ORIGINAL ARTICLE

ACCURACY OF NASOPHARYNGEAL ASPIRATE GENEXPERT COMPARED TO GASTRIC ASPIRATE TB CULTURE AND GENEXPERT IN DIAGNOSING PULMONARY TUBERCULOSIS IN PEDIATRIC PATIENTS

Joy L. Morcilla, MD
Ma. Liza Antoinette M. Gonzales, MD
Anna Lisa T. Ong-Lim, MD

University of the Philippines - Philippine General Hospital

Correspondence:
Dr. Joy L. Morcilla
Email: morcilla_joy@yahoo.com

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.

ABSTRACT

Background: Pulmonary TB in children remains to be a burden in the Philippines. Diagnosis remains to be a challenge for pediatricians due to its paucibacillary nature, difficulty in obtaining specimens, cost of test as well as the varied sensitivity of the different tests available. Gastric aspirate (GA), commonly used for bacteriological diagnosis of pulmonary tuberculosis (PTB) in children, involves an invasive procedure that may cause discomfort and sometimes require admission. Nasopharyngeal aspirate (NPA), on the other hand, can be easily and non-invasively obtained but is currently not a recommended specimen for testing for PTB.

Objectives: This study aims to determine the accuracy of NPA GeneXpert in diagnosing PTB among pediatric patients 0-18 years old with presumptive TB using GA GeneXpert as the initial screening test and GA TB culture as gold standard.

Methodology: This prospective, cross-sectional diagnostic study involved collection of single NPA and GA specimens for GeneXpert and TB culture in 100 patients with presumptive PTB seen at a tertiary government hospital in the Philippines.

Results: Of the one hundred pediatric patients (mean age 6 ± 5.63 years) enrolled, 50 were clinically diagnosed PTB, 16 bacteriologically-confirmed and 34 were not PTB disease. Sensitivity, specificity and predictive values with 95% confidence intervals of the NPA GeneXpert were determined compared to GA GeneXpert and GA culture. Sensitivity, specificity, positive and negative predictive values of the NPA GeneXpert compared to GA GeneXpert were 70%, 96.67%, 70% and 96.67%, respectively. While NPA GeneXpert compared to GA TB culture were 40%, 91.58%, 20% and 96.67%, respectively.

Conclusion: GeneXpert testing on a single NPA specimen is a highly specific and rapid test that can be used to diagnose PTB in pediatric patients, particularly where gastric aspiration or mycobacterial culture is not feasible.

KEYWORDS: *GeneXpert, Gastric Aspirate, Nasopharyngeal Aspirate, TB Culture*

INTRODUCTION

Most cases of tuberculosis (TB) in children occur in TB-endemic countries but the actual burden of childhood TB is unknown. In 2015, the World Health Organization (WHO) estimated that globally, there were 1 million TB cases among children younger than 15 years of age with 210,000 deaths reported as a result of the disease.[1] In 2016, there were approximately 182,200 cases of pulmonary and extrapulmonary TB reported in the Philippines.[2]

The recommended approach in diagnosing pulmonary TB (PTB) in children is based on limited published evidence. A complete history and thorough physical examination remains an important tool. Tuberculin skin testing (TST) and chest X-ray (CXR) are still the most commonly used initial diagnostic examinations and TB culture remains the gold standard. Some of the challenges in the diagnosis of TB in children include the low positivity of cultures due to paucibacillary nature of TB in children, the intricacy of procedures for specimen collection for bacteriologic confirmation of TB (acid fast bacilli (AFB) smear, GeneXpert and TB culture) and high cost of test. The WHO recommends the use of the Xpert MTB/RIF (also known as GeneXpert MTB/RIF or GeneXpert) as the initial test in all children suspected of having pulmonary and extrapulmonary TB.[1,3] The test is useful because of the rapid and early detection of causative mycobacteria in clinical samples.[4]

Gastric aspiration, an invasive procedure that sometimes requires hospital admission, is commonly used for collecting specimens for TB diagnosis in pediatric patients. The gastric aspirate (GA) consists of swallowed respiratory secretions from the stomach. Nasopharyngeal aspiration is a less invasive and an easier method which could be done in the outpatient clinics. In the study of Zar in 2012, NPA GeneXpert had a sensitivity of 56% and specificity of 98.2% compared to culture-positive tuberculosis using either induced sputum or NPA.[5] There are no local data on the use of nasopharyngeal aspiration as a procedure for collection of specimens in children suspected of PTB. Furthermore, there is limited data comparing nasopharyngeal aspirate (NPA) GeneXpert with GA GeneXpert to diagnose PTB in children.

This study aims to determine the diagnostic accuracy of NPA GeneXpert in confirming PTB among pediatric patients compared to GA GeneXpert as the screening test and GA TB culture as the gold standard. The clinico-demographic profile, radiologic and laboratory findings of patients with pulmonary TB were likewise determined.

METHODOLOGY

Study Design and Population

This prospective, cross-sectional study enrolled pediatric patients aged 0-18 years old seen and diagnosed with presumptive TB at the outpatient department (OPD), emergency room (ER) or admitted in the different wards at the University of the Philippines - Philippine General Hospital (UP-PGH) from January 2017 to August 2017. The UP-PGH is a government tertiary hospital with a 1,500-bed capacity. It is the largest government referral center in the country and receives patient referrals from other institutions all over the archipelago.

The Department of Health – National Tuberculosis Control Program (DOH-NTP) guidelines and case definitions were used for the diagnosis of TB.[6] Presumptive TB is diagnosed in any adult or child with clinical signs and/or symptoms suggestive of TB or those with CXR findings suggestive of active TB.[6] In children aged 0-14 years old, a clinical diagnosis of presumptive TB is made when either one of the following is present: (1) at least 3 of the 6 signs and symptoms suggestive of TB (coughing or wheezing for 2 weeks or more, especially if unexplained; unexplained fever of 2 weeks or more, after common causes such as malaria and pneumonia have been excluded; loss of weight/failure to gain weight/weight faltering/loss of appetite; failure to respond to 2 weeks of appropriate antibiotic therapy for lower respiratory tract infection; failure to regain previous state of health 2 weeks after a viral infection or exanthema, and; fatigue, reduced playfulness, or lethargy, or; (2) any one of the above signs and symptoms in a child

who is a close contact of a known TB case. For those 15 years old and above, a presumptive TB case has any one of the of the following: (1) cough of at least 2 weeks duration with or without symptoms (significant and unintentional weight loss, fever, bloody sputum, chest or back pains not referable to any musculoskeletal disorders, easy fatigability or malaise, night sweats, shortness of breath or difficulty of breathing); (2) unexplained cough of any duration in any of the following: close contact of known active TB case or high-risk clinical groups (HIV-AIDS, end-stage renal disease, cancer, connective tissue disease, autoimmune diseases, prolonged systemic steroids). CXR findings suggestive of PTB regardless of age include perihilar or mediastinal lymphadenopathy, parenchymal abnormalities, patchy consolidation, cavitary lesions, fibrotic scarring, atelectasis, calcified nodules and pleural effusion.

Included in the study were newborns suspected to have congenital TB and patients with presumptive PTB with or without associated TB involving other organs (e.g., larynx, pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, meninges). Since gastric aspiration was used as the reference specimen, only those who were unable to expectorate sputum spontaneously were included in the study, regardless of age. Excluded were patients with orofacial abnormalities that prevented the performance of nasopharyngeal aspiration, patients who were hemodynamically unstable, and patients who had received anti-TB treatment for more than 2 weeks except those who have received isoniazid preventive therapy (IPT).

Based on a prevalence of 8% of confirmed TB cases among presumptive pediatric patients and a sensitivity of nasopharyngeal samples in detecting TB at 39.3% with a width of the confidence interval of 0.34, a minimum of 100 subjects were required for this study to achieve 80% power at a level of significance of 5%.[7-9]

Study Procedures

Patient enrolment and Data Collection

Patients diagnosed to have presumptive TB were screened for enrolment using the inclusion and exclusion criteria. General information, demographic data, clinical signs and symptoms, results of the TST, radiographic findings, imaging, and other laboratory findings were recorded in a predesigned data collection form.

Gastric Aspirate and Nasopharyngeal Aspirate Collection

All enrolled patients underwent both gastric aspiration and nasopharyngeal aspiration performed by the primary investigator on the same day using standardized procedures. As recommended by the WHO, only single specimens of GA and NPA were submitted for GeneXpert and TB culture due to unclear advantages in resource-limited settings for testing multiples pecimens.[10] For cases where the specimen was assessed to be inadequate or unsatisfactory, the patient was asked to return for repeat collection and the specimen was submitted and processed using the same method.

Gastric Aspiration. Gastric aspiration was performed in the child early in the morning following an overnight fast of at least 4-6 hours prior to the procedure. With the child kept in recumbent position, the expected distance from nose to stomach was measured and a Fr.8 orogastric tube (OGT) was inserted into the stomach. About 5 to 10ml of gastric contents was aspirated. When the initial attempt to aspirate was unsuccessful, 10 to 20mL of sterile water was instilled and the aspirate was added to the first collection. The minimum acceptable volume of gastric aspirate collected was 10ml. Aspirated gastric fluid was placed in a leak-proof, sterile container and transported to the laboratory at room temperature. When transport will be delayed for more than one hour, the specimen was refrigerated at a temperature of 2-8^{o}C.

Nasopharyngeal aspiration. Nasopharyngeal aspiration was performed using a sterile Fr.8 suction catheter connected to a sterile 10ml syringe. The distance from the tragus of the ear to the tip of the nose was measured and the suction catheter was carefully inserted into the nostril, parallel to the palate. The catheter was rotated gently, and the nasopharyngeal specimen

aspirated while the catheter was slowly withdrawn. This was repeated in the other nostril as necessary. NPA were successfully collected in all subjects. After NPA collection, the suction catheter was flushed with 10ml sterile water and the aspirate was placed in a labelled leak-proof, sterile specimen bottle.[11] In all patients, nasopharyngeal aspiration was performed before gastric aspiration with the two procedures separated by an interval of at least 5 minutes. Patients were monitored during the procedures and for 30 minutes after the procedures were completed for development of any adverse events or complications of the procedure such as intense pain, epistaxis, nasal mucosa trauma, cyanosis or respiratory distress.

Handling, Transport and Processing of Specimens

Both the GA and NPA specimens were sent immediately to the TB-DOTS laboratory in a government infectious disease facility, the San Lazaro Hospital for processing of GeneXpert. Processing of the specimens for GeneXpert was coursed through the UP-PGH Responsive Integrated Multidisciplinary Enhance (UP-PRIME) TB-DOTS where the nurse explained the procedures on specimen transportation to the San Lazaro TB laboratory for GeneXpert. The GA sample for culture was sent to the Microbiology and Infectious Disease Center (MIDC), a private DOH-certified and accredited microbiology laboratory. Both laboratories were not aware of the clinical diagnosis of the patients, and the tests each laboratory performed were interpreted independently of the results of the other's tests.

All GA and NPA specimens were kept in ice and transported in an ice box maintained at 4-8^{o}C temperature to the San Lazaro Hospital TB laboratory for GeneXpert and to the MIDC laboratory for TB culture. All specimens were processed for GeneXpert and culture within 7 days after specimen collection. The adequacy of the specimen was determined by the medical technologist who processed the specimen. In case the specimen was not adequate or unsatisfactory, the primary investigator was informed. The patient was contacted and new specimen was collected. Only one subject was asked to come back for specimen collection due to indeterminate results. TB GeneXpert results were released after 3 days and TB culture results were released after 6-8 weeks.

TB GeneXpert

Xpert MTB/RIF is an automated polymerase chain reaction (PCR) test (that is, a molecular test) utilizing the GeneXpert platform (Cepheid, Sunnyvale, CA, United States). Xpert MTB/RIF is a single test that can detect both *Mycobacterium tuberculosis* complex and rifampicin resistance within 2 hours after starting the assay, with minimal hands-on technical time. PCR amplification and detection of sample are integrated into a single self-enclosed test unit, which is the Xpert MTB/RIF cartridge. Following sample loading, all steps in the assay are automated and contained within the cartridge. In addition, the assay's sample reagent, used to liquefy sputum, is tuberculocidal (that is, it has the ability to kill the TB bacteria) which largely eliminates concerns about biosafety during the test procedures. The test procedure may be used directly on clinical specimens which are obtained after decontamination and concentration. The test material is combined with the reagent, mixed by hand or vortex, and incubated at room temperature for 15 minutes. After incubation, 2ml of the treated sample are transferred to the cartridge, and the run is initiated. Xpert MTB/RIF uses molecular beacon technology to detect rifampicin resistance. The test releases the following results: no TB; TB detected, rifampicin resistance detected; TB detected, no rifampicin resistance detected; TB detected, rifampicin resistance indeterminate; and an invalid result.

In this study, a patient was reported to be a case of bacteriologically diagnosed TB once the specimen tested positive in GeneXpert and/or TB culture. Repeat sample collection and processing of GeneXpert test was done in patients with indeterminate results.[10]

Treatment for TB Disease

All patients with presumptive TB and enrolled in the study were registered with the UP-PRIME TB-DOTS, a DOH accredited center in UP-PGH that facilitates intra- and inter-hospital referral of TB patients for start or continuation of treatment. The patients and their parents or guardians were informed of the GeneXpert and culture results. If the patient was positive for TB on any of the tests, the primary physician or the referring physician was immediately informed for initiation, continuation or revision of treatment. The primary investigator did not interfere with the medical management of the patient. All patients diagnosed to have active TB were referred by the UP PRIME TB-DOTS to the nearest TB-DOTS center upon discharge.

Ethical Consideration

This study underwent approval from the Philippine General Hospital Expanded Hospital Research Office (EHRO) Technical Review Panel and the University of the Philippines Manila Research Ethics Board (UPMREB). The study was conducted in accordance with the principles that have their origin in the Declaration of Helsinki and was consistent with the International Conference on Harmonization Tripartite Guidelines and the Good Clinical Practice Guidelines (ICH-GCP). Written informed consent and assent was obtained by the primary investigator at the OPD clinic for outpatient consults and bedside for admitted patients. Patients enrolled aged 18 years old was asked to sign the informed consent, and 15 to 17 years old was asked to co-sign the informed consent. An assent form was secured for children 12 to 14 years old. Verbal assent was taken for patients 2-7 years old. Parental consent was secured for all patients below 18 years old. The patients and their parents/guardians were informed that they could refuse participation in the study at anytime. All patient information was kept anonymized and confidential. The results of the diagnostic examinations were retrieved by the primary investigator and a copy released only to the parents/guardian of the patient and to the attending physician. Neither the principal investigator nor the co-investigators had any conflicts of interest in relation to the study. This study was partially supported by research funds obtained from the Pediatric Infectious Disease Society of the Philippines (PIDSP), Philippine Pediatric Society (PPS), and UP-PGH–Extended Hospital Research Office (EHRO). The Department of Health-National Center for Disease Prevention and Control (DOH-NTP) and San Lazaro Hospital provided GeneXpert cartridges and processed all specimens for GeneXpert.

Data Analysis

Descriptive statistics were used to summarize the clinical characteristics of all enrolled patients and analyzed using STATA13. Frequencies and proportions were used for nominal variables, mean and SD for interval/ratio variables. Two-way classification table and OpenEpi, a computer application were used to determine and compute the sensitivity, specificity, positive and negative predictive values with 95% confidence intervals (95% CI) of NPA GeneXpert to diagnose PTB using GA GeneXpert results as initial screening test and GA TB culture as the gold standard. Null hypothesis was rejected at 0.05 alpha level of significance.[12]

RESULTS

Demographic Profile

A total of 100 pediatric patients with a mean age of 6 ± 5.63 (SD) years and age range of 0 to 18 years old were enrolled during the study period. Most patients belonged to the age group below 15 years old (86%) and majority were male (64%). Forty-eight (48%) of patients had normal BMI, 29 patients (29%) were severely wasted.

History of TB exposure

Fifty-seven patients (57%) had exposure to known TB, of whom 41 had exposure within the household and 16 patients had exposure outside the household. Among those with household exposure, the primary guardians were the most common TB source whereas outside the household, neighbours were identified as the TB source. Fifty-three (92.98%) patients who had TB exposure were below 15 years old, while 4 (7.02%) patients were 15 years old and above. All 57 patients had daily exposure for more than 3 months duration. Forty-three patients (43%) had no identified TB exposure.

Table 1: Demographic data of Presumptive TB patients

	N=100	%
Sex		
M	64	64.00%
F	36	36.00%
Age		
<15	86	86.00%
>15	14	14.00%
BMI		
Normal	48	48.00%
Wasted	22	22.00%
Severely wasted	29	29.00%
Overweight	1	1.00%
School attended		
None	56	56.00%
Primary	21	21.00%
Secondary	23	23.00%
Tertiary	0	0.00%

Clinical signs and symptoms, TST and CXR of patients 0-14 years old with presumptive PTB

Majority (94.19%) of patients below 15 years of age presented with cough of at least 2 weeks duration. The most common associated symptoms were weight loss (90.70%), followed by unexplained fever of 2 weeks duration or more (77.91%), failure to respond to 2 weeks of appropriate antibiotic therapy for lower respiratory tract infection (30.23%), and fatigue (15.12%). Majority of these patients aged 0-14 years old had perihilar adenopathy (46.51%) on radiologic examination, followed by presence of parenchymal infiltrates (23.26%), pleural effusion (8.13%) and nonspecific pneumonitis (5.81%). Other findings were bronchiectasis, miliary TB and atelectasis. Among these 86 patients, only 26 patients (30%) had positive TST result.

Table 2: Characteristics of Patients 0-14 years old with Presumptive TB

Characteristics	N = 86	%
Clinical Presentation		
Cough 2 weeks or more	81	94.19
Loss of weight/failure to gain/weight faltering/loss of appetite	78	90.70
Fever of 2 weeks or more	67	77.91
Fulfilled 3 out of 6 clinical signs/symptoms suggestive of TB	44	51.16
Failure to respond to 2 weeks of appropriate antibiotic therapy	26	30.23
Fatigue, reduced playfulness, or lethargy	13	15.12
Known TB exposure	53	61.63
Tuberculin skin test		
>10 mm	26	30
<10mm	60	70
Radiologic Chest Findings		
Perihilar adenopathy	40	46.51
Parenchymal infiltrates	20	23.26
Pleural effusion	8	9.30
Pneumonitis	7	8.13
Normal	5	5.81
Bronchiectasis	3	3.49
Miliary	2	2.33
Atelectasis	1	1.16

Clinical signs and symptoms, CXR and exposure of patients >15 years old with presumptive PTB

A total of 14 patients aged 15 years old and above presented with cough of at least 2 weeks duration. The most common associated symptoms were weight loss (78.57%), followed by easy fatigability (71.43%), shortness of breath (71.43%), fever (64.29%) and chest or back pain (64.29%).

None of the enrolled patients presented with bloody sputum. 5 (35.71%) had pleural effusion on the radiologic examination, followed by perihilar adenopathy (21.43%) and presence of parenchymal infiltrates (21.43%). Other findings were nonspecific pneumonitis and pulmonary mass. There were only 4 patients (28.57%) that were identified to have PTB exposure.

Table 3: Characteristics of Patients ≥ 15 years old with Presumptive TB

Characteristics	N=14	%
Clinical Presentation		
Cough 2 weeks or more	14	100
Significant and unintentional weight loss	11	78.57
Easy fatigability or malaise	10	71.43
Shortness of breath or difficulty of breathing	10	71.43
Fever of 2 weeks or more	9	64.29
Chest or back pains	9	64.29
Chest X-ray		
Pleural effusion	5	35.71
Perihilar adenopathy	3	21.43
Cavity	3	21.43
Pneumonitis	1	7.14
Pulmonary mass	1	7.14
Normal	1	7.14
Known TB exposure	4	28.57

Table 4: Final diagnosis of enrolled subjects

Final Diagnosis	<15 years old	≥15 years old	Total
PTB, clinically diagnosed	46 (53%)	4 (29%)	50
PTB, bacteriologically diagnosed	10 (12%)	6 (42%)	16
Not TB disease	30 (35%)	4 (29%)	34
Total	86	14	100

Clinically diagnosed PTB

Fifty patients were clinically diagnosed PTB, of whom46 patients (92%) were below 15 years old and 4 (8%) were 15 years old and above. Of those 46 patients aged below 15 years old, 26 (57%) presented with at least 3 of the 6 clinical signs and symptoms suggestive of TB, had CXR findings compatible with TB and had known TB exposure. Eleven (24%) patients had at least 3 of the 6 clinical signs and symptoms suggestive of TB, had CXR findings compatible with TB, had known TB exposure and a positive TST. Eight (17%) patients had at least 3 of the 6 clinical signs and symptoms suggestive of TB, had CXR compatible with TB and had a positive TST. Only 1 (2%) patient had positive 3 of the 6 clinical signs and symptoms suggestive of TB, known TB exposure, and a positive TST.

All 4 patients 15 years old and above who were clinically diagnosed PTB presented with prolonged cough, 3 (75%) had weight loss and 1 (25%) had extrapulmonary TB (TB osteomyelitis on his foot). All 4 patients had radiologic findings suggestive of PTB but negative GeneXpert and negative TB culture results.

Bacteriologically-confirmed PTB

Out of 100 patients, there were 16 bacteriologically-confirmed TB of which 10 were positive on GeneXpert alone, 3 were positive on TB culture alone, and 3 were positive on both GeneXpert and TB culture.

Of the 13 patients who were positive on GeneXpert, 3 were positive only on NPA GeneXpert, 3 were positive only on GA GeneXpert, while 7 were positive on both NPA and GA GeneXpert.

A total of 6 specimens were positive on TB culture, of which 1 was positive on NPA TB culture but not on GA TB culture, 3 were positive on GA TB culture but not on NPA TB culture and 2 were positive for both GA and NPA TB culture. There were no rifampicin resistant isolates detected on GeneXpert and no drug resistant *Mycobacterium* tuberculosis isolates detected on TB culture. Two patients among these bacteriologically confirmed TB had CNS tuberculosis; one patient had tuberculoma, while another patient had TB meningitis.

Not TB disease

There were 34 patients diagnosed to have no PTB disease, 30 of them were less than 15 years old and 4 were more than 15 years old. These patients were referred back to their referring physician for further work up and management.

Diagnostic accuracy of NPA GeneXpert in detecting PTB

A total of 100 paired NPA and GA specimens were processed for GeneXpert; another 100 GA specimens were submitted for TB culture. Thirteen (13) specimens were positive on GeneXpert and 6 specimens were positive on TB culture. The diagnostic accuracy of NPA GeneXpert compared to GA GeneXpert and GA culture is summarized in Table 5 and Table 6.

The sensitivity of the NPA GeneXpert in detecting PTB compared to GA GeneXpert is 70% (95% CI: 39.68%, 89.22%), specificity is 96.67% (95% CI: 90.65%, 98.86%), positive predictive value is 70% (95% CI: 39.68%, 89.22%) and negative predictive value is 96.67% (95% CI: 90.65%, 98.86%).

Table 5: Diagnostic accuracy of NPA GeneXpert compared to GA GeneXpert as standard screening test

NPA GeneXpert	GA GeneXpert		Total
	Positive	Negative	
Positive	7	3	10
Negative	3	87	90
Total	10	90	100
Sensitivity	70	(95% CI 39.68%, 89.22%)	
Specificity	96.67%	(95% CI 90.65%, 98.86%)	
Positive Predictive Value	70%	(95% CI 39.68%, 89.22%)	
Negative Predictive Value	96.67%	(95% CI 90.65%, 98.86%)	

The sensitivity of the NPA GeneXpert compared to GA TB culture in detecting PTB is 40% (95% CI: 11.76%, 76.93%), specificity is 91.58% (95% CI: 84.25%,95.67%), positive predictive value is 20% (95% CI: 5.668%, 50.98%) and negative predictive value is 96.67% (95% CI: 90.65%,98.86%).

Table 6: Diagnostic accuracy of NPA GeneXpert in diagnosing PTB with GA TB culture as gold standard

NPA GeneXpert	GA Culture		Total
	Positive	Negative	
Positive	2	8	10
Negative	3	87	90
Total	5	95	100
Sensitivity	40%	(95% CI 11.76%, 76.93%)	
Specificity	91.58%	(95% CI 84.25%, 95.67%)	
Positive Predictive Value	20%	(95% CI 5.668%, 50.98%)	
Negative Predictive Value	96.67%	(95% CI 90.65%, 98.86%)	

Safety of Nasopharyngeal aspiration and Gastric aspiration

Both nasopharyngeal aspiration and gastric aspiration procedures were well tolerated. There were no adverse events or complications noted.

DISCUSSION

This study showed that NPA GeneXpert is a rapid and specific test that can be used to diagnose PTB in pediatric patients suspected to have the disease. The GeneXpert test on a single NPA specimen correctly identified majority of the patients with PTB confirmed by either GA GeneXpert or GA TB culture. Nasopharyngeal aspiration is a simple procedure that is easy to perform, is well tolerated and less invasive compared to gastric aspiration. However, since the sensitivity of the NPA GeneXpert remains suboptimal compared with the TB culture results, a negative GeneXpert test cannot be used to rule out TB in children.

PTB in children remains to be a diagnostic dilemma for physician due to its non-specific signs and symptoms and limited laboratory examinations that could establish the diagnosis. This may result in either delayed treatment in those with TB or overtreatment of those who turn out not to have active TB. Bacteriologic tests are important in confirming the diagnosis of PTB in children, however the choice of the respiratory specimen is challenging because of difficulty in collecting sputum in pediatric patients.

Recently, the GeneXpert test has emerged as an important tool in the rapid bacteriologic diagnosis of active TB in both adults and children. This study is the first study conducted among Filipino children with presumptive TB comparing NPA GeneXpert with GA GeneXpert and TB culture.

A total of 100 presumptive TB patients were enrolled and the majority of patients were clinically diagnosed PTB. In this study, presumptive TB patients commonly presented with nonspecific signs and symptoms of fever, chronic cough and inability to gain weight or weight loss. Patients who were clinically diagnosed PTB fulfilled at least 3 out of 6 criteria of clinical signs and symptoms of TB, CXR findings compatible with TB, TB exposure, positive TST and other laboratory tests consistent with TB. Various studies, including a descriptive local study conducted in 2002, support the use of history and clinical features to diagnose childhood TB.[6,13,14] The usefulness of the history of exposure and clinical signs and symptoms suggestive of TB were confirmed in this study. In children living in areas highly endemic for TB, it is important that physicians have a high index of suspicion for the disease. Thorough and comprehensive history and physical examination are necessary to make a correct diagnosis.

The bacteriologic confirmation of PTB in children is challenging due to difficulties in obtaining respiratory samples, particularly in children below 15 years of age. Mycobacterial culture remains to be the gold standard in diagnosing TB, but the procedure has low sensitivity in children. Only about 10 to 50% of tuberculosis cases are culture-proven owing to the paucibacillary nature of the disease and difficulty of specimen collection.[15,16] GeneXpert (Xpert MTB/RIF) may be used rather than conventional microscopy and culture as the initial diagnostic test in all children suspected of having TB.[10] In South Africa, sputum smear microscopy has been replaced with GeneXpert as the initial diagnostic test for tuberculosis.[17] In the Philippines, GeneXpert is currently recommended as the primary diagnostic tool for TB detection in presumptive drug susceptible tuberculosis in children below 15 years old.[18] GeneXpert is useful because of the rapid and early detection of causative mycobacteria in various clinical samples.[4] A systematic review and meta-analysis on the accuracy of GeneXpert to diagnose PTB in children using a variety of respiratory specimens showed that sensitivity varied from 55-90% for samples of expectorated sputum, 40-100% for induced sputum and 40-100% for gastric lavage or aspirate.[10] The reported pooled sensitivity of GeneXpert compared to culture was 66% (95% CI 52-77%) for expectorated or induced sputum and 66% (95% CI 51-81%) for gastric lavage or aspirate. High specificities were reported for all studies using various specimen types, ranging from 93-100%.[10]

Both gastric aspiration and nasopharyngeal aspiration can be performed in children who cannot expectorate sputum, such as infants and young children, patients who are intubated and on mechanical ventilatory support, patients with tracheostomy, and patients with anatomical or structural problems. Compared to gastric aspiration, nasopharyngeal aspiration is less invasive, causes minimal discomfort, and does not require placing patients on 4-6 hours of fasting prior to the procedure. Furthermore, nasopharyngeal aspiration can easily be performed in the outpatient clinic setting, thereby obviating the need for hospitalization and lowering not only healthcare cost but also the risk of acquiring nosocomial infection. This was based on the observation in the institutions where the set up during the study period was admitting patients for gastric aspiration and if unsuccessful the patients need to stay in the hospital for a longer period putting them at risk of acquiring nosocomial infection. Some patients in this study verbalized better tolerance of the NP aspiration.

NPA has been shown to be a useful specimen for the diagnosis of TB in children using GeneXpert. The diagnostic accuracy of the NPA GeneXpert is variable depending on the specimen used as the reference standard. This study showed that NPA GeneXpert had a sensitivity of 70% and specificity of 97% when compared to GA GeneXpert as initial screening test but had a lower sensitivity of 40% and specificity of 91.58% compared to GA culture as the reference standard.

This is the first known study where GeneXpert assay was performed on NPA specimens and compared this with both GeneXpert and TB culture performed on GA specimens. The high specificity found in this study indicates that a positive result on NPA GeneXpert can be used to diagnose PTB; however, the low sensitivity implies that a negative test cannot rule out PTB in

children. In cases of suspected PTB where the GeneXpert is negative, the decision to start anti-TB treatment should still be based on epidemiologic data correlated with findings on clinical history and physical examination.

The lower sensitivity of the GeneXpert compared to TB culture has been reported in several studies.[7,19,20] One possible explanation is the paucibacillary nature of the disease in children.[16,21] When TB culture is used as the reference standard, there is an increased probability that true positive Gene Xpert results will be reported as false positive results, thus underestimating the sensitivity of the GeneXpert test. In this study, 8 patients were positive on NPA GeneXpert but negative on GA culture. These 8 cases were probably true cases of TB since none of the patients received anti-TB treatment in the past, all had clinical manifestations and had chest radiologic findings suggestive of active PTB. Five out of 8 had exposure to a known case of TB. In a study though, four patients with a history of successfully treated TB who presented with lower respiratory tract infections, positive Xpert results, negative liquid TB cultures and clinical improvement without anti- tuberculosis treatment were reported suggesting that the Xpert result was falsely positive.[22]

Other studies have recognized the limitation of TB culture in children and have recommended increasing the number of specimens in order to increase the bacteriologic yield from culture. Zarin found that using 2 specimens of either induced sputum or NPA for TB culture increased positive TB culture results.[7] Nicol et al. reported that using 2 induced sputum samples increased the yield of positive TB cultures in children with smear-negative results.[23] Although studies have shown that processing multiple specimens increases the sensitivity of the GeneXpert assay, the benefit of testing more than 2 specimens has not been shown to consistently increase the diagnostic yield.[24] Due to the additional resources needed for testing multiple specimens with no clear advantages, WHO supports the use of a single specimen for diagnostic testing in resource-limited areas.[10] The use of multiple specimens for GeneXpert and TB culture may be ideal and could increase the diagnostic yield but in resource-limited areas, the decision on the number of specimens to test needs to be guided by the available resources, including considerations for increased transportation costs for the patient.

One limitation of the GeneXpert test for TB is that it cannot be used to detect resistance to all the anti-TB drugs regardless of specimen collected. Hence, it cannot replace TB culture with conventional anti-TB drug susceptibility testing as the gold standard for confirming bacteriologic diagnosis, particularly if data on drug susceptibility is required. However, use of the GeneXpert using NPA may be a more convenient and a more rapid test to diagnose TB in the pediatric population where gastric aspiration is not possible. Another limitation of the GeneXpert may be its variable accuracy in patients with immunosuppression. A meta-analysis to determine the accuracy of GeneXpert in testing samples of expectorated or induced sputum reported sensitivity ranging from 20% to 100% among HIV-positive children and 33% to 100% among HIV-negative children.[5] The wide and overlapping intervals in the results were attributed to the effect of smear positivity status in children regardless of their HIV status. This study was conducted in a low HIV-prevalence country and none of the patients showed clinical manifestations of immunosuppression. Hence, the performance of NPA GeneXpert in the immunosuppressed population cannot be evaluated. In addition, there was only 1 neonate in the study population; this subject was negative for GeneXpert, so it is likewise not possible to draw any conclusions for this age group.

CONCLUSION AND RECOMMENDATIONS

NPA GeneXpert is a highly specific, rapid and early diagnostic test for PTB in children. Compared to GA GeneXpert, NPA GeneXpert had a sensitivity of 70%, specificity of 96.7%, negative predictive value of 96.67% and positive predictive value of 70% while compared to GA TB culture, NPA GeneXpert had a sensitivity of 40%, specificity of 91.58%, positive predictive value of 20%, and negative predictive value of 96.67%. Due to the simplicity and convenience of collection of NPA and more rapid release of results when GeneXpert test is used, a single NPA GeneXpert can be used as an initial screening test to diagnose PTB in pediatric patients. However, a negative GeneXpert test does not exclude a diagnosis of PTB in children. These findings will need to be verified in larger and varied populations including neonates, immuno-compromised patients, and people living with HIV. In order to document the feasibility and acceptability of this test when conducted in a public health setting, NPA GeneXpert test should also be studied in various community and primary care settings.

ACKNOWLEDGEMENTS

Acknowledgement is given to Mary Ann Chiong, MD for her technical review of the protocol and to Salvacion Gatchalian, MD for her support and help to make this research possible.

REFERENCES

1. World Health Organization. Global tuberculosis report 2016 [Internet.] Geneva: World Health Organization; 2016. [Accessed 9th September 2016]. Available from: https://apps.who.int/iris/bitstream/handle/10665/250441/9789241565394-eng.pdf
2. Department of Health (PH). Field Health Service Information System [Internet]. Philippines: Department of Health; 2016. [Accessed 9th September 2016]. Available fromhttp://ro6.doh.gov.ph/index.php/health-statistics/field-health-service- information-system/fhsis-report-cy-2016
3. World Health Organization. Global tuberculosis control: WHO report 2014 [Internet]. Geneva: World Health Organization; 2014. [Accessed 9th September 2016] Available from: https://apps.who.int/iris/bitstream/handle/10665
4. Wilson ML. Recent advances in the laboratory detection of Mycobacterium tuberculosis complex and drug resistance. Clinical Infectious Diseases 2011;52(11):1350–1355.
5. Zar HJ, Workman L, Isaacs W, et al. Rapid molecular diagnosis of pulmonary tuberculosis in children using nasopharyngeal specimens. Clin Infect Dis 2012;55:1088–1095.
6. Department of Health (PH). National Tuberculosis Control Program – Manual of Procedures. Philippines: Department of Health; 2014. [Accessed 9th September 2016]. Available from: http://www.ntp.doh.gov.ph/downloads/publications/guidelines/NTP_MOP_6th _Edition.pdf
7. Zar HJ, Workman L, Isaacs W, Dheda K, Zemanay W, Nicol MP. Rapid diagnosis of pulmonary tuberculosis in African children in a primary care setting by use of Xpert MTB/RIF on respiratory specimens: a prospective study. Lancet Glob Health. 2013 Aug;1(2):e97-104.
8. Chiappini E, Vecchio AL, Garazzino S, et al. Recommendations for the diagnosis of pediatric tuberculosis. Eur J Clin Microbiol Infect Dis. 2016 Jan;35(1):1-18.
9. Buderer NM. Statistical methodology: I. 1996. Incorporating the prevalence of disease into the sample size calculation for sensitivity and specificity. Academic Emergency Medicine [Internet]; 3(9). [cited 9th September 2016]. Available from http://www.ncbi.nlm.nih.gov/pubmed/8870764
10. World Health Organization. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system for the diagnosis of pulmonary and extrapulmonary TB in adults and children. Policy Update [Internet]. Geneva: World Health Organization; 2013. [Accessed 9th September 2016] Available from: https://pubmed.ncbi.nlm.nih.gov/25473701/

11. Perez-Velez CM. Standard operating procedure nasogastric aspiration for the bacteriological confirmation of pulmonary tuberculosis in children [Internet]. Place unknown: Sentinel Project; 2014. [Accessed 9th September 2016]. Available fromhttp://sentinel-project.org/wpcontent/uploads/2014/03/SOP-Gastric_Aspiration_Sentinel_Project.pdf
12. Vianzon R, et al. The tuberculosis profile of the Philippines, 2003–2011: advancing DOTS and beyond. Western Pacific Surveillance and Response Journal. 2013; 4(2)
13. Pama CL, Gatchalian S. Clinical profile of culture-proven tuberculosis cases among Filipino children aged 3 months to 18 years. Pediatric Infectious Disease Society of the Philippines Journal. 2002; 6(1):13-23
14. How, C. Tuberculosis in Infancy and Childhood. Philippine Pediatric Society. 4th Edition. Philippines. 2016. p. 67-80
15. Starke JR, Taylor-Watts KT. Tuberculosis in the pediatric population of Houston, Texas. Pediatrics. 1989: Jul 84(1):28-35.
16. Perez-Velez CM, Marais BJ. Tuberculosis in children. N Engl J Med 2012; 367:348-361
17. Churchyard GJ, Stevens WS, Mametja LD, et al. Xpert MTB/RIF versus sputum microscopy as the initial diagnostic test for tuberculosis: a cluster and randomized trial embedded in South African roll-out of Xpert MTB/RIF. Lancet Glob Health August 2016; 3:e450–57.
18. Department of Health (PH). Implementation of Xpert MTB/RIF test as the primary diagnostic tool for presumptive drug-resistant tuberculosis (DR-TB) and presumptive drug susceptible tuberculosis (DSTB) among selected vulnerable populations. Department Memorandum No. 2016-0825. Philippines: Department of Health; 2016. [Accessed 9th September 2016]. Available from: http://www.ntp.doh.gov.ph/downloads/issuances/dmpdf/dm2016-0285.pdf
19. Zar HJ, Workman L, Isaacs W, et al. Rapid molecular diagnosis of pulmonary tuberculosis in children using nasopharyngeal specimens. Clin Infect Dis 2012; 55:1088–1095.
20. 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)
21. Marais BJ, Schaaf HS, Donald PR. Pediatric TB: issues related to current and future treatment options. Future Microbiol. 2009; 4(6):661-675
22. Boyles TH, Hughes J, Cox V, Burton R, et al. False-positive Xpert MTB/RIF assays in previously treated patients: need for caution in interpreting results. Int J Tuberc Lung Dis 18(7):876–878
23. Nicol MP, Workman L, Isaacs W, 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.
24. Owens S, Abdel-Rahman IE, Balyejusa S, et al. Nasopharyngeal aspiration for diagnosis of pulmonary tuberculosis. Arch Dis Child.2007;92(8):693-6