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Research Report

GeneXpert MTB/RIF and *Mycobacterium tuberculosis* Sputum Culture in Establishing the Diagnosis of Pulmonary Tuberculosis and Rifampicin Resistance in Suspected Childhood Pulmonary Tuberculosis in Soetomo Hospital

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ABSTRACT

The diagnosis of childhood tuberculosis remains a challenge worldwide. The GeneXpert MTB/RIF test, a rapid Mycobacteria tuberculosis diagnostic tool, was recommended for use in children. No pediatric studies of GeneXpert MTB/RIF assessing pulmonary tuberculosis within a hospital setting has been done in Indonesia. We evaluated the performance of the GeneXpert MTB/RIF test compared with sputum culture on Lowenstein-Jensen (LJ) for the diagnosis of childhood pulmonary tuberculosis. This study was conducted in pediatric respirology inpatient and outpatient Dr. Soetomo Hospital, a tertiary care facility in Surabaya between June and August 2015 with a cross-sectional design. We consecutively enrolled 27 children aged 3 months to 14 years who had history of close contact with adult tuberculosis patients and showed symptoms of pulmonary tuberculosis. Sputum collection was performed by induced sputum and three examination methods were performed (microscopic, GeneXpert MTB/RIF and sputum culture) simultaneously followed by a drug sensitivity test for specimens detected with MTB growth. The GeneXpert MTB/RIF test had a sensitivity of 100% (95% CI 100-100) and a specificity of 95% (95% CI 85-100). The positive predictive value for diagnosing pulmonary TB was 89% (95% CI 68-100), the negative predictive value was 100% (95% CI 100-100) and positive likelihood ratio was 20 (95% CI 2.82-128). The GeneXpert MTB/RIF test on one sputum sample rapidly and correctly identified all children with culture-confirmed pulmonary tuberculosis with high specificity. Similar results were obtained between GeneXpert MTB/RIF and sputum culture based on age groups and clinical manifestations. Rifampicin resistance were both detected in GeneXpert MTB/RIF and MTB sensitivity test.

Keywords: *Childhood pulmonary tuberculosis; Sensitivity, Specificity; GeneXpert MTB/RIF*

ABSTRAK

Menegakkan diagnosis tuberkulosis (TB) pada anak sampai saat ini masih sulit dikerjakan. GeneXpert MTB/RIF adalah suatu metode diagnostik baru yang dapat mengidentifikasi Mycobacterium tuberculosis (MTB) dengan cepat. Walaupun metode ini telah direkomendasikan pada anak-anak, namun penelitian tentang GeneXpert MTB/RIF dalam mendiagnosis TB paru anak di lingkungan Rumah Sakit (RS) belum pernah dikerjakan di Indonesia. Kami membandingkan hasil pemeriksaan GeneXpert MTB/RIF dengan kultur dahak MTB pada media Lowenstein Jensen (LJ) dalam menegakkan diagnosis TB paru pada anak yang diduga TB paru. Penelitian ini dilakukan di poli dan bangsal respirologi anak RSUD Dr. Soetomo antara Juni sampai Agustus 2015 secara cross sectional. Dengan sampling konsekutif mengumpulkan 27 anak usia 3 bulan sampai 14 tahun yang mempunyai kontak erat dengan penderita TB dewasa dan menunjukkan gejala TB paru. Pada setiap anak dilakukan pengambilan dahak dengan cara induksi dahak kemudian dilakukan tiga metode pemeriksaan sekaligus yaitu secara mikroskopis, GeneXpert MTB/RIF dan kultur yang dilanjutkan dengan uji kepekaan MTB bagi spesimen yang terdeteksi ada pertumbuhan MTB. Sensitivitas GeneXpert

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MTB/RIF adalah 100% (95% CI 100-100) dan spesifisitas 95% (95% CI 85-100). Nilai duga positif GeneXpert MTB/RIF adalah 89% (95% CI 68-100), sedangkan nilai duga negatifnya adalah 100% (95% CI 100100) dan Likelihood positive nya adalah 20 (95%CI 2,82-128). GeneXpert MTB/RIF mampu mendeteksi semua spesimen yang terdeteksi positif MTB oleh kultur dahak MTB namun dalam waktu yang lebih singkat dan dengan spesifisitas yang tinggi. Kesepadanan hasil antara GeneXpert MTB/RIF dan kultur dahak didapatkan berdasarkan kelompok umur dan manifestasi klinis TB. Selain Dalam mendeteksi resistensi Rifampicin, GeneXpert MTB/RIF memberikan hasil yang sama dengan uji kepekaan MTB.

Kata kunci: *Tuberkulosis paru anak, Sensitivitas, Spesifisitas, GeneXpert MTB/RIF*

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INTRODUCTION

Difficulty to diagnose tuberculosis in children can lead to under and over diagnosis of TB which can cause higher morbidity and mortality. Confirming the diagnosis of childhood TB is a major challenge. However, research on childhood tuberculosis in relation to better diagnostics is often neglected because of technical difficulties, such as the slow growth in culture, the difficulty of obtaining specimens, and the diverse and relatively nonspecific clinical presentation of TB in this age group. While the classic presentation of childhood TB is prolonged cough and weight loss, HIV infection, with its chronic pulmonary manifestations and wasting, may confound the diagnosis of childhood TB. These difficulties are worsened by the increased incidence of multiple drug resistance.[1,2] Therefore, early diagnosis of TB in children is very important in order to control the incidence of TB disease.

Tuberculosis remains a major problem for the health of mankind. In 2012, the estimated incidence of TB cases were 8.6 million cases / year and 58% of these cases occur in Southeast Asia and the western Pacific. Approximately 50-60% of children living with adult pulmonary tuberculosis (PTB) patients who have positive acid-fast bacilli (AFB) sputum results will be infected with TB as well and about 10% of them will get TB disease.[3] World Health Organization (WHO) in 2012 estimated that there were 530,000 new cases of TB in children with a mortality rate of 74,000.[4] Indonesian TB data in 2012 showed the proportion of TB cases in children among all TB cases was 8.2%.[5]

The diagnostic approaches that exist today are less sensitive. Although conventional examination with a microscope has a high positive predictive value for detecting Mycobacterium tuberculosis (MTB), its sensitivity is low. Examination using media culture with Lowenstein-Jensen (LJ) is still the gold standard for diagnosis but this test is difficult and requires a long time (± 6-9 weeks) to get the results with positive results obtained only in 10% - 15% culture examination.[6,7] Polymerase chain reaction (PCR) test provides high sensitivity by multiplying deoxyribonucleic acid (DNA) of bacteria, and has been extensively evaluated in order to detect the DNA of MTB. GeneXpert MTB/RIF is an integrated and automated test with molecular approaches. Sample preparation, amplification and detection is done automatically by PCR. GeneXpert MTB/RIF is able to detect MTB as well as diagnosing resistance to Rifampicin. The results will be obtained in less than 2 hours.[8,9,10,11]

In December 2010, WHO has encouraged the use of GeneXpert MTB/RIF as a tool for the diagnosis of TB due to high sensitivity and specificity but studies on the use of GeneXpert MTB/RIF in children are still rare.[11,12] The aim of this study is to compare the GeneXpert MTB/RIF with MTB sputum culture examination in the diagnosis of PTB and rifampicin resistance in children with suspected PTB in Dr. Soetomo Hospital Surabaya.

MATERIALS & METHODS

This study was analytical observational to compare the GeneXpert MTB/RIF assay with MTB sputum culture for detection of pulmonary tuberculosis and Rifampicin resistance in new pediatric inpatients and outpatients at the Department of Pediatric and Child Health, Dr. Soetomo Hospital, Surabaya, Indonesia, a tertiary referral center. This study was approved by the research ethics committee of Dr. Soetomo Hospital Surabaya. The parents of all study participants provided written informed consent.

Between June 2015 and August 2015, new outpatients and inpatients children, aged 3 months old - 14 years old, with the diagnosis of suspected tuberculosis were eligible for enrollment in the study. A patient with suspected tuberculosis was defined as having a symptom and risk factor screening (one or more of five factors: tuberculosis contact, cough for more than 3 weeks, weight loss, malnutrition, or fever for more than 2 weeks with unknown origin) according to the Indonesian National TB Program. Patients were excluded if they were deemed to have a poor prognosis, congenital heart defects, severe congenital abnormalities, acute hemodynamic disturbances (hypotension, shock, heart failure, decreased consciousness), critical illness (sepsis, renal failure, impaired liver function severe), have received TB treatment > 1 month and patients with HIV infection or if parents or guardians refused the informed consent.

Procedure of sample collection started with history taking and physical examination taken when administered. The clinical manifestations observed in this study were in accordance with TB scores commonly used in Indonesia, including a history of close contact with adult TB patients, the results of TST, fever ≥ 2 weeks are not unexplained, coughing ≥ 3 weeks, enlarged neck lymph nodes, inguinal and axillary, nutritional status, swelling of bones/joints and chest X-rays. Sputum samples were collected from children who could expectorate. In children who could not spontaneously expectorate, sputums were collected by induced sputum procedure. Smear microscopy, culture with LJ media and

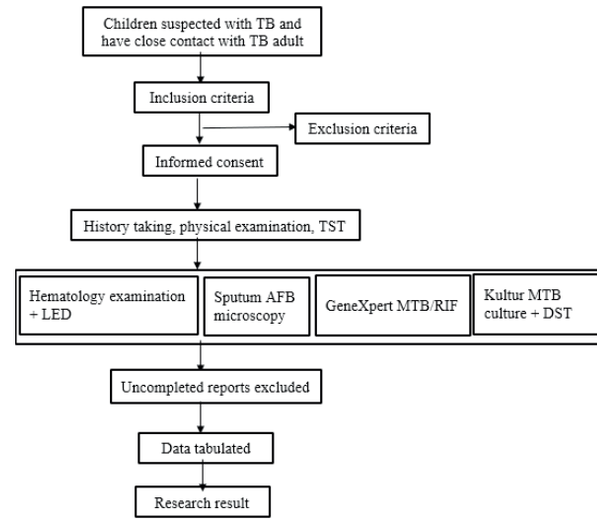

Figure 1. Operational Framework

GeneXpert MTB/RIF were done simultaneously on all samples as described previously. Cultures were classified as negative when no growth were detected after 8 weeks of incubation. Contaminated samples were retreated and re-cultured, and excluded if still contaminated. Drug susceptibility testing was done on LJ media. Sputum was added to the GeneXpert MTB/RIF sample reagent in a 1:1 ratio (1 mL of sputum to 1 mL of the sample reagent). Two mL of this mixture was added to the GeneXpert MTB/RIF cartridge and run in the machine in accordance with manufacturer's instructions.

All clinical and laboratory data were compiled in databases. Selected variables were exported to SPSS (version 21) for analysis. Comparisons of GeneXpert MTB/RIF assay and sputum culture assay were done with Pearson χ^2 or Fishers exact test. The sensitivity, specificity, and predictive values of the assays with 95% CIs were calculated. The equivalence between GeneXpert MTB/RIF assay and sputum culture were analyzed with McNemar test and Kappa. All statistical tests were two-sided with alpha of 5%.

RESULTS

Twenty seven children were recruited and had sputum for analysis. Table 1 shows about half (51.9 %) of the children enrolled were younger than 5 years. The most common clinical

Table 1. Baseline Characteristics

Characteristics	N(%)
Age < 5 years old	14 (51.9)
≥ 5 years old	13 (48.1)
Gender Male	14 (51.9)
Female	13 (48.1)
Contact Identified	18 (66,7)
Not Identified	9 (33,3)
Contact MDR	5 (18.5)
Non MDR	12 (44.4)
Scar BCG Present	21 (77,8)
None	6 (22,2)
Cough > 3 weeks	18 (66.7)
Fever > 2 weeks	19 (70.4)
Lymph node enlargement	9 (33.3)
Nutritional Status Normal	12 (44.4)
Poor	10 (37.0)
Malnutrition	5 (18.5)
TST Positive	18 (66.7)
Negative	9 (33.3)
Bone destruction	1 (3.7)
Chest X-ray Suggestive TB	16 (59.3)
Not Suggestive TB	11 (40.7)
TB Score ≥ 6	23 (85.1)
< 6	4 (14.8)

Table 2. Sputum Results

Sputum Examination	N (%)
MTB culture Positive	8 (29.7)
Negative	19 (70.3)
GeneXpert MTB/RIF Positive	9 (33.3)
Negative	18 (66.7)
Smear microscopic Positive	6 (22.2)
Negative	21 (77.8)

Table 3. Drug Sensitivity Test of Positive Results (8 LJ Culture and 9 GeneXpert MTB/RIF)

Rifampicin Sensitivity Test	N (%)
Drug sensitivity test with LJ	
Rifampicin Sensitive (+)	8 (100)
Rifampicin Resistance	0 (0)
INH Sensitive	7 (87.5)
INH Resistance	1 (12.5)
Etambutol Sensitive	8 (100)
Etambutol Resistance	0 (0)
Streptomycin Sensitive	7 (87.5)
Streptomycin Resistance	1(12.5)
GeneXpert MTB/RIF	
Rifampicin Sensitive (+)	9 (100)
Rifampicin Resistance	0 (0)

manifestation reported was fever with no apparent cause lasting more than 2 weeks. Eighteen subjects (66.7%) showed positive TST and 9 (33.3%) had negative result.

Molecular examination with GeneXpert MTB/RIF provides the highest positive results of 33.3%, followed by microbiological culture (29.7%) and microscopic examination (22.2%) (Table 2). There was no false negative results for GeneXpert MTB/RIF examination.

GeneXpert MTB/RIF correctly identified all 9 rifampicin-sensitive on specimen analysis (Table 3). All 8 LJ culture positive specimens were also analyzed with the LJ drug-sensitivity test and none were Rifampicin resistance. Therefore MTB drug sensitivity cannot be analyzed statistically since the LJ results on solid media were equivalent with the results by the GeneXpert MTB/RIF (Table 3).

Table 4 shows that only 5 (27.8%) of 18 patients with positive TST results were confirmed positive by GeneXpert MTB/RIF, whereas 13 (72.2%) others showed negative results by GeneXpert MTB/RIF. There were 4 (44.4%) of 9 children who showed negative TST result, but confirmed positive on GeneXpert MTB/RIF, and 5 (55.5%) of 9 children showed a negative result on both TST and GeneXpert MTB/RIF.

Table 4. TST Result VS GeneXpert MTB/RIF

		GeneXpert MTB/RIF		Total
		Positive (%)	Negative (%)	
TST	Positive	5 (27,8)	13 (72,2)	18
	Negative	4 (44,4)	5 (55,5)	9
Total		9	18	27

Table 5 shows the equivalence results of GeneXpert MTB/RIF and MTB sputum culture in the age group ≥ 5 years old as many as 12 samples. There is only one sample which showed a positive result on GeneXpert MTB/RIF but confirmed

Table 5. GeneXpert MTB/RIF vs MTB Sputum Culture in Age Group

GeneXpert MTB/RIF		MTB culture (+)	MTB culture (-)	Agreement (%)	McNemar	Kappa
< 5 years old	(+)	2	0	100	p=1,000	1,000 p=0,00
	(-)	0	12			
≥ 5 years old	(+)	6	1	92,4	p=1,000	0,847 p=0,002
	(-)	0	6			

Table 6. GeneXpert MTB/RIF vs MTB Sputum Culture in Clinical TB Group

GeneXpert MTB/RIF		MTB culture (+)	MTB culture (-)	Agreement (%)	McNemar	Kappa
Clinical TB	(+)	8	1	94,2	1,00	0,883 p=0,000
	(-)	0	8			
Non Clinical TB	(+)	0	0	100	-	-
	(-)	0	10			

negative on MTB sputum culture. McNemar test shows no significant difference. Kappa test results shows significant reliability between the results of the GeneXpert MTB/RIF with MTB sputum culture MTB in the age group ≥ 5 years. The agreement between GeneXpert MTB/RIF and MTB sputum culture examination was 92.4%.

Table 6 shows the total positive clinical TB assessment in 17 (63.0%) of 27 children. There were 9 (53.0%) clinical TB children who have positive results of GeneXpert MTB/RIF. All children with clinically negative TB showed negative result on GeneXpert MTB/RIF. McNemar test showed no significant difference between the results of GeneXpert MTB/RIF and MTB sputum culture with positive clinical manifestations of TB. Kappa test showed significant equivalence between GeneXpert MTB/RIF and MTB sputum culture with positive clinically manifestation of TB.

This study showed that GeneXpert MTB/RIF had 100 % sensitivity (95% CI 100-100), specificity of 95% (95% CI 85-100), PPV 89% (95%CI 68-100), NPV 100% (95% CI 100-100), LR +20 (95% CI 2.82-128), LR - 0.

DISCUSSION

In our study, more than 50% of samples had a history of close contact to adult patients with positive AFB smear, cough > 3 weeks, fever of unknown origin > 2 weeks, positive TST results and X-rays which showed PTB process. More than 50% of the sample had a value of TB score > 6. Positive TST results were not always found in children suspected of pulmonary TB. In our study, Positive TST results obtained in 66.67% of the samples, of which only 55.5% of the samples were GeneXpert MTB/RIF positive. Sekadde et al (2013) and Nicol et al (2011) obtained positive results only at ± 30% of the samples[13,14], while Nataprawira et al (2001) get positive TST results only in 9.7% of children who had close contacts with adult TB patients or adults suspected of having TB in Bandung.[15] There are several factors that influence the results of TST, such as malnutrition which effect on phagocytosis, cellular immunity and cytokine production.[16] Malnutrition leads to lymphoid tissue atrophy, thus affecting the development, differentiation and cause a decrease in lymphocytes. Moderate and severe malnutrition lead to decreasing delayed-type hypersensitivity reactions and recall process.[17]

The positive results of microscopic and molecular examination in this study is quite high when compared to previous studies. Giang et al reported positive results of GeneXpert MTB/RIF on 8.6% of samples,[18] Nicol et al reported 12.8%,[14] Sekadde et al reported 14%,[13] Singh et al and Nhu et al reported a respective 16.9% and 16.2%.[19,20] This condition is likely due to several factors, such as the number of MTB in children (paucibacillary) and sputum production capabilities that are lacking in children. Different inclusion criteria with previous studies may also cause these differences. In our study, most of the sample had more than 4 clinical manifestations as well, while these other studies established inclusion criteria of children aged ≤ 14 years with at least 2 clinical manifestations of cough ≥ 2 weeks and one of the symptoms of weight

loss or fever ≥ 2 weeks with unknown origin, or a history of contact with adult TB patients, or a positive result on TST or positive X-ray for TB process.[13,14,20]

Microscopic examination (Olympus CH-20, Olympus Corp., Japan; 1000x magnification) is able to detect 66.7% of specimens with positive GeneXpert MTB/RIF and 75% of specimens positive by MTB sputum culture. Positive smear cases were found mainly in the > 5 years old age group. This occurs because children > 5 years old or adolescents have pathological features of "adult-type" TB that is not paucibacillary with more bacilli accumulated and generally give more positive results on microscopic examination.[21,22] Previous studies reported the same result. Marlowe et al in the US collected 217 sputum specimens and showed that microscopic examination is able to detect 73% of GeneXpert MTB/RIF positive results. Lawn et al (2011) only reported 45% positive smear result of TB cases. This can be explained due to the colony of microscopic detection capabilities is less sensitive than the other two examination modalities. The detection capability of the colony smear microscopy is $5x10^3$ to $5x10^4$ bacilli/ml, the detection capability of GeneXpert MTB RIF is 102-107 CFU/ml and the culture detection capability is 10-100 CFU/ml.[12,23] However, microscopic examination is not specific to diagnose TB because there are several other bacteria that are resistant to acid staining which are Rhodococcus spp, Nocardia spp, Legionella micdadei, cysts and isospora of Crytopsoridium spp, that will give a false-positive smear result.[24]

In this study, the sensitivity of GeneXpert MTB/RIF is 100% and specificity was 94.7%. There is one GeneXpert MTB/RIF positive result that is not detected by MTB sputum culture. PCR concept used in GeneXpert MTB/RIF sequence all of MTB DNA without the capability to detect the viability of the MTB. GeneXpert MTB/RIF false-positive may result from patients who had been treated as well.

Systematic reviews conducted by WHO in 2013 among 13 studies involving 2,603 participants mention pooled sensitivity of GeneXpert MTB/RIF TB was 66% (95% CI 52-77) and pooled specificity was 98%.[25] Meta-analysis conducted in 2012 of 18 studies involving 10,224 specimens reported sensitivity of GeneXpert MTB/RIF amounted to 90.4% (95% CI 89.2 to 91.4) and specificity of 98.4% (95% CI 98-98, 7).[26] Recent meta-analysis of the ability of GeneXpert MTB/RIF in the diagnosis of childhood PTB reported pooled sensitivity of 62% (95% CI 51-73) and a pooled specificity of 98% (95% CI 97-99).[27]

Bates et al reported no significant differences between specimens derived from sputum or liquid gastric washings in the GeneXpert MTB/RIF examination and concluded the use of liquid gastric washings can replace sputum specimens if they are not available.[28] In the study conducted by Nhu et al and Singh et al, stored sputum specimens were used instead of fresh sputum specimens.[19,20] In a sputum that was kept frozen and then thawed, the DNA will be damaged and affect the viscosity of sputum, thus giving bias.[29] Performing GeneXpert MTB/RIF examination twice on one specimen reportedly do not increase the rate of case detection. Repeated examination of GeneXpert MTB/RIF will increase the cost, even though there are still other supporting diagnostic examination. BBLK Surabaya's policy is to do single sputum GeneXpert MTB/RIF examination for each patient.[14,20]

The existence of GeneXpert MTB/RIF machines is not widely available in primary and secondary health facilities, therefore Sekkade et al conducted a study in Uganda and analyze the clinical characteristics associated with GeneXpert MTB/RIF positive results. It is intended to help health workers in limited medical care facilities to predict the likelihood of TB in a suspected TB children. Researchers reported some characteristics of the sample that has a tendency to get positive result of GeneXpert MTB/RIF, such as age group of > 5 years, a positive TST result and a positive TB contacts.[13]

All sputum specimens with positive result of GeneXpert MTB/RIF and MTB sputum culture show sensitive result to rifampicin in this study. A study by Carriquiry et al on 130 patients aged > 18 years in Peru in 2012 reported 100% (95% CI 61-100) and 91% (95% CI 88.7 to 100) for sensitivity and specificity respectively. Predictive result were

66.7% (95% CI 35.4 -87.9) and 100% (95% CI 88.7 to 100) for PPV and NPV respectively.[29] Some researchers have assessed the ability of GeneXpert MTB/RIF in detecting MTB with Rifampicin resistance, but the samples were too small and therefore cannot be assessed.[14,20,30]

Tuberculosis is more progressive and fatal in children aged < 5 years old, while those aged ≥ 5 years old was associated with disease progression being "adult-type TB". Other than that this age group is the most common group of contracting TB in countries with high TB prevalence. This type of "adult-type TB" has the potential to cause extensive damage to lung parenchyma due to calcification and formation of cavities, and this age group is potentially infectious to the community.[21] In our study, the statistical test shows significant equivalence between GeneXpert MTB/RIF and MTB sputum cultures in both age groups. This means that GeneXpert MTB/RIF can be used interchangeably with MTB sputum culture to diagnose PTB in both age groups if there is no MTB sputum culture examination facilities. Beside, molecular methods with GeneXpert MTB/RIF also gives advantage of reading the results quickly (± 2 hours) so clinical decisions to initiate TB treatment can be accelerated. Sekadde et al and Nhu et al reported the same result for sensitivity and specificity of GeneXpert MTB/RIF for > 5 years old age group higher than group age < 5 years old.[19,20]

In our study, statistical analysis suggested there is a link between clinical manifestations and GeneXpert MTB/RIF or MTB sputum culture results. There is equivalence between GeneXpert MTB/RIF results and MTB culture result in the clinical TB group. GeneXpert MTB/RIF and MTB sputum cultures had lower sensitivity in diagnosing TB children clinically than molecularly. This is because children naturally had paucibacillary MTB although demonstrated clinical manifestations of TB and have symptoms improvement after treatment.

Symptoms of TB in children are not specific and more than 50% of children with TB are asymptomatic. Children with TB exhibiting clinical symptoms mostly will experience lung disorders, while 25% - 35% have extra-pulmonary disorders. Systemic disorders such as fever, night sweats, anorexia may also occur. The most common clinical symptoms are cough, body fatigue and weight loss. Specificity of clinical symptoms depends on the tightness of operational definitions used. However there is no cut-off for clinical symptoms that have been validated until now.[22,31,32]

The diagnosis of PTB in children cannot be established by clinical symptoms alone. Laboratory tests need to be done in children with or without clinical symptoms of PTB. However, the negative results of bacteriological examination does not exclude the possibility of TB disease.[22] Patients aged < 5 years, with a positive TST result and history of close contact with adult TB patients but do not show symptoms of PTB, were given INH prophylaxis of 7-15 mg / kg / day, once daily for 6 months, while patients that show symptoms of PTB were given TB drugs according to standard procedures.[33]

CONCLUSION

GeneXpert MTB/RIF has a good sensitivity and specificity to diagnose pulmonary tuberculosis (PTB) in children which give parallel results with MTB sputum culture methods in aiding the diagnosis of PTB in children aged ≥ 3 months old - 14 years old with suspected PTB.

CONFLICT OF INTEREST

There is no conflict of interest of this study.

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