.Treatment of tuberculosis guidelines. Geneva: World Health Organization; 2009 [cited 2018 March 1]. Available from: http://whqlibdoc.who.int/publications/2010/9789241547833_eng.pdf
12. Mallinckrodt B, Abraham WT, Wei M, Russel DW. Advances in testing the statistical significance of mediation effects. J Couns Psychol. 2006;53:372-8.
13. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. JAMA. 1999;282:677-86.
14. Zeka AN, Tasbakan S, Cavusoglo C. Evaluation of the GeneXpert MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in pulmonary and extrapulmonary specimens. J Clin Microbiol. 2011;49:4138-41.
15. Walusimbi S, Bwanga F, De Costa A, Haile M, Joloba M, Hoffner S. Meta-analysis to compare the accuracy of GeneXpert, MODS and the WHO 2007 algorithm for diagnosis of smear-negative pulmonary tuberculosis. BMC Infect Dis. 2013;13:507.
16. Moure R, Munoz L, Torres M, Santin M, Martin R, Alcaide F. Rapid detection of Mycobacterium tuberculosis complex and rifampin resistance in smear-negative clinical samples by use of an integrated real-time PCR method. J Clin Microbiol. 2011;49:1137-9.
17. Bunyasi EW, Tameris M, Geldenhyus H, Schmidt BM, Luabeya AK, Mulenga H, *et al*. Evaluation of Xpert MTB/RIF assay in induced sputum and gastric lavage samples from young children with suspected tuberculosis from the MVA85A TB vaccine trial. PLoS One. 2015;10:e0141623.
18. Tang T, Liu F, Lu X, Huang Q. Evaluation of GeneXpert MTB/RIF for detecting Mycobacterium tuberculosis in a hospital in China. J Int Med Res. 2017;45:816-22.
19. Hasan Z, Shakoor S, Arif F, Mehnaz A, Akber A, Halder M, *et al.* Evaluation of Xpert MTB/RIF testing for rapid diagnosis of childhood pulmonary tuberculosis in children by Xpert MTB/RIF testing of stool samples in a low resource setting. BMC Res Notes. 2017;10:473.
20. Li Y, Pang Y, Zhang T, Xian X, Wang X, Yang J, *et al.* Rapid diagnosis of extrapulmonary tuberculosis with Xpert Mycobacterium tuberculosis/rifampicin assay. J Med Microbiol. 2017;66:910-4.
21. Marais BJ, Gie RP, Schaaf HS, Hesseling AC, Obihara CC, Starke JJ, *et al*. The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. Int J Tuberc Lung Dis.

2004;8:392-402.
22. Marais BJ, Gie RP, Hesseling AH, Beyers N. Adult type pulmonary tuberculosis in children 10-14 years of age. Pediatr Infect Dis J. 2005;24:743-4.