Original Article

The role of induced sputum in the diagnosis of pulmonary tuberculosis

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ABSTRACT

Background: Microbiological confirmation of pulmonary tuberculosis (TB) is of paramount importance in the era of immunocompromised host and emergence of multi-drug resistance. **Objectives:** To assess the value of sputum induction (SI) with hypertonic saline nebulization as a diagnostic tool in patients with suspected pulmonary TB who have no/inadequate sputum or have a sputum smear negative for acid fast bacillus (AFB). **Materials and Methods:** One hundred patients with clinical and radiological evidence of pulmonary TB with no/inadequate sputum or smear negative with spontaneous sputum were studied. Sputum was induced with 20 mL of 3% hypertonic saline solution delivered through ultrasonic nebulizer. The specimens were subjected to Ziehl Neelsen staining and were examined under oil immersion lens for the presence of AFB. The specimens were also subjected to mycobacterial culture in BACTEC 460 TB system. **Results:** Ninety five patients could produce adequate sputum after SI. Sputum from thirty two patients were found to be positive both in smear and culture while sputum from another three patients were smear negative, but culture positive. **Conclusion:** SI is a safe, cheap and non-invasive procedure and provides significant yield in the diagnosis of pulmonary TB; thus, increasing the case detection rate of smear positive pulmonary TB.

KEY WORDS: Induced sputum, pulmonary tuberculosis, sputum smear negative

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INTRODUCTION

Microbiological confirmation of pulmonary tuberculosis (TB) is becoming increasingly important because of emergence of multi-drug resistance and increased incidence of TB among patients with human immunodeficiency virus (HIV) infection. Moreover, rapid and precise diagnosis will reduce the risk of nosocomial transmission of TB. Direct sputum smear microcopy remains a fundamental tool of diagnosis, but may be negative up to 50% case of active pulmonary TB.[1] Alternative methods of obtaining sputum specimens are frequently needed in those patients with radiological suspicion of TB who are unable to expectorate or are smear negative. The methods include – sputum induction (SI), bronchoalveolar lavage (BAL) and gastric washings (GW) specimens. These methods have their own advantages and disadvantages and diagnostic yield vary in respect of safety, tolerability, and feasibility in different set-ups.

GW is reported to reveal the organism only in 25-50% of children with active TB, when a set of three samples are subjected to both microscopy and culture.[2] Bronchoscopy is an invasive procedure, only available in the large hospitals, needs experts for performance, costly and may not be feasible if the large numbers of patients are to be tested. SI is a cheap and non-invasive alternative with a diagnostic yield "same if not better" than bronchoscope.[3] SI with hypertonic saline has been used in the diagnosis of various respiratory disorders. The present study was performed to evaluate the use of SI in establishing the diagnosis in patients with suspected pulmonary TB, who are unable to produce adequate sputum or are found negative on smear examinations.

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MATERIALS AND METHODS

One hundred patients of suspected pulmonary TB with negative sputum smear during the period of 1 year at our

outpatients department were taken into the study. The inclusion criteria were – (i) persistent cough for at least 2 weeks, (ii) sputum either not produced or inadequate for examination (only saliva or sputum quantity < 2 mL) or sputum smear negative for acid fast bacillus (AFB) on two samples, (iii) chest radiograph showing changes consistent with active pulmonary TB, and (iv) age more than 18 years.

Patients with uncontrolled asthma or chronic obstructive pulmonary disease, active hemoptysis, recent eye surgery, unstable angina or arrhythmias and patients already on antituberculous therapy were excluded from the study. Signed informed consent to undergo SI was obtained from the patients. The study was approved by Ethics Committee of the institution.

Protocol of SI – A brief description of the procedure was given to each patient. To avoid contamination, the patients were asked for rinsing and repeated gargling with tap water until the returned fluid was free from debris. The nebulizer chamber was filled with water and the nebulizer reservoir device was placed into the nebulizer chamber. 20 mL of 3% hypertonic saline was injected into the nebulizer reservoir device. The assembly was connected to the ultrasonic nebulizer (Beuer Ultrasonic Nebulizer IH50). The procedure was carried out in a well-ventilated room with windows open and adequate staff protection measures were taken. The patients were instructed to inhale and exhale the mist of the nebulized solution through the month piece only. The inhalation of hypertonic saline was interrupted every 5 min, so that the patient could expectorate the sputum into a clean sterile sputum container. The procedure was continued until an adequate amount of sputum sample (2 mL or more) was obtained or for a maximum of 15 min has passed without success or the patients complained of shortness of breath or wheeze. The patient was closely monitored at all times during the procedure and 1 h post-procedure. The nebulizer equipment was decontaminated after each session by through washing and soaking in glutaraldehyde overnight. The sputum samples were stained with Ziehl Neelsen stain and examined for AFB using the oil immersion lens. The samples were also put on BACTEC 460 TB commercial liquid culture system for culture of *Mycobacterium tuberculosis*.

RESULTS

Out of 100 patients undergoing SI, 65 were males and 35 were females. Most patients were in the age group of 18-40 years (54%). On the total, 42% of patients had cough with no/inadequate sputum while 58% of patients had adequate sputum, but negative for AFB smear. Radiologically, an exclusive upper zone involvement was seen in 67% of patients while 16% of other patients had multi-lobar involvement including upper zones. Patchy non-cavitary opacifications were seen in 91% patients and only 4% patients had cavitary lesions.

SI was successful in 95% of patients who could expectorate adequate sputum (mucoid or purulent sputum of at least 2 mL). Overall, 32% of patients were found positive on smear examination after SI. All of them also showed growth of *M. tuberculosis* in BACTEC 460 TB culture system. Another three samples showed growth of *M. tuberculosis,* but were negative in smear examination. Thus, SI gave an additional yield in diagnosis of pulmonary TB in 35 patients [Table 1]. Out of 58 patients who were smearing negative with spontaneous adequate sputum, 21 (34%) were found positive on induced sputum culture examination. SI culture was successful in confirmation of diagnosis in 14 (33%) out of 42 patients with no/adequate sputum. The sensitivity of smear and culture of induced sputum in diagnosis of pulmonary TB were 80% and 87% respectively. The specificity of both the procedures was 100%.

Out of 83 patients with upper zone involvement 30 (36.1%) patients were sputum culture positive with SI while it was 29.4% (5 out of 17) in patients with non-upper zone involvement. Success rate of SI in patients with non-cavitary and cavitary lesions in radiology was 34% (31 out of 91) and 100% (4 out of 4) respectively.

The procedure was found safe as there was no adverse effect during and immediately after the procedure.

All patients with smear positive induced sputum were treated with anti-tuberculous treatment (ATT) as per national control program. Three patients with smear negative, but culture positive sputum were started with ATT as soon as the culture reports were available. Five smear negative patients were put on ATT before the availability of culture results because they had military TB on clinico-radiological basis. Sixty patients including five patients with failed SI were not given ATT as they were subsequently diagnosed to be having non-TB etiology. All patients on ATT had a successful outcome (cured or treatment completed) except one who was diagnosed as multidrug resistant TB during follow-up.

DISCUSSION

SI was first used by Hensler *et al*., in 1961 in the diagnosis of active TB.[4] Hypertonic saline irritates the airways causing the patient to cough and increases the osmolarity of the airway lining fluid, which produces increased

Table 1: Results of smear and culture examination on induced sputum among patients who had successful sputum induction (*n*=95)

Culture for *Mycobacterium tuberculosis*	Smear results	
	Positive	Negative
Positive	32	03
Negative	00	60[#]
Total	32	63

[#]Five patients among them got anti-tuberculous treatment on clinico-radiological basis and 55 patients were non-tuberculous

vascular permeability, and induces mucus production by submucosal glands.[5,6]

SI has performed well both in resource-poor and resource-rich countries.[7-10] In these studies, SI provided adequate samples for diagnosis was cost-effective and about 25-42% patients were smear positive on SI samples. However, few studies in developed countries showed that SI added little to overall diagnosis and was deemed costly.[11]

GW is regarded as the standard procedure in diagnosis of TB in children who swallow their sputum and cannot expectorate.[12-14] However, this method has yielded variable results in different studies. The main drawbacks are – (1) Limited rate of positive cultures among the smear positive results making positive smear results unreliable,[7,15,16] (2) requirement of 3 samples for evaluation, (3) requirement of overnight fasting, (4) invasive procedure, (5) cannot be easily performed on an out-patient basis, and (6) requires rapid processing to avoid killing by gastric acid. In different studies, the sensitivity of the GW smears was 61-80% while positive cultures were present in only 30%.[16,17]

The disproportionate GW smear and culture results were presumed to be due to non-neutralization of sample, inappropriate decontamination and presence of non-tuberculous mycobateria.[16,17] However, a recent study has reported sensitivity of GW smears and cultures to be 66.07% and 85.7% respectively when problems of transport, neutralization and decontamination were adequately addressed.[18] SI was superior to GW in obtaining a suitable sample for culture, though the two techniques were noted to be complementary.[19,20]

Bronchoscopy for BAL requires special facilities, is invasive, is not accessible in many areas of resource-limited settings. Cost of the procedure, concern regarding infection control, inhibition of mycobacterial growth with the use of local anesthetics during bronchoscopy, sampling from only one portion of the lung are its major drawbacks.[16,21,22] In a study by McWilliams *et al*., the yield of SI (96.3%) was superior to that of BAL (51.9%) and the overall cost of BAL was three times that of performing SI.[23] They suggested to employ SI, followed by BAL only in patients who were negative on SI, but had radiologically active pulmonary TB. In a similar study, the yield of SI and BAL was 19% and 12% respectively, but the direct cost of bronchoscopy was 8 times those of SI.[3] However, a Brazilian study comparing SI with BAL in HIV – positive and negative patients' yielded similar sensitivities.[24]

In comparison with the above two procedures, SI has several advantages, which include less invasiveness, high diagnostic yield, greater patient comfort and safety, low-cost, no age restriction, no need of fasting, out-patient procedure, no need for expert for performance and less time consuming.[3,25] Adequate sputum sample was obtained in 93-97% of patients in different studies.[3,25] The yield of sputum smear positivity varied between 26% and 38%.[9,23,25] The yield can be increased by multiple induced sputum samples.[10,19,23] If the relative yield of SI for previously non-productive cough and previously smear negative sputum is compared, it is seen that the yield is higher in the former group,[8,25] which is similar to our study. However, the yield was similar in both these groups in few studies.[9] SI is reported to be a safe procedure both in children and adults with or without HIV infection.[13,24] Moreover, the SI has also been successfully utilized in patients with pleural TB.[26]

Mycobacterial culture remains the gold standard for the diagnosis of TB. While, smear examination requires at least $10^{[4]}$ bacilli per ml to be positive, culture can detect bacilli as low as 10-100 per ml.[27] Thus, it increases the TB diagnosis by 30-50%. It also establishes the viability of the bacilli helps to identify the species and provides sample for drug susceptibility test (DST). Culture can be carried out on solid media (like Lowenstein Jensen media) or liquid media (like BACTEC 460 TB System, Mycobacteria growth indicator tube). As the mycobacteria are notoriously slow growing, identification of the organism with or without DST is time consuming. Several rapid methods are becoming available for identification and study of resistance pattern of the mycobacteria. X-pert MTB/RIF assay (gene X-pert) is a fully automated nucleic acid amplification that uses real time PCR for detection of *M. tuberculosis* and rifampicin resistance. In 2010 World Health Organization endorsed this test for use in endemic countries and declared it a major milestone for global TB diagnosis. Results are obtained from unprocessed sputum within 90 min with a minimal biohazard and very little technical training for operation. It has a sensitivity of 100% in sputum smear positive cases, 68.6% in smear negative cases.[28] The main drawback is its cost. In resource – limited countries like India where the facilities for culture or molecular methods are not widely available, therapeutic response with ATT in a clinico-radiological suspected TB is the only reliable method for retrospective diagnosis of TB.

Spontaneous sputum is the diagnostic tool of choice in TB control programs throughout the world. SI is not mentioned as an alternative option in our national program. Requirement of electricity – driven ultrasonic nebulizer and a room with negative pressure ventilation are major limitations for its routine use. Moreover, necessity of about 30 min time to complete one SI may not be practical in a high burden country like ours. It has recently been introduced as a source of alternative sample in pediatric pulmonary TB suspect who has persistent non-specific shadows despite a course of antibiotics.[29] On the other hand, high-cost is the main drawback of X-pert MTB/RIF assay for its routine use in national program. A pilot study is in progress to assess its applicability in programmatic management of drug resistant TB in India. It is helpful for rapid diagnosis of both pulmonary and extra-pulmonary TB, species identification and detection of rifampicin resistance. Its routine use in all TB suspect cases can give

the diagnosis within minutes and rifampicin resistance cases can be offered treatment for multi-drug resistant TB immediately.

Our study has few limitations. HIV status of the patients and its impact on the yield of SI were not assessed. Larger studies are needed to assess its utility is different settings, optimal number of samples needed and optimal volume of sample for mycobacterial culture.

CONCLUSION

SI is a safe, cheap, and effective procedure for microbiological confirmation of diagnosis of pulmonary TB in patients who produce no/inadequate sputum or are sputum smear negative. SI should be incorporated in national program at least in selected groups.

REFERENCES

1. American Thoracic Society and Centers for Disease Control and Prevention. Diagnostic standards and classification of tuberculosis in adults and children. Am J Respir Crit Care Med 2002;161:1376-95.
2. World Health Organization. Guidance for National Tuberculosis Programmes on the Management of Tuberculosis in Children. Geneva: World Health Organization; 2006. p. 371.
3. Anderson C, Inhaber N, Menzies D. Comparison of sputum induction with fiber-optic bronchoscopy in the diagnosis of tuberculosis. Am J Respir Crit Care Med 1995;152:1570-4.
4. Hensler NM, Spivey CG Jr, Dees TM. The use of hypertonic aerosol in production of sputum for diagnosis of tuberculosis. Comparison with gastric specimens. Dis Chest 1961;40:639-42.
5. Bell D, Leckie V, McKendrick M. The role of induced sputum in the diagnosis of pulmonary tuberculosis. J Infect 2003;47:317-21.
6. Paggiaro PL, Chanez P, Holz O, Ind PW, Djukanović R, Maestrelli P, *et al*. Sputum induction. Eur Respir J Suppl 2002;37:3s-8.
7. Li LM, Bai LQ, Yang HL, Xiao CF, Tang RY, Chen YF, *et al*. Sputum induction to improve the diagnostic yield in patients with suspected pulmonary tuberculosis. Int J Tuberc Lung Dis 1999;3:1137-9.
8. Wilson D, Nachega J, Morroni C, Chaisson R, Maartens G. Diagnosing smear-negative tuberculosis using case definitions and treatment response in HIV-infected adults. Int J Tuberc Lung Dis 2006;10:31-8.
9. Hartung TK, Maulu A, Nash J, Fredlund VG. Suspected pulmonary tuberculosis in rural South Africa – Sputum induction as a simple diagnostic tool? S Afr Med J 2002;92:455-8.
10. Al Zahrani K, Al Jahdali H, Poirier L, René P, Menzies D. Yield of smear, culture and amplification tests from repeated sputum induction for the diagnosis of pulmonary tuberculosis. Int J Tuberc Lung Dis 2001;5:855-60.
11. Schoch OD, Rieder P, Tueller C, Altpeter E, Zellweger JP, Rieder HL, *et al*. Diagnostic yield of sputum, induced sputum, and bronchoscopy after radiologic tuberculosis screening. Am J Respir Crit Care Med 2007;175:80-6.
12. Singh M, Moosa NV, Kumar L, Sharma M. Role of gastric lavage and broncho-alveolar lavage in the bacteriological diagnosis of childhood pulmonary tuberculosis. Indian Pediatr 2000;37:947-51.
13. Zar HJ, Tannenbaum E, Apolles P, Roux P, Hanslo D, Hussey G. Sputum induction for the diagnosis of pulmonary tuberculosis in infants and young children in an urban setting in South Africa. Arch Dis Child 2000;82:305-8.
14. Shingadia D, Novelli V. Diagnosis and treatment of tuberculosis in children. Lancet Infect Dis 2003;3:624-32.
15. Norrman E, Keistinen T, Uddenfeldt M, Rydström PO, Lundgren R. Bronchoalveolar lavage is better than gastric lavage in the diagnosis of pulmonary tuberculosis. Scand J Infect Dis 1988;20:77-80.
16. Rizvi N, Rao NA, Hussain M. Yield of gastric lavage and bronchial wash in pulmonary tuberculosis. Int J Tuberc Lung Dis 2000;4:147-51.
17. Okutan O, Kartaloglu Z, Kilic E, Bozkanat E, Ilvan A. Diagnostic contribution of gastric and bronchial lavage examinations in cases suggestive of pulmonary tuberculosis. Yonsei Med J 2003;44:242-8.
18. Baghaei P, Tabarsi P, Farnia P, Radaei AH, Kazempour M, Faghani YA, *et al*. Utility of gastric lavage for diagnosis of tuberculosis in patients who are unable to expectorate sputum. J Glob Infect Dis 2011;3:339-43.
19. Brown M, Varia H, Bassett P, Davidson RN, Wall R, Pasvol G. Prospective study of sputum induction, gastric washing, and bronchoalveolar lavage for the diagnosis of pulmonary tuberculosis in patients who are unable to expectorate. Clin Infect Dis 2007;44:1415-20.
20. Zar HJ, Hanslo D, Apolles P, Swingler G, Hussey G. Induced sputum versus gastric lavage for microbiological confirmation of pulmonary tuberculosis in infants and young children: A prospective study. Lancet 2005;365:130-4.
21. Brodie D, Schluger NW. The diagnosis of tuberculosis. Clin Chest Med 2005;26:247-71, vi.
22. Kreider ME, Rossman MD. Pulmonary tuberculosis. In: Schlossberg D, editor. Tuberculosis and Nontuberculous Mycobacterial Infection. 5th ed. New Delhi: Tata McGraw Hill; 2006. p. 177-89.
23. McWilliams T, Wells AU, Harrison AC, Lindstrom S, Cameron RJ, Foskin E. Induced sputum and bronchoscopy in the diagnosis of pulmonary tuberculosis. Thorax 2002;57:1010-4.
24. Conde MB, Soares SL, Mello FC, Rezende VM, Almeida LL, Reingold AL, *et al*. Comparison of sputum induction with fiberoptic bronchoscopy in the diagnosis of tuberculosis: Experience at an acquired immune deficiency syndrome reference center in Rio de Janeiro, Brazil. Am J Respir Crit Care Med 2000;162:2238-40.
25. Gupta KB, Garg S. Use of sputum induction for establishing diagnosis in suspected pulmonary tuberculosis. Indian J Tuberc 2005;52:143-6.
26. Conde MB, Loivos AC, Rezende VM, Soares SL, Mello FC, Reingold AL, *et al*. Yield of sputum induction in the diagnosis of pleural tuberculosis. Am J Respir Crit Care Med 2003;167:723-5.
27. Van Deun A. What is the role of mycobacterial culture in diagnosis and case definition? In: Frieden T, editor. Toman's Tuberculosis Case Detection, Treatment, and Monitoring – Questions and Answers. 2nd ed. Geneva: World Health Organization; 2004. p. 35-43.
28. Zeka AN, Tasbakan S, Cavusoglu C. Evaluation of the GeneXpert MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in pulmonary and extrapulmonary specimens. J Clin Microbiol 2011;49:4138-41.
29. Kumar A, Gupta D, Nagaraja SB, Singh V, Sethi GR, Prasad J. Updated current (2012 national) tuberculosis in India. J Indian Med Assoc 2012;110:840-5.

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