

## Role of gastric aspirate for bacteriological confirmation of pulmonary tuberculosis in hospitalized pre-school children

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Received - 21 December 2016

Initial Review - 27 January 2017

Published Online - 15 April 2017

### ABSTRACT

**Introduction:** Tuberculosis (TB) is an infectious disease caused by *Mycobacterium TB* (MTB). India accounts for one-fourth of the global TB burden, i. e., 2.2 million out of 9.6 million new cases annually. **Objectives:** The aim of this study was to assess the role of gastric aspirate (GA) for bacteriological confirmation of pulmonary TB (PTB) in hospitalized preschool children. **Methods:** A total of 66 consecutive preschool children hospitalized for suspected PTB were included in the study. These patients were prospectively evaluated with a detailed medical history, anthropometric assessment, physical examination and relevant investigations, including complete blood count, erythrocyte sedimentation rate, tuberculin skin test (TST), chest radiograph and GA on three consecutive mornings for smear and culture of MTB. **Results:** Out of 66 TB suspects, 20 (30.3%) cases were discharged on antitubercular treatment (ATT) based on clinical, laboratory, TST radiological and bacteriological criteria while 46 (69.7%) cases were discharged with an alternative diagnosis. Among cases discharged on ATT, 5 (25%) cases were bacteriologically confirmed on GA smear and culture for acid-fast bacilli (confirmed cases), and 15 (75%) cases were smear negative (probable cases). Out of 5 bacteriologically confirmed cases, 4 (80%) were in the age group 0-2 years, and 1 (20%) case was in the age group 4-6 years. **Conclusion:** GA remains a useful diagnostic technique for bacteriological confirmation in young children suspected to have PTB, especially in the inpatient setting. It is cheap, simple to perform and requires no special equipment.

**Key words:** Bacteriological confirmation, Gastric aspirate, Pulmonary tuberculosis

Tuberculosis (TB) is one of the most widespread infections affecting human population. It is estimated that one-third of the world's population is infected with *Mycobacterium TB* (MTB), the bacterium causing TB [1]. The disease is an important cause of morbidity and mortality among both adults and children, especially in developing countries. Children with TB are markers of recent disease transmission, usually from an infectious adult. They provide a reservoir of disease for the future but are rarely infectious themselves, so their treatment, particularly in endemic areas, is often not a priority. TB infection and disease among children are much more prevalent in developing countries, where resources for control are scarce [2]. It is estimated that in developing countries the annual risk of TB infection in children is 2-5%.

The estimated lifetime risk of developing TB disease for a young child infected with MTB as indicated by positive tuberculin test is about 10% [3]. About 5% of those infected are likely to develop disease in the 1<sup>st</sup> year after infection and the remaining 5% during their lifetime. These rates increase about 6-fold in human immunodeficiency virus (HIV)-infected individuals. Nearly 8-20% of the deaths caused by TB occur in children [4]. The age of the child at acquisition of TB infection has a great effect on the occurrence of TB disease.

Approximately 40% of infected children <1 year of age if left untreated develop radiologically significant lymphadenopathy or segmental lesions compared with 24% of children between 1 and 10 years and 16% of children 11-15 years of age [5]. The MTB bacilli can survive in a latent state for many years in children, and it may later progress to active TB in some cases. Therefore, childhood TB is considered an important indicator of public health programs in interrupting and preventing TB transmission [6].

It is difficult to confirm a diagnosis of TB using the methods currently available. Even in industrialized countries, the triad of obtaining a positive tuberculin skin test (TST), identifying radiographic or clinical manifestations consistent with TB, and establishing a recent link to a known infectious TB case is used for diagnosis of childhood TB. Even when these criteria have been met, problems related to bacteriological confirmation often arise [7]. Since infants and young children do not expectorate but instead swallow their sputum, aspiration of the gastric contents is the best procedure for obtaining a specimen from which to culture MTB. We planned this study to assess the role of gastric aspirate (GA) for bacteriological confirmation of pulmonary tuberculosis (PTB) in hospitalized preschool children.

**Table 1: Age-wise distribution of presenting symptoms in TB suspects**

Symptom	Age (years)			Total n=66 (%)
	0-2 (%)	2-4 (%)	4-6 (%)	
Fever	45 (68.2)	12 (18.2)	7 (10.6)	64 (97.0)
Cough	44 (66.7)	11 (16.7)	7 (10.6)	62 (93.9)
Loss of appetite/ refusal to feed	15 (22.7)	7 (10.6)	2 (3)	24 (36.4)
Wheezing	12 (18.2)	0 (0.0)	0 (0.0)	12 (18.2)
Dyspnoea	9 (13.6)	2 (3.0)	0 (0.0)	11 (16.7)
Diarrhoea	8 (12.1)	1 (1.5)	1 (1.5)	10 (15.2)
Wt loss/not gaining wt	4 (6.1)	3 (4.5)	2 (3.0)	9 (13.6)
Vomiting	4 (6.1)	1 (1.5)	1 (1.5)	6 (9.1)
Lethargy	3 (4.5)	2 (3.0)	1 (1.5)	6 (9.1)
Pain abdomen	0 (0.0)	2 (3.0)	1 (1.5)	3 (4.5)
Constipation	1 (1.5)	2 (3.0)	0 (0.0)	3 (4.5)
Convulsions	1 (1.5)	0 (0.0)	1 (1.5)	2 (3.0)
Chest pain	0 (0.0)	1 (1.5)	0 (0.0)	1 (1.5)

TB: Tuberculosis

**Table 2: Age-wise distribution of physical findings in TB suspects**

Physical finding	Age (years)			Total n=66 (%)
	0-2 (%)	2-4 (%)	4-6 (%)	
Pallor	47 (71.2)	12 (18.2)	7 (10.6)	66 (100)
Lymph node enlargement	2 (3.0)	0 (0.0)	2 (3.0)	4 (6.1)
Signs of vitamin deficiency	2 (3.0)	0 (0.0)	0 (0.0)	2 (3.0)
Chest findings	33 (50)	8 (12)	5 (7.6)	46 (69.7)
Abdominal findings	13 (19.7)	2 (3.0)	1 (1.5)	16 (24.4)

TB: Tuberculosis

## MATERIALS AND METHODS

It was a prospective, hospital-based study conducted at a tertiary care institute. The study population included the children aged 0-6 years admitted to the hospital with suspected PTB. The study was conducted for 18 months which included data collection, organization, presentation, analysis, and interpretation. All children with suspected PTB in the age group 0-6 years were enrolled and admitted in the department of pediatrics at our institute after taking informed written consent from the parents/guardians. Any child with persistent fever and/or cough >2 weeks; and/or loss of weight/no weight gain (failure to thrive); and/or history of contact with infectious TB case was taken as clinically suspected TB. The study was commenced after obtaining institutional scientific advisory-cum- protocol review and ethical committee approval. After enrollment, the patients were evaluated with a detailed medical history, anthropometric assessment, physical examination, and relevant investigations, including complete blood count, erythrocyte sedimentation rate, TST, chest radiograph and GA for smear and mycobacterial culture.

The TST was performed by injecting 0.1 ml PPD containing 5 TU intradermally with a tuberculin syringe, with the needle bevel facing upward and injection producing a pale elevation of skin (a wheal) 6-10 mm in diameter over volar aspect of the left forearm. The reaction was read after 72 h. In duration of 10 mm or more in largest diameter was considered positive, irrespective of prior bacillus Calmette-Guérin (BCG) vaccination. In severe malnutrition, the induration of 5 mm and/or necrotic indurations was taken as positive. Chest radiographs were taken in all the patients for radiological changes suggestive of TB like hilar/paratracheal lymphadenitis with or without parenchymal lesions, military TB, and fibro-cavitary pneumonia.

## Bacteriology

An appropriate-sized nasogastric tube was placed transesophageally in the stomach of a fasting patient early in the morning before he/she got out of the bed. A syringe was attached to the end of the tube when it was fully inserted up to the desired length, and 20-30 ml of GA was collected. If there was no aspirate, irrigation of the stomach was done with 50 ml normal saline, and contents aspirated until an optimum aspirate was obtained. The aspiration was collected in a sterile container from each patient for three consecutive days. As specimens were not expected to be processed for at least 4 h till they reached the microbiology laboratory, sodium bicarbonate (50 mg/10 ml of specimen) was added to neutralize the gastric acid and the specimens were kept at a temperature of 4-6°C. In laboratory, the specimens were decontaminated and digested as per standard procedures for 15 min and then centrifuged at 3000 ×g for 30 min.

The concentrates were examined for acid-fast bacilli (AFB) after staining with Ziehl-Neelsen stain. A good binocular light microscope with oil immersion (×100) objective and a ×10 eye piece (total magnification ×1000) was used to examine the smears. A minimum of 100 fields for each smear were examined before reporting it as negative. After digestion/decontamination, 200 µl of the sediment of the GA was inoculated on Lowenstein-Jensen (LJ) medium slant and incubated at 37°C. Culture growths were examined every week. Growths were seen in 3-4 weeks' time in positive cases and media with negative growths were discarded after 8 weeks. Typical colonies of MTB are rough, crumbly, waxy, non-pigmented (cream colored) and slow-growers, i. e., only appearing 2-3 weeks after inoculation.

The data were analyzed using Epi Info statistical package version 6. Chi-square test was applied where appropriate, and two-sided p<0.05 was considered statistically significant.

## RESULTS

The present study included 66 hospitalized patients of suspected PTB aged 0-6 years. Out of these 66 cases, 40 (60.6%) were boy and 26 (39.4%) were girl. All the cases were divided into three age groups, namely, 0-2 years, 2-4 years, and 4-6 years. The

first group (0-2 years) had a total of 47 (71.2%) cases, including 27 (57.4%) males and 20 (42.6%) females. The 2<sup>nd</sup> group (2-4 years) included total 12 (18.2%) cases (8, 66.7% males and 4, 33.3% females). While in the third group (4-6 years), there were a total of 7 (10.6%) cases with males and females being 5 (71.4%) and 2 (28.6%), respectively.

It was found that 6 (9.1%) of the suspected cases had a confirmed case of TB in the household. Among these, 4 (66.7%) were in the first group and 1 (16.7%) each in the 2<sup>nd</sup> and 3<sup>rd</sup> groups. In all cases, the contact person was a case of PTB within the same household. All 66 children had received BCG vaccination in time, and BCG scar was present in 64 (97%) cases.

Out of 66 children, 29 (43.9%) belonged to the upper middle-class families, 25 (37.9%) to the lower middle class, and 12 (18.2%) belonged to the upper lower class families as per modified kuppuswamy scale. All 66 children were assessed for their nutritional status as per the Indian academy of pediatrics criteria. Among these, 36 (54.5%) were found to have malnutrition. Grade wise, there were 18 (27.3%) cases of Grade I, 8 (12.1%) cases of Grade II, 8 (12.1%) cases of Grade III, and 2 (3%) cases of Grade IV protein energy malnutrition (PEM). Within each grade of PEM, maximum numbers of cases were concentrated in the age group 0-2 years.

Fever was the most common presenting complaint being present in 64 (97%) cases, followed by cough and loss of appetite/refusal to feed in 62 (93.9%) and 24 (36.4%) cases, respectively. One patient presented with seizures where neurological examination as well as cerebrospinal fluid, examination was normal, hence, ruled out neuro TB. Pallor was encountered in all 66 (100%) patients while lymph node enlargement was found in only 4 (6.1%) cases. Abnormal findings in respiratory system were present in 46 (69.7%) patients whereas abnormal abdominal finding was observed in 16 (24.4%) cases (Table 1).

Erythrocyte sedimentation rate was raised in 61 (92.4%) children, with a maximum number of 42 (63.6%) children in the age group 0-2 years. The Mantoux test was positive in 18 (27.3%) cases. The chest X-rays were abnormal in 61 (92.4%) children. The most common finding was parenchymal lesions with intrathoracic lymph node enlargement in 35 (53.0%) children followed by isolated parenchymal lesions in 19 (28.8%) children (Table 2). The GA smear for AFB was found positive in 5 (7.6%) out of the 66 suspected cases. In all these cases, GA culture for MTB was also positive. Mantoux test was positive in 2 of 5 (40%) cases that had a positive GA smear and culture for MTB. The negative Mantoux test may be attributed due to malnutrition in these three children.

Out of 5 cases with positive GA smear and culture for MTB 1 had Grade I PEM, 1 had Grade II PEM, 2 had Grade III PEM and 1 had Grade IV PEM. All suspected cases were started on antibiotics till the final diagnosis was established. 46 (69.7%) patients responded to antibiotics while 20 (30.3%) did not. All these 20 (30.3%) cases were finally discharged on ATT based on the clinical, laboratory, TST, radiological, and bacteriological criteria. Out of the 6 (9.1%) cases with a history of contact with confirmed case of PTB, only 1 (16.7%) case had bacteriologically confirmed MTB.

Among the 18 Mantoux positive cases, 2 (11.1%) were diagnosed as smear-positive PTB, 12 (66.7%) were diagnosed as smear negative PTB and 4 (22.2%) were discharged with an alternative diagnosis. Whereas out of 48 Mantoux negative cases, 3 (6.3%) were diagnosed as smear-positive PTB, 3 (6.3%) were diagnosed as smear-negative PTB, and the rest 42 (87.5%) were discharged with an alternative diagnosis.

## DISCUSSION

Children with TB carry a significant disease burden particularly in the endemic areas [8]. The absence of a practical gold standard test complicates the diagnosis of childhood TB. Sputum microscopy is positive only in 10-15% of children with probable TB, and culture yields are also low (30-40%) [9,10]. For this reason in non-endemic areas, the triad of (1) contact with adult index case, (2) positive TST and, (3) suggestive signs on chest X-ray is used in clinical practice for the diagnosis of TB in children [11]. However, the accuracy of this triad is greatly reduced in endemic areas, where most of the population acquire MTB infection during childhood and where transmission is not restricted to the household [12,13]. This limits the diagnostic contribution of both documented household exposure and a positive TST. Consequently, in endemic settings, the diagnosis of childhood TB depends mainly on clinical features and the subjective interpretation of the chest X-rays [14,15].

Children with PTB typically have closed caseous lesions with a relatively small number of mycobacteria [16,17]. This is compounded by the difficulty in collecting sputum in children as they swallow the expectorate coming from the lungs. To obtain the respiratory tract secretions procedures such as GA gastric lavage (GL) and the bronchoalveolar lavage have been used. GL collects the respiratory secretions which are swallowed at night. It is cheap, simple to perform, and requires no special equipment. In BAL, alveolar epithelial lining fluid samples are obtained directly using flexible fiberoptic bronchoscopy. This procedure is extremely invasive, and it requires tertiary care facility.

This study was undertaken in the age group 0-6 years because children in this age group are at increased risk of developing disease after primary MTB infection. They were divided into three age groups to observe the number of cases within each category as the risk of developing disease drops with increasing age. The subject number remained similar to studies done by Somu et al. [18] and Singh et al. [19]. Khan and Starke [20] has shown in their review that in 40-50% of the infants with untreated TB infection, the disease developed within 1-2 years and that the risk decreased to 15% among older children. Marais et al. [21] had also observed that infants were at increased risk to disease after TB infection and the risk dropped in 2<sup>nd</sup> year of life and reached its lowest level in children infected between 5 and 10 years. In the present study also, children aged 0-2 years had a maximum number of TB suspects followed by children aged 3-4 years and then 4-6 years who had the least number of cases.

Lobato et al. [22] compared the bacteriological yield achieved in GA from hospitalized and non-hospitalized children. Inpatients

had a higher proportion of positive GA than that of children who had aspirates collected as outpatients (48% vs. 37%). Singh et al. [19] did a comparative study on 58 children of suspected PTB. Samples from 10 (17.2%) children grew MTB from GL and 12 (20.7%) children had their BAL positive for the bacteria. Cruz et al. [23] in a retrospective study on 280 children with suspected TB reported growth of MTB in GA of 32 (11%) children. This represented 39% of the 82 children who were ultimately treated for TB. Jiménez et al. [24] assessed diagnostic yield from induced sputum (IS) combined with GL for the diagnosis of PTB in 22 children with suspected PTB non-HIV-infected children. Microbiological confirmation was achieved in 10 (58.8%) cases by either GL or IS. MTB was identified by GL in 8 (47.1%) and by IS in 7 (41.2%) cases.

In our study, GA smears were found positive for AFB in 5 (7.6%) out of 66 suspected cases, and all these five cases were also positive for mycobacterial culture on LJ medium. This represented 25% of the 20 children who were ultimately treated for TB. The results pertaining to bacteriological confirmation in the present study were lower than the previous reported studies. The reasons for this difference could be younger age group of patients with paucibacillary disease and presentation to hospital early in the course of disease. In our study, 46 (69.7%) patients responded to antibiotics while 20 (30.3%) did not and these were discharged on ATT based on clinical, laboratory, TST, and radiological and bacteriological criteria. Out of 20 cases, 5 (25%) were bacteriologically confirmed cases and 15 (75%) cases were probable (Smear negative) cases.

Bacteriological confirmation is the only gold standard investigation for the diagnosis of TB infection, and it provides a firm and rational basis to start ATT. AFB microscopy and conventional L-J media culture still remain the cornerstone for the diagnosis of TB in resource-restricted endemic areas. In the present study too, the GA was diagnostic in 5 (25%) out of 20 cases of TB, whereas the classical triad as stated by Starke [11] was present in only 1 out of these 5 cases. The present study, therefore, supports the usefulness of GA as a procedure to obtain specimen for AFB smear and culture. The bacteriological yield from GA can be optimized by standardization of the GA methodology.

## CONCLUSION

GA is a useful method of obtaining samples for bacteriological confirmation in any child suspected to have PTB who cannot expectorate the sputum suitable for culture. It is cheap, simple to perform and requires no special equipment. The bacteriological yield can be optimized by standardization of GA methodology which starts from specimen collection to base neutralization to expedited processing.

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*Funding: None; Conflict of Interest: None Stated.*

**How to cite this article:** Sharma PK, Sharma S, Sharma M, Sharma A. Role of gastric aspirate for bacteriological confirmation of pulmonary tuberculosis in hospitalized pre-school children. *Indian J Child Health.* 2017; 4(2):176-179.