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MEDICINE

Respiratory Specimens for the Diagnosis of Pediatric Pulmonary Tuberculosis: A Comparative Assessment

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Abstract

The objective was to assess the utility of induced sputum (IS) as an alternative or add-on to gastric lavage and aspirate (GLA) as the respiratory specimen to diagnose pulmonary tuberculosis (PTB) in children. After consent, fifty children aged 0–14 years with suspected PTB were enrolled. Sputum/induced sputum and gastric lavage from the participants were collected on two consecutive days. Bronchoalveolar lavage was also done if indicated. The microbiological isolation in these specimens by Smear, MGIT culture, or Xpert MTB/RIF assay was compared. Based on the clinical, radiological, and microbiological profile, 23/50 children were diagnosed with PTB. If GLA was used alone, the yield was 11 cases (47.8%). If sputum (expectorated or induced) was used alone, the yield was 12 cases (52.1%). The difference in the two yields was not statistically significant ($p = 1$). The combined yield of GA and sputa was 69.5%, which was significantly more than when either of the specimens was used alone. Sputum was induced in 11 children, who could not expectorate. GLA and IS were compared, as these are the typical specimens obtainable in children who cannot expectorate. The yield of GLA was 4/11(36.36%) in these samples, and it was 5/11 (45.45%) for IS. Combining GLA and IS increased the yield to 7/11(63.6%) which amounted to a 27.2% increase from using GLA alone. IS is a valuable tool to increase the microbiological yield for the diagnosis of PTB in young children who cannot expectorate and should be considered routinely.

Keywords Gastric lavage · Gastric aspirate · Induced sputum · Children

Introduction

The diagnosis of pulmonary tuberculosis (PTB) is challenging, especially in children, as they are unable to expectorate sputum. Failure of microbiological confirmation can decrease the confidence of the treating physician in the diagnosis as well as perpetuate the rampant misuse of anti-tubercular therapy (ATT). Empirical use of ATT has resulted in the epidemic of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB across the globe, especially in areas with a high TB prevalence. Screening the contacts, accurate diagnostic tests, and timely initiation of therapy are some strategies which are in focus to curb this epidemic. Diagnosing pulmonary tuberculosis (PTB) in children is challenging, as they are unable to expectorate sputum. Respiratory specimen acquisition requires the child to cough out the secretions from the lungs, and if the child is unable to do so, the swallowed respiratory secretions from the stomach are aspirated via a nasogastric tube. Various studies have analyzed these approaches to determine the best one, but the evidence is conflicting. Studies from South Africa have shown that induced sputum (IS) is a feasible, safe, and a more sensitive alternative to gastric lavage and aspirate (GLA) to isolate *Mycobacterium* tuberculosis in children. A study with 149 children with a median age of 9 months and with a suspected diagnosis of PTB was subjected to an early-morning sputum induction and GLA. The sputum induction was successful in 95% of these children. The microbiological yield of IS was 4.3% more than that of GLA [1]. In another study by the same authors, samples from IS and GLA were positive in 87% and 65% children, respectively ($p = 0.018$) [2]. Induction of sputum requires minimum

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equipment and can be performed by trained healthcare workers even in the primary healthcare setting. In a study conducted in the community setting, 270 children were enrolled, with a median age of 38 months. Two sequential specimens by sputum induction were successful in 99% of these children and led to microbiological confirmation in 21.6% of the 83 children diagnosed with PTB, with no significant adverse events associated with the procedure [3]. Another study showed that IS and GLA collected on the same day had a yield comparable to 2 consecutive GLAs, and a better yield than two consecutive IS evaluations [4].

The advantages of IS examination have not been replicated in the Indian scenario. A multicentric study in India compared the yields of IS and GLA in 433 children who were PTB suspects with the median age of 9 years. Overall yield for acid-fast bacilli by either IS and/or GLA was 152 (37.7%). Smear positivity from IS and GLS was 5.7% and 10.4%, respectively. Culture positivity from IS and GL was 17.9% and 32.5%, respectively ($p < 0.001$). IS and GL had and additional yield of 4.2% and 18.1%, respectively, over and above the positivity be the other method. The combined yield of the day 1 GLA and IS was less than that of 2 consecutive morning GLAs, but greater than 2 consecutive IS samples. This study aimed to assess the utility of IS as an alternative or add-on to GLA to increase the microbiological yield of testing.

Study Population and Methods

The study was conducted in a tertiary care teaching hospital in New Delhi, while the specimen processing and lab work were done at the NDTB center, an intermediate reference laboratory (IRL) recognized by the Central Tuberculosis Division of the Government of India. Children with suspected pulmonary tuberculosis were enrolled from the wards and clinics. Pulmonary tuberculosis was suspected if the child had a history of cough and/or fever of greater than a 2-week duration, a significant history of weight loss, or a history of contact with a sputum-positive pulmonary tuberculosis case [5]. The characteristics of the enrolled participants are highlighted in Table 1.

A written informed consent was obtained before the enrollment of participants, and assent was obtained from the children aged 7 seven years and above. The study protocol was approved by the Institutional Ethical Committee of Maulana Azad Medical College prior to enrollment.

Paired sputum/IS and GLA specimen were collected on 2 consecutive days. For sputum collection, the children with a productive cough were given a sterile container to cough out the secretions. For the GLA, 15–30 mL of sterile normal saline was passed into the child's stomach via a nasogastric tube, early in the morning when the child was fasting. It was then aspirated and sent for evaluation.

Table 1 Patient characteristics at the time of entry into the study (n = 50)

Characteristics	Measurement, n (%)
Age, months, mean (range)	63.9 (2–144)
Sex (M:F)	1.27:1
Weight for age, mean Z score ± 2 SD	− 3.06 ± 2.53
Cough (%)	36 (72)
Fever	44 (88)
Weight loss	29 (58)
History of contact	7 (14)
History of immune-suppression	7 (14)
BCG vaccinated	40 (80)
Socioeconomic status (Kuppuswamy scale)	
Lower lower	29 (58)
Upper lower	7 (14)
Lower middle	10 (20)
Upper middle	4 (8)

Sputum induction was performed in a well-ventilated room. Salbutamol nebulization followed by 3 mL hypertonic (3%) saline nebulization was performed, sequentially for 10–15 min each. This was followed by chest physiotherapy to mobilize the secretions. The child was encouraged to cough out the secretions into a sterile container. If the child was unable to cough out the secretions due to age, a nasopharyngeal aspiration was performed after the procedure.

The paired GLA and sputum/IS samples collected on day 1 were transported immediately for Xpert testing as well as for direct smear and MGIT culture. The paired specimens from day 2 were transported only for smear and culture. A chest radiograph and TST were done for all enrolled patients, while fine-needle aspiration cytology, contrast-enhanced computed tomography (CECT) of the chest, and bronchoalveolar lavage were done only if indicated in selected patients.

Laboratory Methods

Direct smear microscopy was done using the Ziehl-Neelsen method, whereas liquid culture using the BACTEC 960 MGIT system (BD, Sparks, MD, USA) was used to isolate the mycobacterium. The paired sputum/IS and GLA samples received on day 1 were subjected to Xpert MTB/RIF assay as well.

Based on clinical findings, CXR, TST, smear, culture, and Xpert MTB/RIF assay, children were diagnosed as pulmonary tuberculosis. We compared the microbiological yields of sputum, induced sputum, and GLA using smear, culture, and Xpert as methods of detection. The sensitivity and specificity of Xpert against the gold standard of culture were also calculated which has been reported separately in another publication [6].

Statistical Analysis

Fisher's exact test was used when comparing the yields of IS and GLA. The means of quantitative variables were compared using the *t* test.

Results

Fifty children were enrolled for this study, with a mean age of 5 years. The male-to-female ratio was 1.27:1. The children were significantly undernourished at baseline with a mean weight for age *Z* score of − 3.06. Cough, fever, and weight loss were present in 72%, 88%, and 58% of the children, respectively. Only 14% had a history of contact with a Kochs patient while 14% had a history of immunosuppression. Eighty percent of the children were vaccinated with the BCG vaccine. Most of the children (58%) belonged to a very low socioeconomic status according to the Kuppuswamy scale. The baseline characteristics of the study population are elaborated in Table 1. Based on the RNTCP guidelines, which combine clinic-radiological findings and microbiological criteria, 23/50 children were diagnosed as PTB. GLA was performed in all 23 of these the children, and used alone; the microbiological point-of-care yield by smear and/or CBNAAT was 11 cases (47.8%). If sputum (expectorated or induced) was used alone, the yield was 12 cases (52.1%). The difference in the two yields was not statistically significant ($p = 1$). The combined yield of GLA and sputa was significantly more than when either of the specimens was used alone, adding 21.7% to the yield of GLA and 17.4% to the yield of sputa (Table 2).

The differential yields of mycobacteria according to the respiratory specimen and the microbiological test used are summarized in Table 3. For IS, 1/11 PTB cases were smear-positive, 2/11 were culture-positive, and 5/11 were CBNAAT-positive. For sputum, 3/12 cases were smear-positive, 8/12 were culture-positive, and 7/12 were CBNAAT-positive. For GLA, 4/23 were smear-positive, 12/23 were culture-positive, and 11/23 were CBNAAT-positive. Bronchoalveolar lavage (BAL) was done only for 6 cases of PTB. Three samples were Xpert-positive. The 3 BAL-positive samples were all positive in alternative samples (1 GLA+; 1 IS+ and 1 both GA+ and IS+). Thus, BAL had no incremental yield over GA and IS/sputum. IS was performed in 11 children, who could not expectorate. The procedure was successful and well-tolerated in all the 11 children and there were no adverse events. GLA and IS were compared, as these are the typical specimens obtainable in children who cannot expectorate. The yield of GLA was 4/11(36.36%) in these samples, and it was 5/11 (45.45%) for IS. The difference in yield was not statistically significant ($p = 0.49$). Combining GLA and IS increased the yield to 7/11 (63.6%) which amounted to a 27.2% increase from using GLA alone.

Table 2 Combining IS and GLA increases the microbiological yield than when either specimen is used alone

Diagnosed by:	IS/S only	GA only	Both
Smear	3/7	3/7	7/7
Xpert MTB/RIF	5/16	4/16	7/16

Table 3 Rates of mycobacterial isolation according to respiratory specimen and the method of detection

	Smear+	Culture+	Xpert MTB/RIF Assay+
IS (11/23)	1/11	2/11	5/11
Spt (12/23)	3/12	8/12	7/12
GA (23/23)	4/23	12/23	11/23
BAL (5/23)	0/6	Not done	3/6

Discussion

Childhood tuberculosis is a cause of significant concern, especially in the developing world. This study aimed to compare the utility of various respiratory specimens for the diagnosis of pulmonary tuberculosis in children. In a recent systematic review, which included 30 studies, with 11,554 children, detection yields for culture ranged between 1 and 30% for IS, 1 and 45% for GA, and 4 and 24% for NPA. For Xpert MTB/RIF, it was between 2 and 17% for IS, 5 and 51% for GA, and 3 and 8% for NPA. There was a tendency of better yields with IS when the pretest probability of ITB was low to moderate and with GA when it was high. Sampling a second specimen contributed for 6–33% of the cumulative yield and combination of different methods significantly increases the detection yields [7]. Thus, both methods are nearly equally efficacious in isolating the bacterium. The number of specimens taken increases the probability of detection and two IS and GLA samples combined enhance the isolation of the tuberculosis bacillus in children with acute severe pneumonia [8]. Gastric lavage has been used traditionally to obtain samples of respiratory secretions. This method has many drawbacks. It is invasive and causes significant distress to the child and caregivers. Although it is possible to conduct GLA in an outpatient basis, there is a possibility of movement of the secretions lower down the intestine if the child is agitated or moved. Insertion of a nasogastric tube often causes agitation in children which

decreases the quality of the specimen obtained. Admission with the insertion of the NG tube the previous night is often recommended to circumvent these issues. Induction of sputum does not require any such precautions. It is easy to conduct, safe, and generally well-tolerated. Studies have reported minor adverse events like an increase in cough, wheeze, epistaxis, and transient desaturations in a few children. In a study on 843 children, the safety of the procedure was assessed by recording clinical signs and symptoms before and for 30 min after sputum induction. The major side effects noted were epistaxis and wheezing, and the maximum desaturation noted during the procedure was by 1% [9]. Induction of sputum was found to be feasible in our study and there were no adverse events following the procedure in any of the children. There is some concern of the spread of AFB to the surrounding patients when sputum is expectorated. This is an unlikely to be a major threat as the majority of children have paucibacillary disease which is unlikely to spread. However, it is recommended that the procedure should be performed in a room with negative-pressure ventilation. This is not feasible in most developing countries where tuberculosis is most prevalent. Hence, a well-ventilated room should be used in such settings.

This study had a few limitations. The sample size was small and larger studies need to be conducted to generalize the results. Secondly, of all the specimen studies, BAL should have had the maximum yields as it is taken directly from the site of infection. However, BAL was done only in six patients and in such a small sample size, meaningful comparisons cannot be drawn.

Conclusion

IS is a valuable tool to increase the microbiological yield for the diagnosis of PTB in young children who cannot expectorate. Adding an IS evaluation to the traditional GLA can lead to an incremental bacteriological yield of 27%. BAL does not seem to have additional value over GLA and sputum/IS in detecting bacilli, but larger studies are required to confirm these findings. Larger studies are warranted to further establish the role of induced sputum examination in the diagnostic evaluation of pulmonary tuberculosis in children.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the institutional ethics committee.

Informed Consent Informed consent was obtained from the parents of all individual participants included in the study. Assent was taken for children aged 7 years and above.

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