



A Study on the Mycobacterial yield from Gastric-Lavage and Broncho-Alveolar Lavage in the Diagnosis of Childhood Pulmonary Tuberculosis in a Tertiary Care Centre in Bhagalpur.

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ABSTRACT **Objective:** Comparison of the mycobacteriological yield from gastric lavage (GL) and bronchoalveolar lavage (BAL), in children with pulmonary tuberculosis.

Methods: 116 children with chest radiograph suggestive of tuberculosis and positive Mantoux test or a positive history of family contact with a case of tuberculosis were prospectively subjected to gastric lavage on three consecutive mornings and bronchoalveolar lavage on the last day. The samples were subjected to bacteriological isolation.

Results: Samples from 20 (17.2%) children grew Mycobacterium tuberculosis from gastric lavage and 24 children had their BAL positive for this bacteria ($p > 0.05$). Overall mycobacterial isolation was possible in 40 patients (34.4%) as four children had grown Mycobacterium tuberculosis in GL as well as BAL. Addition of BAL to the diagnostic work up, increased the mycobacteriological yield from 17.2% with gastric lavage alone to 34.4% when BAL was also performed ($p = 0.013$).

Conclusion: No difference seen in mycobacterial isolation rates from gastric lavage and BAL when studied in isolation. However, when both GL and BAL are used; these procedures complement each other to double the diagnostic yield.

KEYWORDS : Mycobacterium tuberculosis; Gastric lavage; Bronchoalveolar lavage

INTRODUCTION

Increasing incidence of tuberculosis (TB) as well as the escalating trend of resistance to anti-TB drugs has attracted much attention during the past decades and has turned out the need for early microbiological confirmation of TB and drug sensitivity testing more than ever.[1] On the other hand, approximately 50% of patients with suspected active TB are either unable to produce sputum or demonstrate a negative sputum smear for acid fast bacillus (AFB).[2] Isolation of Mycobacterium tuberculosis is difficult in children with pulmonary tuberculosis as compared to adults where sputum is positive in upto two third of patients.[3] Children with pulmonary tuberculosis typically have closed caseous lesions with a relatively small number of Mycobacteria. The large cavitory population of tubercle bacilli seen in adults is usually absent in children. [4,5] 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 like gastric lavage (GL) and the bronchoalveolar lavage (BAL) have been used. Gastric lavage collects the respiratory secretions which are swallowed at night. Bronchoalveolar lavage samples the alveolar epithelial lining fluid directly and has been found to be useful in the diagnosis of several respiratory infections including tuberculosis.[6]

The isolation rate of Mycobacterium tuberculosis from GL cultures in children have been reported as 20-40%[7,8]; it is higher in infants (upto 75%).[9] However, the reported culture positivity rate of BAL in all children is only 10-16%.[10,11] Norman et al. reported that BAL is better than GL in the diagnosis of pulmonary tuberculosis in adults [12] showing just the opposite that GL is better than BAL and BAL does not improve the isolation rate of Mycobacterium tuberculosis in children suspected of pulmonary tuberculosis. The present prospective study was undertaken to compare the yield of M. tuberculosis from gastric lavage and bronchoalveolar lavage from children with pulmonary tuberculosis.

MATERIALS AND METHODS

Children with suspected pulmonary tuberculosis in the age group of 6 months to 14 years, admitted in the Department of Pediatrics, Jawaharlal Nehru Medical College and Hospital, Bhagalpur, Bihar from March 2015 to February 2017 were taken up for the study. Approval was taken from the Ethical Committee. Informed consent was taken from the parents/guardians of the children who were included in the present study.

A provisional diagnosis of pulmonary tuberculosis made in the presence of the following criteria was needed for inclusion of the patient in the study. Abnormal chest X-ray suggestive of tuberculosis like paratracheal gland, hilar adeno-pathy, segmental lesion, miliary appearance, unresolving pneumonia, chronic cavitory lesion with Positive tuberculin test which is taken as 10 mm or more induration at

48-72 hours following 1 Tu of PPD intradermally OR Discovery of an adult source case (usually a family member) with contagious tuberculosis who has contact with the patient. Gastric lavage was done on 3 consecutive mornings after an overnight fast with 30-35 ml of 0.9% saline and the lavaged specimen sent for staining and culture. A minimum of 30 ml of specimen was sent for analysis. Bronchoalveolar lavage (BAL) was obtained by flexible fiberoptic bronchoscopy performed on the last day of gastric lavage by a trained person (MS). The procedure was explained to parents/guardians of all patients and patients more than 8 years of age. All patients were monitored during the procedure clinically and by pulse oximetry for any complications. Patients were sedated with ketamine (1-2 mg/kg) and/or diazepam or midazolam (0.01-0.03 mg/kg). Supplementary oxygen was given during the procedure. Lignocaine jelly (2%) was applied to the nasal passage.

The flexible fiberoptic bronchoscope was inserted transnasally and 1-2 ml of 2% lignocaine was instilled at the larynx. No further lignocaine was used. The bronchoscope was advanced into the trachea and wedged into the most involved area as seen on the chest X-ray or into a segment of right middle lobe if infiltrate was diffuse. After wedging, 1-2 ml/kg (maximum 10 ml) of aliquots of sterile non bacteriostatic 0.9% NaCl solution was instilled through the suction channel of bronchoscope and subsequently aspirated by suction into a mucous specimen trap. After the lavage procedure, BAL samples were immediately submitted for staining and culture. Specimens from gastric lavage and BAL were digested and decontaminated with sodium hydroxide and N-acetyl L-cysteine. After buffering, samples were centrifuged at a rate of 3000 rpm for 20 minutes and the sediments were stained by Zeihl Neelsen technique for acid fast bacilli. After centrifuging, sediments were in-oculated into standard Lowenstein Jensen medium and incubated at 37-38°C for 6 weeks. The culture were checked weekly for any growth. The organism was identified by colony morphology and standard biochemical reactions. [13] Statistical analysis included Chi-square and McNemar tests and statistical significance was defined as $p < 0.005$.

RESULTS

In the present study 116 children were included. The mean age was 6.2 \pm 2.57 years (range 6 months to 14 years). There were 64 boys and 52 girls. Twenty-two patients were less than 3 years of age with four infants less than one year. 84 (76%) children were malnourished. 66 (57%) patients had received BCG in the first year of life. A contact case was identified in 64 (55%) patients. All patients except two were symptomatic at admission. The asymptomatic patients were sibling of a patient. The common symptoms included cough (96%), fever (90%), failure to thrive (48%), and difficulty in breathing (16%). Sixteen patients (15%) had hemoptysis. A positive Mantoux reaction was observed in 80 (68.9%) patients. All patients with a negative Mantoux were malnourished (weight less than 60% of expected) grade III or

below. Mantoux reactors were equally distributed between culture positive and culture negative cases.

78 patients on chest radiography had segmental lesions in the form of collapse and/or consolidation. Two of these patients also had pleural effusion. Primary complex was seen in 10 cases, cavitation in 4 and diffuse miliary infiltrate in 8 cases. Sixteen patients had paratracheal or hilar adenopathy on chest radiograph.

In the present study, all children underwent GL and BAL and tolerated bronchoscopy and BAL without any complications. Gastric lavage culture for *M. tuberculosis* was positive in 20 of the 116 (17.2%) patients and BAL in 24/116. In only four patients were GL and BAL both positive. Thus, there were 44 samples positive in 40 patients (34.5%). The difference in the recovery rates of *Mycobacterium tuberculosis* by GL and BAL technique was not statistically significant ($p > 0.05$). Mycobacterial isolation rate increased from 17.2% to 34.48% by the addition of BAL to gastric lavage in the workup for tuberculosis ($p = 0.013$). There was no statistically significant difference ($p = 0.48$) in the age of children who grew *M. tuberculosis* on gastric lavage or BAL (GL = 6.9 ± 1.6 years, BAL = 6.1 ± 3.3 years). There was no significant difference in age and radiological lesions of patients in whom both BAL and GL were positive in comparison to those in whom only one of the investigations yielded positive result for *M. tuberculosis*. Taking culture positivity as the gold standard for confirming tuberculosis, the sensitivity of gastric lavage was 50% and that of BAL was 60%. Smear for acid fast bacilli (AFB) was positive in 8 patients only on GL specimens. All were subsequently confirmed by culture. Four of these patients had miliary tuberculosis and the other four had cavitary tuberculosis.

DISCUSSION

The results of this study indicate that both BAL and GL cultures are complementary to each other for isolation of *Mycobacterium tuberculosis* in children clinically diagnosed to be suffering from pulmonary tuberculosis. Mycobacterial isolation rate increased from 17% to 34% by addition of BAL as an investigation.

Our study results are contrary to earlier reports [10,11], where gastric lavage proved better than BAL and BAL did not improve the yield of *Mycobacterium tuberculosis*. The first study with similar inclusion criteria as ours, sampled 20 children and found that gastric lavage was positive in 10 (50%) patients and BAL in only 2 (10%) patients (gastric lavage cultures were also positive). Younger age (Mean age 2.5 years in comparison to 6.2 years in our study) could account for this difference. Starke and Taylor Watts [8] reported that infants with pulmonary tuberculosis have a higher yield of Myco-bacterium tuberculosis from gastric lavage samples (75%) than did older children with tuberculosis. This has also been confirmed by others. [9] The method of collecting gastric lavage samples also affect the yield. Ideally, it is recommended that, the nasogastric tube be left in situ overnight and the contents have to be aspirated followed by a lavage, before the patient wakes up. Once the patient is awake, the gastric peristalsis increases and the gastric contents may not be available for analysis. [14] The nasogastric tube could not be left in situ in most of our children because they were older and active and invariably pulled out the tube at night. In spite of this the positivity rate in our study is well within the reported limits (20-40%). In another study [10], out of the ten cases positive on GL only two had BAL positive for *M. tuberculosis*. In the present study also only four patients had both the investigations positive for *M. tuberculosis*. A factor that can influence positivity of bacteria on GL is the timing of BAL. If GL is done after BAL there are more chances of GL being positive because BAL facilitates flux of secretions from the airways upwards which are swallowed into the stomach. This point however has not been discussed in the previous studies. In our study protocol, BAL was performed on one of the three days when GL was performed.

Somu et al. [11] reported that of the 50 cases with suspected pulmonary tuberculosis, *Mycobacterium tuberculosis* was grown in 6 (12%) BAL samples and 16 (32%) of the gastric lavage samples making a total of 17 (34%) culture proven cases. Out of the 6 BAL positive cases, gastric lavage was also positive in 5 cases. The rate of isolation of *M. tuberculosis* is similar in the present study but the proportion of patients positive on BAL are higher. The mean age in their study was 5.1 ± 3.8 years and the age group ranged from 7 months to 12 years. The reason for the higher yield of mycobacteria in BAL in the present study could be BAL fluid centrifugation at 2500g which increases the

recovery rate. [15] All specimens were centrifuged at 3000 rpm for 20 minutes. On the contrary, in the study by Somu et al. [11] samples were decontaminated by modified Petroff's method, in which samples are centrifuged at 1500 g for 30 minutes. However, in the study by Abadco and Steiner [10] this was not a factor as they centrifuged the specimen at 2500 g.

Some studies have suggested that anti-bacterial activity of lignocaine reduces the yield of *Mycobacterium tuberculosis*. Kvale et al. [16] reported that the culture of bronchial washings was negative in upto two third of their adult patients with pulmonary tuberculosis. They used a maximum 600 mg of lignocaine during the procedure and suggested that this accounted for the low recovery of mycobacteria in their patients. Most studies in adults which reported better yield with BAL used lesser amount of lignocaine (200-320 mg). Schmidt and Rosenkranz [17] have demonstrated the inhibition of *Mycobacterium tuberculosis* by varying concentrations of lignocaine. Lignocaine was used only at the time of entering the larynx to anesthetize the vocal cords and was not used after entering the trachea. This could have resulted in better yield with BAL in our study. In our study, midazolam or ketamine were used for conscious sedation which permitted a smooth performance of the procedure. The yield on smears for AFB from BAL fluid was nil. A low yield has been reported by previous studies also. [10,11] This is possibly because of low bacillary load in childhood pulmonary tuberculosis. The detection of *Mycobacterium tuberculosis* by acid fast staining requires about 10,000 bacilli per ml of specimen which may not be available from BAL fluid of children due to closed caseous lesions.

Our study also carried some limitations. The study is undertaken in the hospital, so at least in part, the findings may not be the exact representation of the general population.

CONCLUSION

From the present study we conclude that when both GL and BAL are used for isolation of *M. tuberculosis* these procedures are complementary to each other. Bacteriological yield is doubled by introduction of BAL in the diagnostic armamentarium. Bronchoalveolar lavage (BAL) is a useful investigation to aid the bacteriological diagnosis of pulmonary tuberculosis in children. Bronchoalveolar lavage (BAL) when performed in addition to gastric lavage improves the isolation rate of *Mycobacterium tuberculosis* in children with pulmonary tuberculosis. In spite of the fact that tuberculosis stands as one of the most ancient illness of mankind, there is considerable controversy regarding diagnosis and management. Till date there is no quick and easy diagnostic modality and the recently recommended diagnostic tests are not available in resource-poor setting like that of ours. Gastric Lavage and induced sputum has been studied by many workers as a diagnostic tool since long time.

This present study will definitely help us to know the diagnostic value of these tests, though further large scale studies in this regard is needed ; because there is a pressing need and ample scope of continual research in this field.

REFERENCES

- Mirsaeidi M, Tabarsi P, Farnia P, Ebrahimi G, Morris M, Masjedi M, et al. Trends of drug resistant *Mycobacterium tuberculosis* in a tertiary tuberculosis center in Iran. *Saudi Med J*. 2007;28:544-50. [PubMed]
- Sputum-smear-negative pulmonary tuberculosis: Controlled trial of 3-month and 2-month regimens of chemotherapy. *Lancet*. 1979;1:1361-3. [PubMed]
- Daniel TM. tuberculosis In: Harrison's Principles of Internal Medicine, 13th edn, Vol I. Eds Isselbacher KJ, Braunwald E, Wilson JD, Martin JB, Fauci AS, Kasper DL. New York, McGraw Hill, Inc, 1994; pp 710-717.
- Jacobs RF, Starke JR. Tuberculosis in children. *Med Clin North Am* 1996; 77(6): 1335-1351.
- Starke JR. Tuberculosis In: Nelson Textbook of Pediatrics, 15th Edn, Vol1. Eds. Nelson WE, Behrman RE, Kliegman RM, Arvin AM Bangalore, Prism Books Pvt Ltd, 1996; pp 834-846.
- Deblie J, Azevedo I, Burren CP, Le Bourgeois M, Lallemand D, Scheinmann P. The value of flexible bronchoscopy in childhood pulmonary tuberculosis. *Chest* 1991; 100: 688-692.
- Lincoln EM, Harris LC, Brovornkitti S, Carretero RW. Endobronchial tuberculosis in children: A study of 156 patients. *Am Rev Tuberc* 1958;177: 39-61.
- Starke JR, Taylor-Watts KT. Tuberculosis in pediatric population of Houston, Texas, *Pediatrics* 1989; 84: 28-33.
- Vallejo JG, Ong LT, Starke JR. Clinical features, diagnosis and treatment of tuberculosis in infants. *Pediatrics* 1994; 94: 1-7.
- Abadco DL, Steiner P. Gastric lavage is better than bronchoalveolar lavage for isolation of *Mycobacterium tuberculosis* in childhood pulmonary tuberculosis. *Pediatr Infect Dis J* 1992; 11: 735-738.
- Somu N, Swaminathan S, Paramasivam CN, Vijaysekaran D, Chandrabhooshanam A, Vijayan VK, et al. Value of bronchoalveolar lavage and gastric lavage in the diagnosis of pulmonary tuberculosis in children. *Tuber Lung Dis* 1995; 76: 295-299.
- Normann E, Keistinen T, Uddenfeldt M, Rydstrom P, Lundgren R. Bronchoalveolar

- lavage is better than gastric lavage in the diagnosis of pulmonary tuberculosis. *Scand J Infect Dis* 1988; 20: 77-80.
13. Laidlaw M. Mycobacterium: Tubercle bacilli. In: Mackie and McCartney Practical Medical Microbiology, 13th Edn, Vol 2. Eds. Colle JG, Duguid JP, Fraser AG, Marimon BP, New York, Churchill Livingstone, 1989; pp 399-416.
 14. Singh S, Agni R, Rattan A. Laboratory diagnosis of Childhood tuberculosis, In: Tuberculosis in Children, 1st Edn. Eds Seth V, Puri RK, Sachdev HPS, New Delhi, Indian Pediatrics, 1991; pp133-142.
 15. Rickman TW, Moyer NP. Increased sensitivity of acid fast smears. *J Clin Microbiol* 1980; 11: 618-620.
 16. Kvale PA, Johnson MC, Wroblewski DA. Diagnosis of tuberculosis: Routine cultures of bronchial washings are not indicated. *Chest* 1979; 76: 140-142.
 17. Schmidt PM, Rosenkranz NS. Antimicrobial activity of local anesthetics: Lidocaine and procaine. *J Infect Dis* 1970; 121: 597-607.