The Yield of Acid-Fast Bacilli (AFB) and Tuberculosis (TB) Culture in the Microbiologic Diagnosis of Childhood Tuberculosis Using Sputum Induction: A Randomized Controlled Trial with Interrupted Time Series

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ABSTRACT

Objectives. This study aims to determine the diagnostic yield and safety of sputum induction with hypertonic saline in the microbiologic confirmation of childhood tuberculosis (TB) in a tertiary hospital in the Philippines.

Methods. This is a randomized controlled trial with an interrupted time series in the control group. One hundred twelve (112) pediatric patients (4-18 years old) with clinical findings suggestive of TB were enrolled in the study. Patients were randomized into two groups composed of 56 patients each. Group A patients underwent sputum induction. Group B patients underwent spontaneous expectoration followed by sputum induction. The microbiologic yield for acid-fast bacilli and TB culture were determined and analyzed.

Results. Among the patients randomized to Group A, microbiologic confirmation for TB was 8/56 patients (14.3%) after sputum induction. For patients randomized to Group B, microbiologic yield was 4/56 patients (7.1%) from spontaneous expectoration; after sputum induction, the microbiologic yield increased to 5/56 patients (8.9%). There is insufficient evidence of statistical significance in microbiologic yield on parallel analysis of the two separate groups (p=0.22). Furthermore, for patients randomized to Group B, the increase in microbiologic yield after sputum induction compared to spontaneous expectoration did not reach statistical significance (p=1.000). The procedure was well-tolerated among children; no serious adverse events were observed.

Conclusion. Sputum induction is a feasible and safe method of specimen collection for microbiologic diagnosis of TB among children. While the microbiologic yield increased after sputum induction compared to spontaneous expectoration, the additional yield does not seem to be significant.

Key Words: tuberculosis, children, sputum induction

INTRODUCTION

Tuberculosis (TB) remains to be a major threat to children in developing countries. The Philippines ranked ninth worldwide for having the most number of TB cases in 2008.[1] Clinical parameters and radiologic evidence can serve as the basis for the diagnosis of TB. However, microbiologic confirmation through acid-fast bacilli (AFB) smear and TB culture is important to facilitate drug sensitivity and resistance testing.[2-3]

Due to the paucibacillary nature of TB and because of the tendency of children to swallow sputum and their inability to expectorate it, there is difficulty in obtaining appropriate specimens for microbiologic testing for TB.[4] Furthermore,

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the diagnosis of drug-resistant TB cannot accurately be made on clinical basis alone and an adequate specimen for culture or molecular testing is warranted.[5-6]

An effective and safe method of specimen collection for the microbiologic diagnosis of TB among pediatric patients should be explored. Several studies in developing countries have reported that sputum induction using hypertonic saline has enabled microbiologic diagnosis of childhood TB.[2,5,7] Presently, there are no studies on the microbiologic yield of sputum induction for TB diagnosis among Filipino children.

This study aimed to compare the diagnostic yield and safety of sputum induction using hypertonic saline versus spontaneous expectoration (or no sputum induction) for microbiologic confirmation for pulmonary TB among children (aged 4 to 18 years) with clinical features of pulmonary and extrapulmonary TB in the University of the Philippines - Philippine General Hospital (UP-PGH).

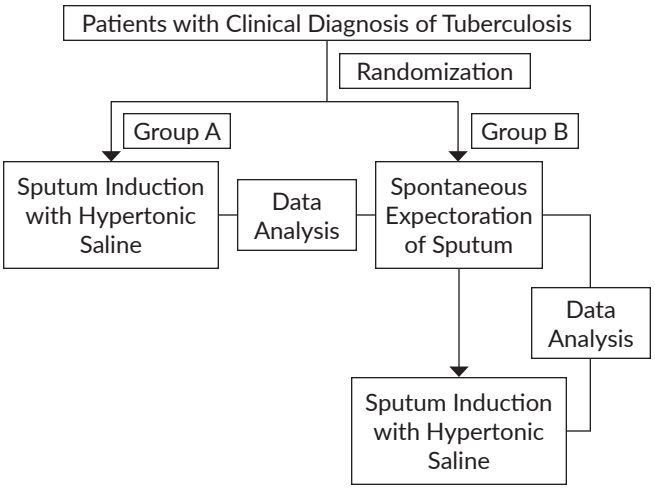

Figure 1. Flowchart of Methodology for Randomization of Patients.

METHODOLOGY

Study Design

This was a randomized trial with an interrupted series in the control group.

Setting

This prospective study involved pediatric patients from the outpatient and inpatient service areas (pediatric wards and pediatric emergency room) of the Department of Pediatrics, UP-PGH. The study was conducted from September 2015 to May 2016.

Participants

One-hundred twelve (112) pediatric patients with ages 4 to 18 years old and with clinical and/or radiologic features of pulmonary or extrapulmonary TB were included in this study. Similarly, pediatric patients with possible drug resistant TB were included. We excluded patients with lack of consent for procedure, patients previously started on treatment with anti-TB medications for 2 weeks or more, and patients with severe or uncontrolled bronchial asthma or a recent asthma exacerbation within the past month.

Procedure and Outcomes

Clinical summaries including history and physical examination findings of study participants were prepared and recorded in a separate data collection form. Anthropometric measurements were obtained and weight and height measurements were plotted on appropriate WHO z-scores growth charts. Patient background and demographic data including hospital case number, name, age, location (inpatient/outpatient), and home address and contact number were collected and kept confidential in a separate database.

The following data were evaluated and recorded: (1) signs and symptoms suggestive of TB, (2) history of exposure to close contact with active TB disease, (3) tuberculin test result, if any (reactive/nonreactive), (4) chest radiograph findings, (5) results of prior histological, cytological, biochemical, immunologic, and/or molecular tests for TB, if available.

All enrolled patients with clinical and/or radiologic diagnosis of TB were randomized into two groups: Group A - patients who underwent sputum induction with hypertonic saline; and Group B – patients who underwent spontaneous expectoration, and after 3 days, underwent sputum induction with hypertonic saline. This is to facilitate randomized analysis and interrupted time series analysis.

To ensure randomization, a study research assistant performed randomization and allocation was concealed using opaque and sealed envelopes that were drawn consecutively and given to the subjects' parents or guardian with corresponding assigned groups in letters A or B.

After obtaining informed consent, the procedure was performed in the Pediatric Pulmonary Function Laboratory at PGH. The room was kept well ventilated with an exhaust fan. Strict respiratory airborne protection measures were observed and appropriate personal protective devices (N95 face masks) were used by the investigator and a trained nurse during the sputum induction. Disposable gloves were used in handling of specimens. All nebulizing kits were discarded after each patient use.

Sputum induction using a standardized protocol developed by Zar[8] et al. was employed. Enrolled patients were placed on nothing per orem (NPO) 2 to 3 hours prior to sputum induction. To prevent bronchoconstriction, children were nebulized with one nebule of salbutamol combined with 2 mL of plain normal saline solution and delivered using a compression nebulizer. This was then followed by nebulization of five mL of hypertonic saline for 15 minutes. After every 5 minutes of nebulization with hypertonic saline, children were asked to expectorate sputum with a volume of 2 mL into a sterile specimen bottle. The sputum

specimen was inspected to ascertain that it is not a salivary specimen (the latter appears thin and watery). Gentle chest physiotherapy by chest tapping was done to encourage sputum expectoration.

Pulse oximetry monitoring was done during and after the procedure. The child was observed by the primary investigator or trained nurse for 30 minutes. Any untoward reactions such as vomiting, abdominal pain, dyspnea, or other symptoms were recorded. A pulse oximetry monitor and an oxygen source were made readily available.

Three sputum samples were obtained on three consecutive days. Three specimens were sent for sputum AFB, and the first specimen was also examined for sputum TB culture. However, if it was not possible for the child to return the next day, three samples were taken on the same day, with minimum interval of 4 hours between specimen collections.

For patients randomized to Group B, the patients were first asked to spontaneously expectorate sputum specimen which were placed into a sterile specimen bottle. Spontaneous sputum samples were collected for three consecutive days and submitted for AFB smear tests. The first specimen was also tested for sputum TB culture. The patients were subsequently asked to return after three days for sputum induction with hypertonic saline using the protocol described above.

Collected specimens were submitted to the PGH Central Laboratory and the Lung Center of the Philippines for processing for AFB smear and TB culture and sensitivity testing. The sputum samples were stained with Ziehl Neelsen stain and examined for AFB. A portion of the first sample sent for sputum AFB was also examined on BACTEC 460 TB commercial liquid culture system for *Mycobacterium tuberculosis* culture. For potential drug-resistant TB cases, specimens were sent for sputum GeneXpert testing in the laboratory of the Philippine Tuberculosis Society, Inc. in Tayuman, Manila. Results of the AFB smear and culture and sensitivity were recorded and patients with microbiologically-confirmed TB as well as their attending physicians were notified of results.

Definitions

A clinical diagnosis of TB was based on the presence ≥ 3 of the following: (1) exposure to an adult or adolescent with TB, (2) symptoms suggestive of TB, (3) positive tuberculin test, (4) chest radiograph suggestive of TB, or (5) other laboratory findings suggestive of TB (histological, cytological, biochemical, immunologic, and/or molecular).[9]

Clinical forms of TB were classified as pulmonary or extrapulmonary. Pulmonary TB included the following forms: latent TB infection (positive tuberculin skin test, no symptoms, or normal chest x-ray), primary TB, progressive primary TB, pleurisy with effusion, endobronchial TB, or miliary TB. On the other hand, extrapulmonary TB included the following: TB of cervical lymph nodes (scrofula), central nervous system TB (meningitis, tuberculoma, abscess), skeletal TB (bones, joints, spine), gastrointestinal TB (TB enteritis, peritonitis, hepatobiliary, pancreas), cutaneous TB (scrofuloderma, erythema nodosum), ocular TB, genitourinary TB (renal, genital TB), and TB of the middle ear.[9] Drug-resistant TB was considered if there was lack of clinical improvement despite 2 months of antimicrobial treatment for TB.[9]

Microbiologic confirmation was defined as at least one positive sputum AFB smear and/or culture with growth of *Mycobacterium tuberculosis*.

Statistical Analysis

The z-score test for proportions in 2 populations was employed to analyze difference in microbiologic yield among patients randomized to spontaneous expectoration compared to another group of patients who underwent sputum induction. Fisher Exact Test was used in the analysis of the increase in microbiologic yield in the group of patients who underwent spontaneous expectoration followed by sputum induction. To determine the significant factors associated with positive TB culture, binary logistic regression analysis was used. Null hypothesis was rejected at 0.05α-level of significance. STATA 12.0 was used for data analysis.

Ethical Considerations

This study was reviewed and approved for implementation by the University of the Philippines Manila Research Ethics Board (UPMREB) PGH Review Panel [UPMREB Code: (PED) 2015-332-01]. Written consent from the parent or guardian as well as assent for participants ≥ 7 years old were obtained. All personal patient information was kept anonymous and confidential.

RESULTS

Demographic Characteristics

A total of one hundred twelve (112) children with clinical features of TB were randomized to either Group A (sputum induction) or Group B (spontaneous expectoration followed by sputum induction). Fifty-six (56) patients were included each for Groups A and B. Eighty percent (80%) of the patients included in our study belonged to adolescent age group (11-18 years old); the median age of the patients was 14 years old. Most of the patients were males (57%). While most of the patients had normal WHO z-scores for weight and height, 40% of patients were wasted or severely wasted and 25% were stunted or severely stunted. There was no statistical difference in the characteristics of both groups ($p<0.05$).

Clinical presentation of children with TB Disease

Pulmonary TB (71%) was the most common form of TB among children included in our study. The presence of cough for more than 2 weeks (79%), fever of more than 2 weeks (71%), and failure to gain weight (63%) were the

Table 1. Demographic and clinical profile of the patients

	Group A (n=56) Frequency (%)	Group B (n=56) Frequency (%)	p-value
Age			0.957
4 to 6 years old	5 (8.93)	5 (8.93)	
7 to 10 years old	7 (12.5)	6 (10.71)	
11 to 18 years old	44 (78.57)	45 (80.36)	
Sex			0.252
Male	29 (51.79)	35 (62.50)	
Female	27 (48.21)	21 (37.50)	
Weight z-scores			0.386
Normal	38 (67.86)	31 (55.36)	
Wasted	10 (17.86)	13 (23.21)	
Severely wasted	8 (14.29)	12 (21.43)	
Height z-scores			0.629
Normal	43 (76.79)	40 (71.43)	
Stunted	9 (16.07)	9 (16.07)	
Severely stunted	4 (7.14)	7 (12.50)	

Statistical test used: Chi-square test

most frequently reported symptoms. Sixty-five percent (65%) of patients had a history of close contact or exposure to an adult or adolescent with pulmonary TB. More than half of the patients had a reactive tuberculin skin test. Most of the patients (37%) had non-specific or normal chest x-ray findings; among those with findings suggestive of TB, the presence of the pleural effusion and pulmonary infiltrates were the most commonly reported radiographic abnormalities (Table 2).

Mycobacterial yield in patients with Presumptive TB

Yield of AFB and TB Culture from Induced Sputum (IS) and Spontaneous Expectoration (SE) in separate groups of children

In the IS group, of the 8 patients with microbiologic yield, 6 patients had positive growth on culture; 5 patients had positive AFB, none in the first specimen, 1 in the second specimen, and 4 in the third specimen. No patient had positive AFB smear in all three specimens from the IS group. Over-all, eight patients (14.2%) had microbiologic yield from IS and 4 (7.14%) patients from SE in separate groups of children (Table 3). Using the z-score test for proportions, there was insufficient evidence of statistically significant difference in yield on parallel analysis of the two separate groups of children (p=0.22).

Difference in Yield of AFB and TB Culture in the same group of patients who underwent both Spontaneous Expectoration (SE) and Induced Sputum (IS)

Follow-up with IS after SE increased the microbiologic yield from 4 patients (7.14%) to 5 patients (8.9%) (Figure 2). The additional patient had a positive AFB and a positive culture in the first sample. However, using the Fisher Exact test, the increase in microbiologic yield after IS compared to SE in the same group of patients did not reach statistical significance (p=1.00).

Table 2. Clinical and Radiologic Characteristics of Children with TB

	Group A (n=56) Frequency (%)	Group B (n=56) Frequency (%)	Total (n=112) Frequency (%)
Diagnosis			
Pulmonary tuberculosis	40 (71)	39 (70)	79 (71)
Extrapulmonary tuberculosis	16 (29)	17 (30)	33 (29)
Signs and Symptoms			
History of cough >14 days	45 (80)	44 (79)	89 (79)
Fever >14 days	41 (82)	38 (69)	79 (71)
Weight loss/failure to gain weight	32 (70)	38 (68)	70 (63)
Painless cervical lymphadenopathy	7 (13)	4 (7)	11 (10)
Failure to make a quick return to normal after an infection	3 (5)	1 (2)	4 (4)
Failure to respond to antimicrobial therapy	2 (4)	–	2 (2)
History of TB Exposure			
Positive	41 (73)	32 (57)	73 (65)
Negative	15 (27)	24 (43)	39 (35)
Tuberculin Skin Test			
Reactive	26 (46)	31 (55)	57 (51)
Non-reactive	15 (27)	12 (21)	27 (24)
Not done	15 (27)	13 (23)	28 (25)
Chest x-ray Finding			
Pleural effusion	11 (20)	17 (30)	28 (25)
Pulmonary infiltrate	18 (32)	8 (14)	26 (23)
Pulmonary mass	2 (4)	1 (2)	3 (3)
Lymphadenopathy	5 (9)	3 (5)	8 (7)
Normal/Non-specific findings	19 (34)	22 (39)	41 (37)

Table 3. Microbiologic yield of Induced Sputum and Spontaneous Expectoration in separate groups of children

	Induced Sputum (n=56)	Spontaneous Expectoration (n=56)
AFB smear (three separate specimens taken)		
Positive in the first specimen	0	2
Positive in the second specimen	1	0
Positive in the third specimen	4	0
Positive TB culture (taken in the first specimen only)	6	2
Total positive microbiologic yield	8	4

Table 4. Microbiologic yield of Induced Sputum and Spontaneous Expectoration in the same group of children

	Spontaneous Expectoration (n=56)	Induced Sputum (n=56)
AFB smear (three separate specimens taken)		
Positive in the first specimen	2	3
Positive in the second specimen	0	0
Positive in the third specimen	0	0
Positive TB culture (taken in the first specimen only)	2	5
Total positive microbiologic yield	4	5

Sensitivity and resistance patterns of culture-confirmed TB cases

Table 5 summarizes the clinical presentation and drug sensitivity/resistance patterns of culture-confirmed TB cases from specimens obtained using sputum induction. Eleven patients had positive culture from sputum induction; 6 had growth of *Mycobacterium tuberculosis* while there were 5 isolates of non-tuberculous Mycobacteria (NTM).

Two cases, both adolescents with pulmonary TB, had positive *Mycobacterium tuberculosis* culture with documented multiple drug resistance to first line anti-TB medications. The first patient is an 18 year old boy with cough, fever, poor weight gain, and tuberculous pleural effusion, while the second case is that of a 14 year old girl with cough, fever, and weight loss and chest x-ray of pulmonary infiltrates suggestive of TB.

Variables associated with positive TB culture

Among the clinical characteristics of children with TB in our study, no variables were noted to be statistically significantly associated with positive TB culture. However, the presence of cough was the noted to be significantly associated with a negative TB culture result (Odds Ratio 0.11, 95% CI 0.02 to 0.67) (Table 6).

Adverse Events

Sputum induction with hypertonic saline was well tolerated among participants in this study with no major or life-threatening adverse events noted. One patient had transient desaturation (peripheral oxygen saturation of 90%) while undergoing nebulization with hypertonic saline, however this was not associated with respiratory distress or cyanosis and resolved spontaneously after nebulization. One patient experienced nausea and abdominal pain after nebulization with hypertonic saline. No other adverse events were observed.

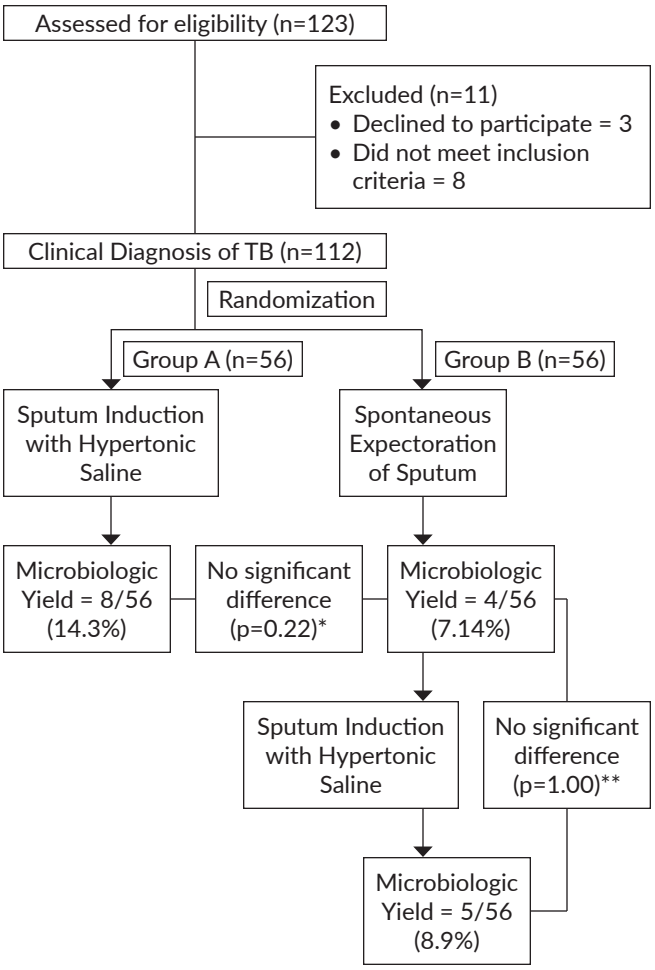

Figure 2. Flow diagram of patients and microbiologic yield of sputum induction and spontaneous expectoration followed by sputum induction groups.

* *Statistical test: Z-score test (p<0.05),*
** *Statistical test: Fisher Exact Test (p<0.05).*

DISCUSSION

Summary of Main Results

Our study is the first randomized controlled trial in the Philippines that determined the microbiologic yield of AFB and TB culture for children with presumptive TB using sputum induction (IS) using hypertonic saline or spontaneous expectoration (SE) in separate groups of children. The microbiologic yield in separate groups of patients was

Table 5. Profile and drug sensitivity patterns of culture-confirmed TB from induced specimens

Patient Code	Culture Isolate	Age/Sex	Symptoms	Exposure	TST[1]	Radiologic Findings	Sensitivity	Resistance
A-1	MTB[2]	16/M	cough, fever, poor weight gain	(+)	reactive	pleural effusion	R[4]	H[6]E[7]S[8]
A-9	NTM[3]	15/M	poor weight gain	(+)	non-reactive	pulmonary infiltrates	Z[5]	HRES
A-10	MTB	15/M	fever, weight loss	(+)	not done	pulmonary infiltrates	HRZES	-
A-11	MTB	15/F	cough, fever, weight loss	(+)	not done	pulmonary infiltrates	HRZES	-
A-39	NTM	14/F	fever, weight loss	(+)	reactive	pulmonary infiltrates	HRZES	-
A-55	NTM	5/M	cough, fever, weight loss	(+)	non-reactive	pulmonary infiltrates		HRZES
B-1	NTM	17/M	fever, poor weight gain	(-)	not done	pleural effusion	HRZES	-
B-6	MTB	13/M	weight loss	(-)	non-reactive	pulmonary infiltrates	HRZES	-
B-9	MTB	14/F	cough, fever, weight loss	(+)	reactive	pulmonary infiltrates	-	HRZES
B-26	MTB	16/F	cough, fever, poor weight gain	(+)	not done	pleural effusion	HRZES	-
B-55	NTM	12/F	cough, fever, weight loss	(+)	reactive	pleural effusion	HRZES	

[1]TST = Tuberculin Skin Test; [2]MTB = Mycobacterium tuberculosis; [3]NTM = Non-Tuberculous Mycobacteria; [4]R = Rifampicin; [5]Z = Pyrazinamide; [6]H = Isoniazid; [7]E = Ethambutol; [8]S = Streptomycin

Table 6. Clinical variables associated with positive TB culture from induced specimens

	Culture positive (n=11) Frequency (%)	Culture negative (n=101) Frequency (%)	Odds Ratio (95% CI)	p-value
Age				
4 to 6 years old	1 (9)	9 (9)	(reference)	-
7 to 10 years old	0	13 (13)	-	-
11 to 18 years old	101 (90)	78 (77)	1.21 (0.10 to 14.68)	0.882
Female	4 (36)	44 (44)	0.50 (0.11 to 2.37)	0.386
Pulmonary TB	9 (81)	72 (71)	1.57 (0.24 to 10.36)	0.639
Wasted and severely wasted	4 (36)	39 (39)	0.38 (0.07 to 2.04)	0.256
Stunted and severely stunted	6 (55)	23 (23)	3.74 (0.74 to 18.99)	0.112
Signs and symptoms				
Cough	7 (63)	82 (82)	0.11 (0.02 to 0.67)	0.016
Fever	10 (75)	70 (69)	4.44 (0.02 to 0.67)	0.208
Weight loss	9 (81)	61 (60)	1.35 (0.23 to 7.79)	0.739
Poor weight gain	3 (27)	20 (20)	1.71 (0.28 to 0.46)	0.561
Exposure	10 (91)	63 (63)	4.23 (0.57 to 1.12)	0.157
Skin test	5 (45)	3 (2.97)	1.17 (0.39 to 3.54)	0.780
Positive Chest X-ray result	11 (100)	94 (93)	0.51 (0.03 to 7.74)	0.626

p-value = 0.3231; R^2 = 21.13%

8/56 (14.3%) and 4/56 (7.1%) from IS and SE, respectively; there is insufficient evidence of statistically significant difference in yield on parallel analysis of the separate groups (p=0.22). On the other hand, in the same group of patients, follow-up with IS after SE increased the microbiologic yield from 4 patients (7.14%) to 5 patients (8.9%), but this, too, did not reach statistical significance (p=1.00). With minimal observed side effects, sputum induction with hypertonic saline is a feasible and safe method of specimen collection for microbiologic diagnosis of TB among children.

Diagnostic Utility of Sputum Induction

The use of sputum induction utilizing hypertonic saline is a simple and inexpensive method of collecting respiratory specimens.[10] The mechanism is unknown but hypothesized to be an increased osmolarity of the airway lining fluid, which subsequently increases vascular permeability in the bronchial mucosa.[11] Sputum induction has enabled isolation of causative organisms for pneumonia,[12-13] measurement of airway hyperresponsiveness,[14] and TB diagnosis in young children.[15]

In our setting, however, current practice guidelines do not specifically list sputum induction as a method of specimen collection for TB diagnosis.[16] The basis for TB diagnosis often relies on clinical parameters, which may be nonspecific.[5] This strengthens the need for a practical and safe method of obtaining sputum specimens for the microbiologic diagnosis of TB in children.

Comparison of Study Results with Other Studies

The microbiologic yield of our study is comparable to those reported in other studies on the use of sputum induction for childhood TB diagnosis, which ranges from 6-47%.[7,17-20] History of excessive sweating and painless cervical lymphadenopathy were significantly associated with positive TB culture from induced specimens in the study by Iriso.[19] Among the variables analyzed in our study, the presence of cough was significantly associated with a negative TB culture. This may be attributed to cough being not a prominent symptom of TB among children, or because of co-existing conditions which also present with this respiratory complaint.

Advantages of Sputum Induction

In our study, we were able to identify drug sensitivity and resistance patterns in 11 cases; two children had drug-resistant TB. Moreover, it is worthwhile to note that TB culture results from our study yielded five isolates of non-tuberculous mycobacteria (NTM) from sputum induction. A study by Seong, et al. also reported yields of NTM isolates from induced sputum specimens.[20] These findings further highlight the need for microbiologic confirmation for isolate speciation, drug sensitivity testing, and identification of drug-resistant cases.

Previous reports recorded no statistically significant difference in yield based on the number of days sputum induction was performed.[21] Sputum induction enabled one-day testing among several patients in our study who were unable to come back on subsequent days for the procedure. We observed that sputum induction is a feasible method of specimen collection among children across different age groups and in various clinical settings.

Procedural Risks and Adverse Events of Sputum Induction

Necessary precautions to ensure the safety of study participants during sputum induction were observed in our study. As bronchoconstriction may be a concern, the patients were nebulized with salbutamol prior to nebulization with hypertonic saline. As in other studies[8,21], our patients were monitored for potential adverse events. While few side effects were reported[12,20] in our study, there were no serious adverse events. With the recent COVID-19 pandemic, airborne precautions should be strictly observed during sputum induction to reduce aerosolization risks.[22]

Limitations of the Study

The results of our study reveal that the use of sputum induction with hypertonic saline did not show a significant increase in the microbiologic yield compared to spontaneous expectoration. Previous studies also did not demonstrate significant differences in yield of sputum induction versus self-expectoration[20] and sputum induction versus gastric lavage.[23] In our study, possible contributory factors for the lack of significant difference include the study design, the sample size of patients, and the relatively lower microbiologic yield in the group who underwent spontaneous expectoration first followed by sputum induction. The study design may be further refined to include a cross-over arm and sequential randomization of patients. Furthermore, due to resource limitations, the methods involved testing only the first specimen for TB culture. Increasing the number of specimens sent for TB culture may also affect diagnostic yield.

Recommendations for Further Research

The use of sputum induction for microbiologic diagnosis of childhood TB in various health care settings among high-TB burden countries has been explored in other studies.[5,8,16] The feasibility of the use of sputum induction in the diagnosis of childhood TB in primary care or multi-center settings in the Philippines may be recommended for further research.

CONCLUSION

Sputum induction with hypertonic saline is a feasible and safe method for microbiologic diagnosis of childhood TB. This procedure enabled identification of MTB and drug sensitivity and resistance patterns among children, with no serious adverse events observed. While the microbiologic yield increased after sputum induction compared to spontaneous expectoration, the additional yield obtained after sputum induction did not seem to be significant.

Statement of Authorship

The final paper has been approved for submission by both authors.

Author Disclosure

Both authors declared no conflicts of interest.

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APPENDIX

Sample Size Computation

The sample size was calculated by a statistician as follows:

$$n = (Z_{\alpha/2}+Z_{\beta})^2 \times \frac{[p_1(1-p_1)+p_2(1-p_2)]}{(p_1-p_2)^2}$$

Where:
$Z_{\alpha/2}$ is the critical value of the normal distribution at $\alpha/2 = 1.96$ at $\alpha = 0.05$
Z_{β} is the critical value of the normal distribution at $\beta = 0.84$ at $\beta = 0.2$ or 80% power
p_1 = % positive AFB yield with gastric lavage = 0.65*
$q_1 = 1 - p_1 = 0.35$
p_2 = % positive AFB yield with sputum induction = 0.87*
$q_2 = 1 - p_2 = 0.13$

**based on results of the study of Zar et al.[15]*

Calculation

$$n = (1.96 + 0.84)^2 \times \frac{[(0.65 \times 0.35) + (0.87 \times 0.13)]}{(0.65 - 0.87)^2}$$

$n = 56$

Thus, 56 patients were randomized to each group of patients, with a total of 112 patients.