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Ancheta JB and Garcia RD. A Retrospective Study on Sensitivity, Specificity, Negative Predictive Value, Positive Predictive Value of TB PCR Versus TB Culture in Diagnosing Tuberculosis in Filipino Children Aged 3 Months to 18 Years at a Tertiary Care Center

ORIGINAL

A RETROSPECTIVE STUDY ON SENSITIVITY, SPECIFICITY, NEGATIVE PREDICTIVE VALUE, POSITIVE PREDICTIVE VALUE OF TB PCR VERSUS TB CULTURE IN DIAGNOSING TUBERCULOSIS IN FILIPINO CHILDREN AGED 3 MONTHS TO 18 YEARS AT A TERTIARY CARE CENTER

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The authors declare that the data presented are original material and has not been previously published, accepted or considered for publication elsewhere; that the manuscript has been approved by all authors, and all authors have met the requirements for authorship.

4TH PRIZE PIDSP RESEARCH

ABSTRACT

Objectives: This study aimed to establish the accuracy of TB PCR versus TB culture and rifampicin resistance detection by PCR versus conventional susceptibility testing of body fluids in diagnosing tuberculosis in pediatric patients 3 months to 18 years with suspected tuberculous disease at a tertiary care center.

Methods: This is a retrospective analytical study of patients seen between January 1, 2012 to May 31, 2017, with clinical and radiographic features suggestive of tuberculosis, who had diagnostic testing of body fluids for TB PCR and TB culture.

Results: Among 159 patients suspected of TB, 46 (28%) tested positive by PCR, of which one was rifampicin-resistant. The sensitivity, specificity, positive predictive value and negative predictive values of TB PCR, using TB culture as the gold standard were 90%, 91.6%, 78.3%, and 96.5% respectively. The sensitivity, specificity, positive predictive value, and negative predictive values of TB PCR for detecting rifampicin resistance, using TB culture and sensitivity as the gold standard, were 33%, 100%, 100%, and 95%, respectively. Overall, the accuracy of TB PCR in detecting TB disease is 91.2% and the accuracy of TB PCR in detecting rifampicin resistance is 95%.

Conclusion: Findings in our study suggest that TB PCR play an important role in TB disease diagnosis, but clinical and radiological assessment continue to be essential in the diagnosis of childhood tuberculosis. The accuracy of TB PCR in detecting TB disease in children is 91.2% and the accuracy of TB PCR in detecting Rifampicin resistance is 95%.

KEYWORDS: *TB PCR, tuberculosis, Filipino, pediatrics, accuracy*

INTRODUCTION

Tuberculosis (TB) is both a preventable and treatable illness. In children it is infrequently confirmed bacteriologically due to the lack of effective diagnostic tools.[1] Early identification of TB is very important, as it can help in the initiation of adequate treatment for patients and in the prevention of further spread of drug-resistant strains.[2] Tools for the diagnosis of active disease include clinical suspicion, chest radiographs, staining for acid-fast bacilli (AFB), culture for mycobacteria, nucleic acid amplification assays, and response to treatment.[3] The current gold standard for the diagnosis of tuberculosis is the combination of culture and clinical diagnosis.[4] However, there are shortcomings to the standard diagnostic methods. The direct smear for acid-fast bacilli has low sensitivity, while mycobacterial culture usually requires two to six weeks to yield a result. [2] TB Polymerase Chain Reaction (TB PCR) can detect a 3-fold greater number of confirmed tuberculosis cases compared to AFB smear microscopy but with equal rapidity. [5] The TB PCR has the advantage of high sensitivity and specificity for the diagnosis of TB, and has the capacity to detect resistance to rifampicin.[6] An ideal test for active TB is one that would produce rapid results, has high sensitivity and specificity, with low-cost, easily performed without the need for excessive sample preparation or technical expertise, and able to provide drug-susceptibility data.[3]

Despite the development of quick and more sensitive diagnostic techniques, the high cost has limited their use in many resource-poor countries. Due to the rapidly growing TB problem in developing countries like the Philippines, there is an urgent need to assess alternative methodologies in settings with high disease prevalence.[4] It is necessary to investigate the diagnostic accuracy of TB PCR in the local setting since positive predictive value (PPV) and negative predictive value (NPV) are directly related to the prevalence of the disease in the population.

This study aims to determine the diagnostic accuracy of TB PCR versus TB culture in terms of its specificity, sensitivity, positive predictive value and negative predictive value in the diagnosis of TB in all in-patient and out-patient Filipino children 3 months to 18 years with suspected active TB seen in a tertiary care center. [7.] Moreover, this study sought to determine the accuracy of TB PCR in establishing rifampicin resistance in comparison to conventional mycobacterial susceptibility testing. It also intended to determine the yield of TB PCR and TB Culture among specimens submitted and tested, according to body fluid sampled.

MATERIALS AND METHODS

Study Design and Setting

This was a retrospective analytical study of all patients (out-patients and in-patients) seen at a tertiary care center between January 1, 2012 to May 31, 2017. During the study period, all patients suspected to have TB disease based on clinical features and radiograph findings, who had diagnostic TB sampling (TB PCR, culture) of body fluids (sputum, endotracheal, gastric lavage, cerebrospinal fluid) were included in the study.

Subject selection

Inclusion criteria

All in-patient and out-patient Filipino children aged 3 months to 18 years who underwent TB PCR and mycobacterial culture and susceptibility testing (Lowenstein Jensen and MGIT culture media) were included. The following data were obtained: sex, age, weight, height, body mass index (BMI), chest radiograph results, and CSF and MRI/CT scan results, if with suspected TB meningitis, and clinical findings relevant to MTB disease. A person with TB disease is someone with presumptive TB who, after clinical and diagnostic evaluation is done, is confirmed to have TB disease.[8] A definitive diagnosis of TB was made by positive TB culture result. For those patients with a negative culture for *M.*

tuberculosis, a clinical diagnosis was made with respect to the clinical and radiological presentation and tuberculin skin tests, hematological findings, histological findings (when available), and clinical response to anti-TB treatment.[6]

Exclusion criteria

All patients (out-patients and in-patients) aged 3 months to 18 years who underwent mycobacterial culture (Lowenstein Jensen and MGIT culture media) without TB PCR or underwent TB PCR without mycobacterial culture, were not included in the study.

Data Collection

TB PCR results from the hospital's molecular laboratory and TB culture and susceptibility results from the hospital's bacteriology laboratory of suspected pediatric TB cases were reviewed and analyzed in relation to signs and symptoms. Clinical, radiologic and culture information for each pediatric in-patient were collected from the medical records ArchiveOne database, Radiologic information system and Picture Archiving and Communication System (RIS PACS) and the laboratory. Clinical, radiologic and culture information for each pediatric out-patient were retrieved from the medical records of pediatric infectious disease and pediatric pulmonology specialists who requested for TB PCR and TB Culture, as well as the Radiologic information system and Picture Archiving and Communication System (RIS PACS) and the laboratory.

The presence or absence of the following clinical findings were noted: 1) fever, 2) cough, 3) pleuritic or retrosternal pain of gradual onset, 4) anorexia, 5) night sweats, 6) weight loss, 7) lymphadenopathy, and 8) signs and symptoms suggestive of non-pulmonary TB.[5] Moreover, exposure to an adult/adolescent with active TB disease, positive tuberculin skin test, abnormal chest radiograph suggestive of TB and laboratory findings suggestive of TB (TST, smear microscopy, culture and TB quantiferon result) were also analyzed.

The TB PCR is an automated, cartridge-based system, which makes use of a closed amplification system that reduces the potential for cross-contamination between specimens.[9] A region of the mycobacterial 16S DNA, conserved in all members of the MTB complex, is amplified and detected by a flurogenic probe. The assay system contains, in one master mix, all reagents and enzymes for the specific amplification and detection of a 155-base pair region of the MTB genome.[6] A sample is considered positive when a fragment of the 155-base pair is observed in the ultraviolet transilluminator.[10]

This assay is used for the rapid identification of MTB-complex (i.e., M. tuberculosis, M. bovis, M. africanum, M. canettii, M. microti, M. caprae, M. pinnipedi, M. mungi, and M. orygis) in clinical samples and the detection of mutations affecting resistance to rifampicin.[7] It also provides information about potential rifampicin resistance, by detecting mutations in an 81-base pair region of the rpoB gene that is responsible for conferring approximately 96% of rifampin resistance in the MTB complex. Rifampicin resistance is a predictor of multi-drug resistant TB since majority of rifampicin-resistant isolates are also isoniazid-resistant.[11]

Statistical Methods

Sample size determination

A minimum of 156 subjects were required based on a level of significance of 5%, a prevalence of 12%, sensitivity of 62% with a width of the confidence interval of 0.22. The values for the prevalence of a positive TB culture, and sensitivity of the TB PCR (Xpert) test for tuberculosis detection were based on the study by Detjen et al., 2015[12].

Legend:

n = minimum sample
P = Prevalence of a positive TB culture test = 12%
S= Sensitivity of TB PCR test for tuberculosis detection = 62%
L = width of the confidence interval = 51% to 73% = 22%
$z_\alpha = 1.96$

Sample size formula[21]:

$$n \geq \frac{Z^2{}_\alpha \times S_N \times (1 - S_N)}{L^2 \times \text{Prevalence}}$$

$$n \geq \frac{1.96^2 \times 0.62 \times (1 - 0.62)}{0.22^2 \times 0.12}$$

$$n \geq 155.83 \approx 156$$

Statistical Analysis

Descriptive statistics were used to summarize the clinical characteristics of the patients. Frequency and proportion were used for nominal variables, median and range for ordinal variables, and mean and SD for interval/ratio variables. Sensitivity, specificity, PPV, NPV and likelihood ratio of TB PCR were compared to TB culture as the gold standard. Crude and adjusted odds ratios with corresponding 95% CI were determined via binary logistic regression. All valid data were included in the analysis. Missing variables were neither replaced nor estimated. Null hypotheses were rejected at 0.05 α-level of significance. STATA 15.0 was used for data analysis.

Ethical consideration

The protocol of this study adhered to the ethical considerations and ethical principles set out in relevant guidelines, including the Declaration of Helsinki, WHO guidelines, International Conference on Harmonization-Good Clinical Practice, and National Ethics Guidelines for Health Research.

IRB approval and informed consent

The study commenced upon the approval of the Institutional Review Board of the hospital.

Data safety and confidentiality

Subject information was kept in a secure office, with access available only to members of the research team. Computerized study information was stored in a secured network with password access. All identifiable information and data were given a code number. A master list linking the code number and subject identity was kept separately from the research data. Only members of the research team had access to the list. The research records are to be stored for at least five years following completion of the study. Individually identifiable research data was not shared with others outside of the research team. The investigator and all key personnel were able to complete the Good Clinical Practice (GCP) training on the responsible conduct of research with human data.

Compensation

This study was initiated and funded wholly by the principal investigator.

Adverse events

No adverse events were seen in this retrospective study.

Vulnerability

We recognize the vulnerability of our subjects and extra care was done to assure the confidentiality of their identities.

Conflict of Interest

There are no potential conflicts of interest.

RESULTS

We reviewed the charts of 159 pediatric patients suspected with TB disease. Forty-six (28%) were found positive on PCR, of whom one was rifampicin-resistant. Forty (25%) were TB culture-positive, four (2%) of whom were PCR-negative. The overall pediatric Rifampicin resistance was 1.8% (Figure 1).

Figure 1. Diagnostic pathway results among children suspected to have TB Disease.

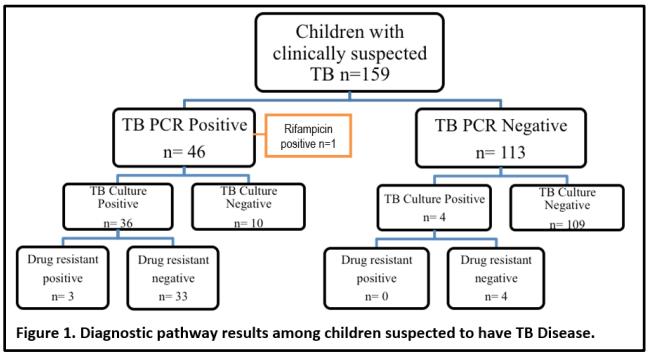

Figure 1. Diagnostic pathway results among children suspected to have TB Disease.

Our patients had a median age of 15 years (range 3 months to 18 years) and were more commonly male (54%). The most common presenting signs and symptoms were fever (87%), cough (67%) and anorexia (47%). Active TB exposure was disclosed in 31%. Other diagnostic results are tabulated in Table 1.

Table 1. Demographic and clinical profile of pediatric patients suspected of TB Disease (n=159)

	Frequency (%); Mean ± SD; Median (Range)
Age (years)	15 years (3 months – 18 years)
Sex	
Male	86 (54.09)
Female	73 (45.91)
Height (cm)	155 (58 – 185)
Weight (kg)	47 (3.1 – 85.5)
BMI	18.56 (9.22 – 28.98)
Active TB exposure	50 (31.45)
Chest x-ray	
Positive	29 (18.2)
Negative	130 (81.8)
Signs and symptoms	
Fever	139 (87.4)
Cough	108 (67.9)
Anorexia	76 (47.8)
Weight loss	54 (33.9)
Vomiting	32 (20.1)
Lymphadenopathy	26 (16.4)
Pleuritic or retrosternal pain	23 (14.5)
	22 (13.8)
Crackles	16 (10.1)
Headache	8 (5.0)
Abdominal pain	4 (2.5)
Night sweats	77 (48.4)
Others	
TST (n=131)	
Positive	27 (20.6)
Negative	104 (79.4)
AFB smear (n=157)	
Positive	4 (2.6)
Negative	153 (97.5)
TB quantiferon (n=33)	
Positive	15 (45.5)
Negative	18 (54.6)
TB PCR	
Positive	46 (28.9)
Negative	113 (71.1)
RIFAMPICIN RESIS by PCR	
Positive	1 (0.6)
Negative	158 (99.4)
TB Culture	
Positive	40 (25.2)
Negative	119 (74.8)
Drug susceptibility (n=40)	
Positive for Rifampicin resistance	3 (7.5)
Negative for Rifampicin resistance	37 (92.5)

Specimens were mostly pulmonary (59.1%), coming from sputum and pleural fluid.

Table 2. Specimen type, pediatric patients (n=159)

	Frequency (%)
Pulmonary	94 (59.12)
Sputum	79 (84.04)
Pleural fluid	15 (15.96)
Non-pulmonary	65 (40.88)
Gastric/NGT aspirate	11 (16.92)
Others	54 (83.08)

We assessed for the diagnostic accuracy of TB PCR in comparison to TB culture (Table 3). With TB culture as a gold standard, TB PCR had good sensitivity and specificity to detect TB disease in children. Among patients who were

TB culture-positive, there was a 90% probability that PCR would be positive (sensitivity). Among patients without TB, there was a 91.6% probability that PCR would be negative (specificity). Patients who were TB culture-positive were 10.71 times more likely to yield a positive PCR compared to patients who were TB-negative (LR+), and were 89% less likely to yield a negative PCR result (LR-). When PCR was positive, there was a 78.26% probability that TB culture was positive (PPV). When PCR was negative, there was a 96.46% probability that the patient was TB culture-negative (NPV). Overall, the accuracy of TB PCR in detecting TB disease was 91.19%

Table 3. Diagnostic accuracy of TB PCR versus conventional TB culture in detecting presence of TB in pediatric patients (n=159).

	TB culture positive	TB culture negative	Total
	Frequency (%)		
TB PCR positive	36 (22.6)	10 (6.3)	46 (28.9)
TB PCR negative	4 (2.5)	109 (68.6)	113 (71.1)
Total	40 (25.2)	119 (74.8)	159 (100)
Sensitivity	90% (76.3% - 97.2%)	Positive LR	10.71 (5.9 – 19.6)
Specificity	91.6% (85.1% - 95.9%)	Negative LR	0.11 (0.04 – 0.28)
PPV	78.26% (66.4% - 86.8%)	Accuracy	91.19% (85.7% – 95.1%)
NPV	96.46% (91.5% - 98.6%)		

PPV, positive predictive value; NPV, negative predicted value; LR, likelihood ratio.

With conventional mycobacterial susceptibility as the gold standard, TB PCR was highly specific in detecting rifampicin resistance. Among patients who were TB culture-positive, there was a 33% probability that PCR would be positive (sensitivity). Among patients without TB, there was nearly 100% probability that PCR would be negative (specificity). That is, patients who were rifampicin-resistant by conventional mycobacterial susceptibility testing were 33% less likely to have a negative RIF-PCR result (LR). When PCR rifampicin resistance was present, there was a nearly 100% probability that TB conventional rifampicin resistance was present (PPV). When PCR rifampicin resistance was not present, there was a 94.87% probability that the patient is did not have rifampicin resistance (NPV). Overall, the accuracy of TB PCR in detecting TB disease was 95% (Table 4).

Table 4. Diagnostic accuracy of RIF versus drug susceptibility as a gold standard in pediatric patients (n=40)

	Drug susceptibility testing		Total
	Resistant/positive	Sensitive/negative	
	Frequency (%)		
RIF resistance	1 (2.5)	0	1 (2.5)
RIF negative	2 (5)	37 (92.5)	39 (97.5)
Total	3 (7.5)	37 (92.5)	40 (100)
Sensitivity	33.33% (0.84% - 90.57%)	Positive LR	-
Specificity	100% (90.51% - 100%)	Negative LR	0.67 (0.30 to 1.48)
PPV	100%	Accuracy	95% (83.1% - 99.4%)
NPV	94.87% (89.25% - 97.63%)		

PPV, positive predictive value; NPV, negative predicted value; LR, likelihood ratio.

The following clinical characteristics were associated with a positive TB culture in children (Table 5): older age (cOR 1.093, 95% CI 1.01 – 1.18, p value = 0.019), an active TB exposure (cOR 3.43, 95% CI 1.62 – 7.25, p value = 0.001), a positive chest x-ray (cOR 19.56, 95% CI 7.3 – 52.39, p value <0.0001), presence of fever (cOR 0.275, 95% CI 0.10-0.72, p value = 0.009), weight loss (cOR 2.88, 95% CI 1.38 – 6.03, p value = 0.005), presence of pleuritic/retrosternal pain (cOR 3.382, 95% CI 1.35 – 8.45, p value = 0.009), a positive TST (cOR 20.07, 95% CI 7.1 – 56.6, p value <0.001), and a positive TB quantiferon test (cOR 25.5, 95% CI 2.65 – 245.83, p value = 0.005).

We performed a stepwise backward elimination logistic regression to determine the

predictors of positive TB culture in children (Table 5.1). These were age, active TB exposure, positive chest radiograph findings, and positive TST. For every unit increase in age, the odds of a positive TB culture increases by approximately 16.6%. Patients with active TB exposure were 3.94 times more likely to have a positive TB culture. Patients with positive chest radiograph were 6.51 times more likely to have a positive TB culture. Patients with positive TST were 25.96 times more likely to have a positive TB culture. This model predicts 47.67% in the variation of positivity of TB cultures, and is statistically significant at p = 0.0001.

Table 5. Predictors of positive TB culture in children

	TB Culture Positive (n=40)	**TB Culture Negative (n=119)**	**Crude Odds Ratio (95% CI)**	**P-value**
	Frequency (%); Mean ± SD			
Age (years)	17 (4 mos – 18 years)	14 (3 mos – 18 years)	1.093 (1.01 – 1.18)	**0.019**
Sex (Male)	22 (55)	64 (53.78)	1.05 (0.51 – 2.16)	0.894
BMI (kg/m^2)	18.5 (13.4 – 28.4)	18.6 (9.2 – 28.98)	0.953 (0.86 – 1.05)	0.344
Active TB exposure	21 (52.5)	29 (24.4)	3.430 (1.62 – 7.25)	**0.001**
Chest x-ray				
Positive	22 (55)	7 (5.88)	19.56 (7.3 – 52.39)	**<0.0001**
Negative	18 (45)	112 (94.12)	(reference)	-
Signs and symptoms				
Fever	30 (75)	109 (91.6)	0.275 (0.10 – 0.72)	**0.009**
Cough	29 (72.5)	79 (66.39)	1.335 (0.60 – 2.95)	0.474
Anorexia	21 (52.5)	55 (46.22)	1.286 (0.63 – 2.64)	0.492
Weight loss	21 (52.5)	33 (27.73)	2.88 (1.38 – 6.03)	**0.005**
Vomiting	6 (15)	26 (21.85)	0.631 (0.24 – 1.67)	0.353
Lymphadenopathy	9 (22.5)	17 (14.3)	1.742 (0.71 – 4.29)	0.228
Pleuritic/Retrosternal pain	11 (27.5)	12 (10.1)	3.382 (1.35 – 8.45)	**0.009**
Crackles	3 (7.5)	19 (16)	0.427 (0.12 – 1.53)	0.190
Headache	2 (5)	14 (11.8)	0.395 (0.09 – 1.82)	0.223
Abdominal pain	3 (7.5)	5 (4.2)	1.849 (0.42 – 8.11)	0.415
Night sweats	3 (7.5)	1 (0.84)	9.57 (0.97 – 94.77)	0.054
TST (n=131)				
Positive	19 (63.33)	8 (7.92)	20.08 (7.1 – 56.6)	**<0.0001**
Negative	11 (36.67)	93 (92.08)	(Reference)	-
AFB smear (n=157)				
Positive	3 (7.5)	1 (0.85)	9.41 (0.95 – 93.18)	0.055
Negative	37 (92.5)	116 (99.15)	(reference)	-
TB quantiferon (n=33)				
Positive	9 (90)	6 (26.1)	25.5 (2.65 – 245.83)	**0.005**
Negative	1 (10)	17 (73.9)	(reference)	-

Table 5.1. Predictors of positive TB culture in children

	Adjusted Odds Ratio	95% CI	P-value
Age	1.166	1.02 – 1.33	**0.024**
Active TB exposure	3.94	1.12 – 13.9	**0.032**
Positive Chest x-ray	6.51	1.59 – 26.69	**0.009**
Positive TST	25.96	6.37 – 105.8	**<0.0001**

R^2 = 47.67%, p = 0.0001

We had insufficient evidence to demonstrate an association between age, sex, BMI, and other clinical findings with drug resistant TB (Table 6). Two of the three drug resistant TB patients presented with crackles, which was a higher proportion compared to the one of 37 patients who were rifampicin-susceptible (p = 0.007).

Table 6. Association of select clinical features with rifampicin resistant TB in children (n=40)

	Rifampicin-resistant (n=3)	Rifampicin-susceptible (n=37)	Crude Odds Ratio (95% CI)	P-value
	Frequency (%); Mean ± SD			
Age (years)	14 (4 months – 18 years)	17 (1 year – 18 years)	0.927 (0.77 – 1.12)	0.425
Sex (Male)	3 (100)	19 (51.35)	(omitted)	-
BMI (kg/m^2)	14.2 (13.7 – 28.4)	18.56 (13.4 – 24.98)	1.028 (0.73 – 1.44)	0.872
Active TB exposure	1 (33.33)	20 (54.05)	0.425 (0.04 – 5.11)	0.500
Chest x-ray				
Positive	2 (66.7)	20 (54.05)	1.7 (0.14 – 20.42)	0.676
Negative	1 (33.3)	17 (45.95)	(reference)	-
Signs and symptoms				
Fever	3 (100)	27 (72.97)	(omitted)	-
Cough	3 (100)	26 (70.27)	(omitted)	-
Anorexia	2 (66.7)	19 (51.35)	1.895 (0.16 – 22.75)	0.614
Weight loss	2 (66.7)	19 (51.35)	1.895 (0.16 – 22.75)	0.614
Vomiting	0	6 (16.22)	(omitted)	-
Lymphadenopathy	2 (66.7)	7 (18.92)	8.571 (0.68 – 108.4)	0.097
Pleuritic/Retrosternal	1 (33.3)	10 (27.03)	1.35 (0.11 – 16.57)	0.815
pain	2 (66.7)	1 (2.70)	72.0 (3.19 – 1624.3)	**0.007**
Crackles	0	2 (5.41)	(omitted)	-
Headache	0	3 (8.1)	(omitted)	-
Abdominal pain	1 (33.3)	2 (5.41)	8.75 (0.54 – 142.68)	0.128
Night sweats				
TST (n=30)				

	Rifampicin-resistant (n=3)	Rifampicin-susceptible (n=37)	Crude Odds Ratio (95% CI)	P-value
	Frequency (%); Mean ± SD			
Positive	1 (33.3)	18 (66.7)	0.25 (0.02 – 3.14)	0.283
Negative	2 (66.7)	9 (33.3)	(reference)	-
AFB smear (n=40)				
Positive	0	3 (8.11)	(omitted)	-
Negative	3 (100)	34 (91.89)	-	-
TB quantiferon (n=10)				
Positive	1 (100)	8 (88.89)	(omitted)	-
Negative	0	1 (11.11)	-	-
Rifampicin (resistant)	1 (33.3)	0	(omitted)	-

DISCUSSION

This retrospective study of childhood TB seen at a private tertiary care center showed the following key findings: of 159 children clinically suspected to have TB disease, the TB culture yield was 25% and TB PCR yield was 28%. Specimen samples were mostly pulmonary (59.1%), consisting of 84% sputum and 16% pleural fluid, while 40.9% were non-pulmonary specimens. The sensitivity, specificity, PPV and NPV of TB PCR, using conventional TB culture as the gold standard were 90%, 91.6%, 78.3%, and 96.5% respectively. Rifampicin resistance for TB-PCR positive and TB culture-positive children were 2% and 8%, respectively. The sensitivity, specificity, PPV and NPV of TB PCR rifampicin resistance detection, using conventional TB susceptibility as the gold standard, were 33%, 100%, 100% and 95%, respectively. Overall, the accuracy of TB PCR in detecting TB disease is 91.2% and the accuracy of TB PCR in detecting rifampicin resistance is 95%. Among selected demographic and clinical variables, the following were found to be significant predictors for a positive TB culture: older age, known TB exposure, a chest radiograph compatible with TB disease; presence of fever, weight loss, pleuritic or retrosternal pain; a positive TST and a positive TB quantiferon test. Presence of rales was significantly associated with having a rifampicin-resistant isolate.

The TB culture yield was 25% and TB PCR yield was 28%. In a study done in Philippine General Hospital (PGH) and Research Institute for Tropical Medicine (RITM), the TB culture yield of all pediatric samples ranged from 40-50%[13]. In a study done in New York City, culture was positive for TB in 12% of all children assessed and TB PCR was positive in 11%[12]. In a meta-analysis, the yield of culture in childhood TB ranged from 20% to 70% depending on factors such as age, disease severity, and type and quality of the specimen, and culture method used[12]. TB culture has a generally low yield in children due to the difficulty in specimen collection especially in those under seven years. Also, childhood TB is a paucibacillary illness, so that even with the best effort to submit samples, there may be too small a population in the child's diseased organ to produce a culture yield [14]. There is no local study on TB PCR in children as this is a relatively new test and is only available at national reference centers in the Philippines. The diagnosis of TB in children is difficult in many cases. The factor of age, stage of development and the vulnerability of the young are considered important. A Shortened time to detection of TB is an important comparative

advantage of TB PCR over conventional culture. It also offers the opportunity for prompt clinical management of pediatric TB cases[15].

Specimen samples were obtained mostly from respiratory specimens (59.1%), consisting of 84% sputum and 16% pleural fluid, while 40.9% were non-pulmonary specimens. Studies in children showed an improved yield of TB PCR from various specimens. A systematic review and meta- analysis in the accuracy of TB PCR to diagnose TB in children showed sensitivities of 55-90% from sputum samples, and 40-100% for gastric lavage or aspirate specimens. Specificities for all specimen types ranged from 93-100%[16]. In a study done at the National Institute of Health in New Orleans, specimen samples were obtained from sputum (25%), gastric aspirate (25%) and non-pulmonary (50%) sites.[13] The WHO recommends the use of TB PCR in the following specimens: processed or unprocessed sputum, gastric lavage or aspirate, CSF, lymph node and other tissues[16]. This suggests that no specimen type is superior.

The sensitivity, specificity, PPV and NPV of TB PCR, using TB culture as the gold standard were 90%, 91.6%, 78.3%, and 96.5% respectively. In a meta-analysis, sensitivity and specificity of TB PCR for TB detection were 62% and 98%, respectively[12]. In a more recent meta-analysis, sensitivity and specificity of TB PCR compared to TB culture were 62% and 98%, respectively with use of sputum samples, and 66% and 98%, respectively, with the use of gastric lavage samples[12]. With these good results, TB PCR has been shown to be a valuable test that can produce rapid results that will be useful in children suspected to have TB disease, where resources are available. In situations where the sample volume is low, or additional specimen cannot be obtained, TB PCR can be helpful because its accuracy is comparable to that of TB culture[16].

Rifampicin resistance for TB PCR-positive and TB culture-positive children were 2% and 8%, respectively. Rifampicin resistance can be used to suggest multi-drug resistance since rifampicin mono-resistance is uncommon and most isolates that are rifampicin-resistant are also isoniazid-resistant[17]. In this study, TB PCR underestimated the real rifampicin resistance rate when compared to conventional culture, consistent with findings from previous studies[1]. TB PCR can detect DNA from both viable and non- viable bacilli and is not recommended for monitoring the treatment response of patients[7]. This suggests that conventional culture is still needed to monitor treatment response; it is also necessary if data on resistance to drugs other than rifampicin is desired.

The sensitivity, specificity, PPV and NPV of TB PCR rifampicin resistance detection, using TB culture susceptibility as the gold standard, were 33%, 100%, 100% and 95%, respectively. In a meta-analysis, sensitivity and specificity of TB PCR rifampicin resistance detection were 86% and 98%, respectively[12]. In a study done in Geneva, rifampicin resistance showed sensitivity and specificity of 83.3% and 99.1% respectively[16]. TB PCR rifampicin resistance in this study showed that it is a method with high specificity and positive predictive value. The sensitivity in this study was low in relation to the above reported results. The paucibacillary nature of childhood TB may not be blamed for this low sensitivity, as the sensitivity of TB PCR versus culture as shown above is a high 90%, so it is unclear why the PCR rifampicin resistance sensitivity is low at 33%. The false positive results, on the other hand, may be explained by the detection of non-viable MTB that would not be detected on culture. For reliable results a good quality of specimen collection is very important[1].

The following were demographic factors found to be significant predictors for a positive TB culture: older age, known TB exposure, a chest radiograph compatible with TB disease; presence of fever, weight loss, pleuritic or retrosternal pain; a positive TST and a positive TB quantiferon test.

For every unit increase in age, the odds of a positive TB culture increases by 16.6%. In a study done at PGH and RITM, majority were 11-15 years of age (33% were 1-5 years old, 22% were 11-15 years old, 16.5% were less than 1 year old and 15% were 16-18 years old) [13]. Age is the most important risk factor that determines the progression to disease following primary infection among immune-competent children[18].

Patients with active TB exposure were 3.94 times more likely to have a positive TB culture. In the PGH and RITM study, 56.7% of patients had a history of TB exposure[13]. According to the WHO, after prolonged exposure with a sputum smear-positive source, 60-80% of children become infected. When the household contact is smear-negative, 30-40% of children become infected. Children with history of contact exposure are 2.5 times more likely to develop TB disease than in children without known exposure[18]. Young children are most vulnerable among household members who are exposed to an adult or adolescent source case. These children are at greater risk of developing infection, disease and its complications, with dissemination or even death[19].

A chest radiograph compatible with TB disease was a significant predictor for a positive TB culture. Children with a chest radiograph indicating TB disease were 6.5 times more likely to have a positive TB culture. Radiologic examination is often equivocal, needing consensus among radiologists. A chest radiograph is the most basic and widely used radiologic investigation for TB. There are no pathognomonic radiograph findings but the most common findings are lymphadenopathy, parenchymal abnormalities and millet seeds. In the PGH and RITM study, hazy densities were the most frequently seen chest x-ray finding at 23.6%, followed by cystic lucencies at 21.8%[13]. In a cross-sectional study done among Filipino children 6 months to 18 years old at the Philippine Heart Center, 64% of patients showed lymphadenopathies as part of Ghon focus in 64% and 7% had normal radiographs[13].

The presence of fever, weight loss and pleuritic or retrosternal pain were significant predictors for a positive TB culture. Weight loss can be used as a red flag in TB disease case-finding[19]. In a study done in PGH and RITM, the most frequently seen symptoms were fever in 89.6%, cough in 76.1%, weight loss in 50.7%, anorexia in 44.8% and difficulty in breathing 28.4%. On physical examination, cervical lymphadenopathy was found in 62.7% followed by hepatomegaly at 37.3%[13].

A positive TST and a positive serum TB quantiferon test were significant predictors for a positive TB culture. Children with positive TST results were 25.96 times more likely to have a positive TB culture. In a study done in PGH and RITM, of the subjects who tested positive for TST, 28% had pulmonary TB while 57.1% had disseminated TB[13]. TST has become the standard method in demonstrating TB infection. Tuberculin reactivity provides a general measure of a person's cellular immune responsiveness[20]. The tuberculin skin test reaction should always be correlated with history of exposure to an infectious TB source, presence of clinical signs and symptoms suggestive of TB and chest radiograph findings in order to diagnose TB infection and disease.

In clinical practice, TB PCR is an invaluable tool that can provide rapid diagnosis of TB disease and detect possible drug resistance, thereby allowing a more prompt and confident start of treatment. In childhood TB in particular, the paucibacillary nature of TB causes a low initial AFB smear-positive rate.

Limitations of the study

This was a single-site study done at an urban tertiary care hospital with a predominantly middle class to affluent market, so that the results may not be applicable to children in other settings. TB disease affects the poor and malnourished

individuals in the community,[21] so the results herewith may potentially underestimate the rifampicin resistance rate.

CONCLUSION

The accuracy of TB PCR in detecting TB disease is 91.2% versus conventional TB culture, and the accuracy of TB PCR in detecting rifampicin resistance is 95% versus conventional TB susceptibility testing. The findings in our study suggest that TB PCR plays an important role in rapid diagnosis, but clinical and radiological assessment and contact tracing are still essential in the diagnosis of childhood TB. This assay allows patients to be treated promptly and to identify those who need second-line drug treatment. However, conventional culture is still needed to monitor treatment and to detect resistance to drugs other than rifampicin.

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