

# SPUTUM STATUS IN CHILDHOOD TUBERCULOSIS AND OTHER METHODS TO INCREASE THE YIELD

## Paediatrics

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### Abstract:

**Introduction:** Tuberculosis continues to be major health problem in India and diagnosis of tuberculosis in paediatric age group is difficult hence this study was undertaken to know yield of sputum smear examination as per diagnostic algorithm under RNTCP and to compare the yield among different methods (gastric lavage, laryngeal swab, sputum induction).

**Material & Methods:** The study was done on 50 cases suspected of having pulmonary TB. The study was conducted in children between 3 months to 18 years to 18 years of age admitted to Department of Pediatrics and Tuberculosis & Respiratory Diseases, Govt Medical College, Patiala. Children, who were not able to expectorate, were subjected to three gastric lavage on consecutive mornings for AFB, one laryngeal swab and one sputum induction was done. Expecterated sputum was collected in children who were able to expectorate and one laryngeal swab and induced sputum was also taken for comparison.

**Results:** The present study included 50 cases suspected of having pulmonary tuberculosis between 3 months to 18 years of age. Out of 50 cases, 20(40%) children were not able to expectorate and 30(60%) were able to expectorate. 33 were males and 17 were females. Sputum induction has maximum smear positivity (33.3%) followed by gastric lavage (16.6%), simple sputum (13.3%) and laryngeal swab (10%). Statistical difference between simple sputum and sputum induction was highly significant ( $p < 0.005$ ), gastric lavage and sputum induction was significant ( $p < 0.05$ ) and laryngeal swab and sputum induction was highly significant ( $p < 0.001$ ) respectively. Out of 30 children who were able to expectorate, 13 (43.3%) were smear positive and 17 were smear negative. Out of 13 smear positive cases, 10 cases (62.5 %) belonged to urban population and 2(21.4 %) belonged to rural area, difference is statistically highly significant ( $p < 0.001$ ).

**Conclusion:** It can be concluded from present study that sputum expectoration is problem in children. None of the child < 3 years was able to expectorate. In non-expectorated cases, gastric lavage, laryngeal swab and sputum induction are not effective method for yield of mycobacterium tuberculosis. In expecterated cases, sputum induction in comparison to simple sputum, gastric lavage and laryngeal swab is best method for yield of mycobacterium tuberculosis.

**Keywords:** Tuberculosis, gastric lavage, sputum induction, laryngeal swab,

### Introduction:

Tuberculosis is the leading cause of death worldwide, with over 1.5 million

deaths per year. This disease is caused by *Mycobacterium tuberculosis*, which is an acid-fast bacilli, and it is transmitted mainly by the airway.<sup>1</sup> TB in childhood is frequently more difficult to diagnose

due to the atypical radiological features and the difficulty to expectorate Whereas adult TB cases are often easily recognizable, due to typical symptoms (radiological features and a positive sputum smear).<sup>2</sup> There is significant morbidity and mortality in children worldwide,<sup>3</sup> with majority of cases of latent TB infection (LTBI) and active disease occurring in developing countries.<sup>4</sup> Childhood tuberculosis is commonly extra pulmonary, disseminated, and severe, especially in children under 3 years of age, and it is associated with high morbidity and mortality.<sup>5</sup> Approximately, 15–20% of all TB cases in sub-Saharan Africa are in children.<sup>6,7</sup>

Tuberculosis is categorized into 1) pulmonary tuberculosis which can presents as primary complex, pneumonia, pleural effusion, endobronchial tuberculosis and military tuberculosis; 2) Extra pulmonary tuberculosis which presents as lymph node tuberculosis, tubercular meningitis, pericardial effusion, enteritis ,peritoneal effusion, genitourinary, spine, bones and joint tuberculosis.<sup>8</sup>

Diagnosis of pulmonary tuberculosis (consensus statement under revised national tuberculosis programme) in children is based on, clinical profile (fever and cough > 3 weeks with or without loss of weight/ no gain in weight), mantoux test (1 Tuberculin unit of purified protein derivative RT 23 with Tween 80 was injected intradermally on the flexor surface of forearm after cleaning with 70% alcohol. Maximum transverse diameter of the site perpendicular to long axis of forearm was measured after 48-72 hours. Induration  $\geq$  10 mm was suggestive of positive Mx test) and chest x-ray showing evidence of primary complex, pneumonic pattern, military mottling, pleural effusion, cavitory suggestive of PTB, Sputum examination wherever possible.<sup>9</sup> Diagnosis of pulmonary tuberculosis is difficult in infants and young children in whom clinical and radiological signs can be nonspecific and variable.<sup>10</sup>

Microbiological confirmation of tuberculosis is desirable for definitive diagnosis, for best use of antituberculosis medication, and for epidemiological tracing of isolates.<sup>11</sup> Isolation of mycobacterium tuberculosis is difficult in children with pulmonary tuberculosis as compared to adults where sputum is positive in up to two third of patients. Children with pulmonary tuberculosis typically have closed caseous lesion with relatively small number of mycobacteria. The large cavitory population of tubercle bacilli seen in adults, is usually absent in children.<sup>12</sup> Expectoration of sputum is a problem in majority of

children especially in younger ones because forceful cough is not possible, effective cough is not present till 5 years, child may not follow command and specimen is usually small .Under such conditions we can use methods like gastric lavage, laryngeal swab, sputum induction, tracheal lavage and bronchoalveolar lavage.

In order to increase the diagnostic yield newer methods need to be applied. Hence the present study was undertaken to see the role of gastric lavage, laryngeal swab and sputum induction by nebulisation with 3% saline to increase the diagnostic yield apart from the diagnostic algorithm.

### **Material and methods:**

The present study was done on 50 cases suspected of having pulmonary tuberculosis. The study was conducted in children between 3 months to 18 years of age admitted to Department of paediatrics and tuberculosis & respiratory diseases, Govt Medical College, Patiala. Pulmonary tuberculosis was suspected on basis of clinical profile (fever and cough > 3 weeks with or without loss of weight/ no gain in weight) , mantoux test (1 Tuberculin unit of purified protein derivative RT 23 with Tween 80 was injected intradermally on the flexor surface of forearm after cleaning with 70% alcohol. Maximum transverse diameter of the site perpendicular to long axis of forearm was measured after 48-72 hours. Induration  $\geq$  10 mm was suggestive of positive Mx test. Induration <10mm was considered to be negative. Catrgorization of size of tuberculin test was done as 1) 0-4 mm 2) 5-9 mm 3) 10-14 mm 4) 15-19 mm 5) 20mm and chest x-ray.<sup>11</sup>

Sputum collection was done on 3 successive early mornings after forceful coughing.<sup>12</sup> Sputum specimen were evaluated in terms of quantity (>2 ml) and quality (thick and viscous or saliva alone). Smear was prepared and stained by Ziehl Nelson staining and graded according to RNTCP criteria.

Irrespective of the results of sputum examinations all the patients were subjected to Gastric lavage, laryngeal swab, sputum induction by nebulisation with 3 % saline to increase the sputum yield.

Gastric lavage was collected on 3 consecutive mornings. Ryles tube was put in stomach at night after last feed. In morning when child was asleep, 30-50 ml of normal saline was infused in stomach and same was aspirated.

Petroff's method was performed for decontamination and concentration.<sup>13</sup>

Laryngeal swab was taken with swab stick. Tongue was depressed with disposable tongue depressor. Swab stick was introduced up to the level of entrance of larynx.

Sputum induction by nebulisation with 3% saline was done only once. The child was kept on fast for at least 3 hours before. Sputum was induced by nebulising 5-10 ml of 3% saline for 10-20 minutes using a nebulizer.

**Ziehl Nelson staining:** Smear prepared from specimen obtained by each method was air dried for 15-30 minutes and fixed by passing it over a flame 3-5 times for 3-4 seconds each time. Carbol fuchsin was poured on the slide and slide was gently heated until vapour rise. Carbol fuchsin was left for 5 minutes on the slide and was gently rinsed with tap water until all free carbol fuchsin was washed away. 25 % sulphuric acid was poured on the slide and it was allowed to stand for 2-4 minutes. It was then rinsed with tap water and counter staining was done with 0.1% methylene blue for 30 seconds. Slide was rinsed with tap water and allowed to dry.<sup>13</sup> Slide was examined under microscope using X40 lens to select suitable area and then was examined under X100 lens using a drop of immersion oil. It was graded according to RNTCP criteria. The data so obtained was subjected to statistical analysis using paired t test.

## Results:

The present study included 50 cases suspected of having pulmonary tuberculosis between 3 months to 18 years of age. Out of 50 cases, 20(40%) children were not able to expectorate and 30(60%) were able to expectorate. 33 were males and 17 were females. Table- 1 is showing distribution of cases on the basis of expectoration of sputum the statistical difference was not significant ( $p>0.05$ ).

**Table-1**  
**Distribution Of Cases On The Basis Of Expectoration Of Sputum**

	No of cases	% age
Non expectorated cases	20	40
Expectorated cases	30	60
Total	50	100
$X_2$	2	
P value	>0.05	
Sig	NS	

Out of 20 cases that were not able to expectorate none of sputum collected by gastric lavage, laryngeal swab and induced sputum was smear positive as shown in Table-2.

**Table-2**  
**Comparison Of Gastric Lavage, Laryngeal Swab & Sputum Induction In Non Expectorated Cases**

	No of cases	Smear positive	% age
Gastric lavage	20	0	0
Laryngeal swab	20	0	0
Sputum induction	20	0	0
Total	20	0	0
$X_2$		-	
P value		-	
Sig		-	

Out of 30 cases that were able to expectorate sputum induction has maximum smear positivity (33.3%) followed by gastric lavage (16.6%), simple sputum (13.3%) and laryngeal swab (10%). Statistical difference between simple sputum and sputum induction, gastric lavage and sputum induction and laryngeal swab and sputum induction was highly significant ( $p<0.005$ ), significant ( $p<0.05$ ) and highly significant ( $p<0.001$ ) respectively as shown in table -3 &4.

**Table-3**  
**Comparison Of Simple Sputum, Gastric Lavage, Laryngeal Swab & Sputum Induction Expectorated Cases**

S.No.	Method	No. of cases	Smear +ve	% age
I	Simple sputum	30	4	13.3
II	Gastric lavage	30	5	16.6
III	Laryngeal swab	30	3	10
IV	Sputum induction	30	10	33.3
	<b>Total</b>	<b>30</b>	<b>13</b>	<b>43.3</b>

**Table -4**  
**Statistical Analysis**

Comparison	X <sup>2</sup>	'p'	Significance
I & II	0.36	>0.05	NS
I & III	0.46	>0.05	NS
I & IV	8.58	<0.05	HS
II & III	1.64	>0.05	NS
II & IV	5.59	<0.05	S
III & IV	12.54	<0.01	HS

### Discussion:

Sputum expectoration is a problem in children because forceful cough is not possible, effective cough is not present till 5 years of age, child may not follow command to expectorate and specimen is small.

In all non-expectorated cases, gastric lavage, laryngeal swab and sputum were negative for acid fast bacilli (Table-2). This was in contrast to the studies conducted by Jesus et al (1994), Ambedkar (2005) and Zar et al (2000) and Zar et al (2005). In the study conducted by Jesus et al, gastric lavage was positive for acid fast bacilli in 75% of cases. In their study, culture was done for acid fast bacilli which are more sensitive than smear examination. In the report mentioned by Ambedkar (2005), tubercle bacilli can be detected in 70% of infants in gastric lavage. Zar et al (2000) had observed that gastric lavage was culture in 6.3% (9/142) of cases. Zar et al (2005) had reported that gastric lavage was culture positive in 15 % of cases and smear positive in 7% of cases. Combined culture and smear positivity was 16% in their study.

None of sputum induction in non expectorated cases was positive in present study (Table-2). This was in contrast to study conducted by Zar et al (2000) and Zar et al (2005). In study conducted by Zar et al (2000), Mycobacterium tuberculosis was grown from induced sputum in 10.6% (15/142) of cases. Zar et al (2005) found tubercle bacilli in induced sputum specimens in 20% cases (51/250) by culture method, 10% (25/250) by smear examination and 22% (54/250) by combined culture and smear examination. In their study, sputum induction method used was different from that of present study. Concentration of saline used in these studies for nebulisation was 5% as compared as compared to 3% in present study. It was followed by chest percussion and collection of secretion by mucus extractor by suctioning through nasopharynx and sputum induction was conducted on 3 consecutive days. Staining method was auramine O staining in above studies. Whereas in our study,

sputum induction was done only once without any chest percussion and secretions were collected from oral cavity and no suctioning was done. Smear were stained with ziehl Nelson staining in present study. No data was available in literature regarding isolation of acid fast bacillus in laryngeal swab in non-expectorated children. The entire laryngeal swab in nonexpectorated cases was negative for acid fast bacilli in present study as shown in table-2.

In expectorated cases, simple sputum smear was positive for acid fast bacilli in 4 cases (13.3%) as shown in table-3. It was similar to study conducted by Ambedkar (2005) who had observed the yield of AFB in <20 % in children by ZN stain. The result of present study was in contrast to the study conducted by Kiwanuka et al (2001). According to him, culture positivity rate of sputum was 27.8% (5/18). But in the present study smear examination was done and culture was not done.

Gastric lavage was positive for AFB in 16.6% (5/30) cases in expectorated cases in the present study (Table-3). It was similar to that observed by Singh et al (2000), Jeffrey (2000), Gomez (2002) and Dickson et al (2003). In the study conducted by Singh et al (2000) gastric lavage was positive for AFB in 17.2% (10/58) of cases by culture method. The mean age of children in their study was  $6.2 \pm 2.57$  years, although culture method was not used in present study but mean of children was higher ( $10.80 \pm 4.43$  years) which could account for similarity between two studies. Jeffrey (2000) has reported a yield of 0-20% in gastric lavage by staining method. The yield of AFB from gastric lavage by Gomez (2002) was 13% and by Dickson et al (2003) gastric lavage was positive in 21% of cases.

In the present study, in expectorated cases maximum yield of AFB was by sputum induction i.e. 33.3% (Table-3). This was in contrast to the study conducted by Shata et al (1996). In their study 4/29 (14%) were smear positive by ZN staining and 8 were positive by culture method (28%). The difference may be due to difference in the age group. They did sputum induction in children up to 15 years whereas in the present study children up to 18 years were taken.

Laryngeal swab smear positivity rate was 10% in expectorated cases (Table-3) and 6% in total number of cases, it was close to the study conducted by Kiwanuka et al (2001) who had reported positivity in 6.6 % (4/60) of children aged 4 month to 14 years.

Difference in the smear positivity of simple sputum and gastric lavage was comparable and non significant statistically ( $p>0.05$ ) (Table- 3) in the present study. This was similar to study conducted by Laven et al (1997).

The statistical difference in the yield of AFB between sputum induction (33.3%) and simple sputum (13.3%) and sputum induction and laryngeal swab was highly significant ( $p<0.001$ ) (Table-3) in the present study. No data was available in literature regarding the comparison in the yield of AFB by simple sputum and sputum induction and sputum induction and laryngeal swab.

The statistical difference in yield of AFB between sputum induction (33.3%) and gastric lavage (16.6%) was significant ( $p<0.05$ ) (Table-3). In the study conducted by Hensler et al (1961) and Brown et al (2007) sputum induction had been found to give a better yield than the gastric lavage. In the study conducted by Hensler et al (1961), gastric lavage was culture positive in 43% and induced sputum was culture positive in 65% of cases. The difference was statistically significant ( $p<0.002$ ). In the study by Brown et al (2007), 39% cases were positive by sputum induction as compared to 30% by gastric lavage, the difference being statistically significant ( $p<0.05$ ). All above studies had been conducted in adults. No data was available in literature to make comparison in children.

### **Conclusion:**

It can be concluded from present study that sputum expectoration is problem in children. None of the child < 3 years was able to expectorate. In non-expectorated cases, gastric lavage, laryngeal swab and sputum induction are not effective method for yield of mycobacterium tuberculosis. In expectorated cases, sputum induction in comparison to simple sputum, gastric lavage and laryngeal swab is best method for yield of mycobacterium tuberculosis.

### **References:**

1. J.L. Flynn and J chan. Tuberculosis, Latency and reactivation. *Infection and immunity*.2001;(69): 4195-4201
2. MP Nicol and HJ Zar. New specimen and laboratory diagnostics for childhood pulmonary tuberculosis. *Progress and Prospects*.2011; 1(12): 16-21.
3. MP Nicol, D. Piennar, K. Wood et al. Enzyme-linked immunospot assay response to early secretory antigenic target, culture filtrate protein 10 and PPD among children with Tuberculosis: implication for diagnosis and monitoring of therapy. *Clinical infectious diseases*. 2005; 9(40): 1301-1308.
4. S Dogra, P Narang, DK Mendiratta et al. Comparison of whole blood interferon gamma assay with tuberculin skin testing for detection of tuberculosis infection in hospitalized children in rural India. *Journal of infection*.2007; 3(54): 267-276.
5. ALalvani, KA millington. Screening for tuberculosis infection prior to initiation of anti-TNF therapy. *Autoimmunity Review*. 2008; 2(8):147-152.
6. DJ Edwards, F Kitetele and A Van Rie. Agreement between clinical scoring systems used for diagnosis of pediatric tuberculosis in HIV era. *Int jou of Tuberculosis and lung disease*. 2008; 3(11):263-269.
7. Gladys Guadalupe Lopez A valosi, Ernesto Prado Montes de Oca. Classic and New Diagnostic Approaches to childhood Tuberculosis. *Journal of Tropical Medicine*. Volume 2012, Article ID 818219, 12 pages.
8. Ambedker YK, Sachdev HPS, Nair MKC, Mukherjee D, Shah NK, Sangani A et al. Consensus statement of IAP working group: Status report on diagnosis of childhood tuberculosis. *Indian Pediatrics* 2004 Feb; 41:146-55.
9. Zar HJ, Hanslo D, Appoles P, Swingleer G, Hussy G. Induced sputum versus gastric lavage for microbiological confirmation of pulmonary tuberculosis in infant and young children : A prospective study. *Lancet* 2005; 365(9454); 130-134.
10. Zar HJ, Tannenbaum E, Appoles P, Roux P, Hanslo D, Hussy G. Sputum induction for the diagnosis of pulmonary tuberculosis in infant and young children in an urban setting in South Africa. *Arch Dis Child*.2000 April; 82(4): 305-8.
11. Singh M, Moosa NVA, Kumar L, Sharma M. Role of gastric lavage and bronchoalveolar lavage in the bacteriological diagnosis of childhood pulmonary

- ttuberculosis. Indian Pediatrics 2000 Sept; 37:947-51.
12. Chadha VK, Jagnath PS, Kumar P. Tuberculin sensitivity among children vaccinated with BCG under universal immunization programme. Indian journal of Pediatrics 2004; 71:1063-8.
  13. Taneja DK National Tuberculosis control Programme. Health Policies and Programme in India. Doctor's publication. Delhi 2004 July; 4:57-70.
  14. Laidlaw M, Collee JG, Duguid JP, Frase AG, Marmion BP. Mycobacterium: tubercle bacilli. Mackie and McCartneys Practical medical microbiology. 13th ed London Churchill Livingstone; 2002.399-415.
  15. Jesus GV, Lydia TO, Jeffrey RS. Clinical features, diagnosis and treatment in infants. Pediatrics 1994 July; 94(1): 1-7.
  16. Kiwanuka J, Graham SM, Coulter JBS, Gonwe JS, Chilewani N, Carty H et al. Diagnosis of pulmonary tuberculosis in children in an HIV endemic area, Malawi. Annals of Tropical Pediatrics; Int child health. Carfax publishing, part of the Taylor & Francis Group. March 2001; 21(1):5-14.
  17. Jeffrey RS. Diagnosis of tuberculosis in children. Pediatrics infec Dis J.2000; 19:1093-6.
  18. Gomez DPD. Bacilloscopia Y Cultivo en Muestras de jugo gastric en el diagnosis de tuberculosis pulmonary infantil. Articulos publicado. Enviar Correspondencia a. 2002; 1-2.
  19. Shata AMA, Coulter JBS, Parry CM, Chingani G, Broadhead RL, Hart CA. Sputum induction for diagnosis of tuberculosis. Archives of Diseases in childhood 1996; 74:535-7.
  20. Hensler NM, Spivey CG, Dees TM. The use of hypertonic aerosol in production of sputum for diagnosis of tuberculosis. American college of chest Physicians 1961; 40:639-42.
  21. Dickson SJ, Brent A, Davidson RN, Wall R. Comparison of bronchoscopy and gastric washings in the investigation of smear-negative pulmonary tuberculosis. Clin Infect Dis. 2003 Dec 15;37(12):1649-53.