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Diagnostic yield and safety of sputum induction with nebulized racemic salbutamol *versus* hypertonic saline in smear-negative pulmonary tuberculosis

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Abstract

Objectives: The aim of the study was to compare the diagnostic yield and safety profile of sputum induction (SI) with nebulized racemic salbutamol solution *versus* hypertonic saline in smear-negative pulmonary tuberculosis (TB).

Methods: The prospective study was conducted at Songklanagarind Hospital, Thailand. Suspected smear-negative pulmonary TB cases were recruited and randomized to receive SI with either nebulized racemic salbutamol solution or 3% sodium chloride (NaCl) solution. Induced sputum was examined with the acid-fast bacilli (AFB) smear test and cultured for *Mycobacterium tuberculosis*. The efficacy and adverse events of SI were analyzed.

Results: A total of 59 patients received SI with nebulized racemic salbutamol solution and 53 received 3% NaCl solution. There was no significant difference between the two groups in the average quantity of induced sputum (1.3 $\pm$ 0.1 *versus* 1.2 $\pm$ 0.2 ml, $p = 0.5$). The percentages of positive AFB smear and TB cultures in the salbutamol group were 15% and 22%, and 13% and 17% in the 3% NaCl group ($p = 0.5$), respectively. Racemic salbutamol solution could increase the TB diagnostic yield similarly to 3% NaCl, but incurred less chest tightness (5% *versus* 15%) and bronchospasm (0% *versus* 11.3%, $p = 0.02$) compared with 3% NaCl.

Conclusions: SI by nebulized racemic salbutamol solution offers equal benefits to 3% NaCl solution in increasing both sputum quantity and diagnostic yield in smear-negative patients suspected of having pulmonary TB. Nebulized racemic salbutamol does not produce bronchospasm and chest tightness occurs less frequently than with 3% NaCl. Therefore, SI with nebulized racemic salbutamol solution should be considered as a good alternative noninvasive diagnostic tool for the diagnosis of pulmonary TB when hypertonic saline is unavailable or contraindicated.

Keywords: hypertonic saline, salbutamol nebulizer, smear-negative pulmonary tuberculosis, sputum induction

Introduction

Pulmonary tuberculosis (TB) continues to be a major worldwide health problem. Delayed diagnosis and untreated patients lead to further disease progression and transmission [WHO, 2012; Golub *et al.* 2006]. Intensified case finding is therefore a key intervention and still presents a major challenge. There are many tools for making a TB diagnosis, for example, gastric washing, bronchoscopy with bronchoalveolar lavage (BAL), and lung biopsy. These procedures have their own limitations such as invasiveness, need for patient cooperation, cost, and availability of the institutional and local expertise needed. Therefore sputum collection for Ziehl–Neelsen staining for acid-fast bacilli (AFB) detection remains the mainstay of diagnosis [American Thoracic Society and the Centers of Disease Control and Prevention, 2000]. However,

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40–60% of active pulmonary TB cases have negative AFB staining by self-expectoration (smear-negative pulmonary TB) due to a dry cough, scarce sputum, or paucibacillary TB [Paggiaro *et al.* 2002; Hong Kong Chest Survice/Tuberculosis Research Center Madras/British Medical Research Council, 1979; Narain *et al.* 1971; Hensler *et al.* 1961], thus noninvasive sputum induction (SI) with ultrasonic nebulized hypertonic saline (3% sodium chloride [NaCl]) is a good alternative method for diagnosis in this patient group.

SI was first used by Hensler and colleagues [Hensler *et al.* 1961], and hypertonic saline was used to stimulate cough reflex, increase osmotic pressure in the lower airways, draw more water into the lumen, dilute mucins in the airways, and facilitate sputum expectoration. Previous studies showed that SI with hypertonic saline could improve both the quantity and quality of the collected sputum in 70–90% of cases [Paggiaro *et al.* 2002]. Moreover, SI has been reported to be as effective as gastric washing and bronchoscopy with BAL for TB diagnoses, and gives a diagnostic yield 2–41% higher than self-expectorated sputum collection [Gonzalez-Angulo *et al.* 2012; Hepple *et al.* 2012; Hatherill *et al.* 2009; Brown *et al.* 2007; Gupta and Seema, 2005; Bell *et al.* 2003; Li *et al.* 1999; Kawada *et al.* 1996; Shata *et al.* 1996; Anderson *et al.* 1995; Kim *et al.* 1984; Narain *et al.* 1971]. However, SI with hypertonic saline itself can produce chest pain, tightness, rapid breathing, and bronchospasm by activation of airway mast cells and sensory nerve endings with an incidence of 6–32% (especially in patients with asthma or chronic obstructive pulmonary disease [COPD]), despite pretreatment with beta2-agonist inhalation [Geldenhuys *et al.* 2012, 2014; Dunleavy *et al.* 2008; Makker and Holgate, 1993]. There is a report of death in one patient with asthma undergoing ultrasonic nebulized hypertonic saline [Saetta *et al.* 1995], thus SI with hypertonic saline was contraindicated for patients with a history of bronchospasm, and prophylactic nebulized salbutamol is suggested in cases of moderate to severe asthma where SI is required [Carlsten *et al.* 2007; Delvaux *et al.* 2004; Pizzichini *et al.* 2002; Jones *et al.* 2001].

Beta2-agonists have been demonstrated to enhance mucociliary transport in healthy subjects as well as patients with asthma and chronic bronchitis. It decreases the tenacity of sputum and thereby facilitates easy expectoration [Bennett, 2002; Yazdani *et al.* 2002; Mortensen *et al.* 1991]. In 2002, 1 mg of oral salbutamol three times a day for 3 days was used to induce sputum in 289 chest-symptomatic patients who had either a dry cough or scanty sputum [Yazdani *et al.* 2002]. It showed that 88% of cases could produce adequate sputum after induction with oral salbutamol and there was no reported adverse drug reaction (ADR). Recently, Ansari and colleagues used nebulized levosalbutamol (R-isomer salbutamol) at a dosage of 1.26 mg for 2 consecutive days prior to collecting three sputum samples in 40 clinically suspected TB patients who had a dry cough or scant sputum [Ansari *et al.* 2013]. Results showed sputum collection was successful in 90% of patients and gave a positive AFB smear in 77.5% of cases. Nebulized racemic salbutamol (S-isomer salbutamol) was not only cheaper than levosalbutamol but was also a widely used beta2-agonist in Thailand. Its efficacy and safety profile was not considered to be inferior to levosalbutamol for the treatment of acute asthma attack [Jat and Khairwa, 2013]. Therefore, the present study was conducted to evaluate the diagnostic yield and adverse events of SI in nebulized racemic salbutamol *versus* hypertonic saline (3% NaCl) in Thai patients who were suspected of smear-negative pulmonary TB.

Material and methods

A prospective, randomized, patient-blinded, comparative study was conducted from August 2008 to February 2012. The inclusion criteria were: (a) patients at least 15 years old in Songklanagarind Hospital, Songkhla, Thailand, who presented with a nonproductive cough persisting for at least 3 weeks or a dry cough or scanty sputum ($<$ 2 ml saliva) with prolonged fever; (b) radiologic findings compatible with active pulmonary TB; (c) negative AFB smears on 3 consecutive days of self-expectorated sputum after being well instructed by a certified respiratory therapy technician. Patients diagnosed with asthma, COPD, pneumonia, lung cancer, bronchiectasis, uncontrolled hypertension, cardiac arrhythmias, pregnancy, allergy to bronchodilators, undiagnosed cause of chest symptoms and abnormal imaging, had received antituberculosis drugs within the previous 2 months, or had a positive AFB smear from self-expectoration were excluded from the study. All patients signed informed consent and were randomized (using block randomization) into two groups to receive SI *via* a face mask with either nebulized racemic salbutamol solution (1 ml of 0.5% salbutamol solution plus 3 ml of

0.9% NaCl), or 4 ml nebulized 3% NaCl (without prophylactic bronchodilator) under 100% oxygen compressor nebulizer at a flow rate of 15 L/min for up to 20 min in a standard isolation negative pressure room. Within 60 min of nebulization, all patients were instructed to expectorate and a spot sputum specimen was collected in a sterile container and measured. It was then examined with Ziehl–Neelsen staining and cultured for *Mycobacterium tuberculosis* (using Lowenstein–Jensen medium) by the blinded experienced laboratory technicians. Vital signs (by automated device), electrocardiography, and SI-related adverse events, for example, palpitation, cardiac arrhythmia, chest tightness, bronchospasm, tachypnea, and oxygen desaturation (by finger pulse oximeter) were closely observed at baseline, continuously during the nebulization phase, and at 15-min intervals for up to 1 h postnebulization by the primary physicians who were blinded to the composition of the SI. If symptomatic bronchospasm (expiratory wheezing with tachypnea or oxygen desaturation, $SO_2 < 95\%$) occurred, the rescue bronchodilator (nebulized salbutamol solution) was given, and the SI procedure stopped for safety reasons and interpreted as a negative AFB smear result.

Patients with a negative AFB smear by SI underwent bronchoscopy with BAL with or without transbronchial lung biopsy (TBB) within 1 week for a definite diagnosis. The definitive diagnosis of active pulmonary TB cases depends on either the detection of *M. tuberculosis* from culture specimens (by induced sputum or bronchoscopy with BAL) or TBB showing granulomatous inflammation with clinical response to a full treatment course of antituberculosis drugs. The study protocol was approved by the ethics committee of the institution (IRB#50/372-045), and was conducted in accordance with the World Medical Association Declaration of Helsinki and 2013 good clinical practical guideline. [WMA, 2013].

Statistical analysis

The sample sizes were calculated using two independent proportions with 80% power of detection. Previous studies showed that diagnostic yield in pulmonary TB using nebulized 3% NaCl solution for SI ranged from 2% to 41%. Thus an expected smear-positive SI by nebulized 3% NaCl in this study was 15%, whereas the diagnostic yield of pulmonary TB using nebulized levosalbutamol was 61% in the study of Ansari and colleagues [Ansari *et al.* 2013] However, negative AFB smears on 3 consecutive days of self-expectorated sputum had not been excluded before SI in this study. Therefore, an expected smear-positive SI by nebulized racemic salbutamol was 40% (at $\alpha = 0.05$). The total calculated sample size was 49 patients per group. The mean + standard deviation was used to describe continuous data. Proportion (%) was used to describe categorical data. Student's *t*-test and the chi-square test were used to analyze continuous and categorical data, respectively. Outcomes of interest were analyzed with SPSS version 11 software and the results were considered as statistically significant if the p value was less than 0.05.

Results

A total of 147 participants were initially recruited into the study and randomized into two groups: 74 patients received SI with nebulized racemic salbutamol solution and 73 received nebulized 3% NaCl solution, respectively. A total of 35 cases were excluded due to negative TB results by bronchoscopy or other conditions were proven to be the cause of pulmonary disease (i.e. 12 lung cancers, eight pneumonias, two bronchiectasis, and 13 inconclusive), which meant that 112 patients with pulmonary TB were analyzed in the study. Of these 59 received SI with nebulized racemic salbutamol solution and 53 received nebulized 3% NaCl solution. There was no difference in the baseline characteristics between the two groups in terms of age, sex, underlying diseases, and pattern abnormalities on chest X-ray (Tables 1 and 2). A total of 84 cases (75%) were diagnosed as pulmonary TB by positive culture of induced sputum or BAL, and 25% were diagnosed by lung biopsy specimens (15 cases in the salbutamol group and 13 cases in the hypertonic saline group, respectively). There was no significant difference between the two groups in the average quantity of induced sputum (1.3 ± 0.1 *versus* 1.2 ± 0.2 ml/case, $p = 0.5$). The percentages of positive AFB smear and positive TB culture from spot SI with the nebulized racemic salbutamol solution were 15% (nine cases) and 22% (13 cases), and 13% (seven cases) and 17% (nine cases) with the 3% NaCl solution, respectively ($p = 0.5$) (Table 3). There was no statistically significant change in body temperature, heart rate, blood pressure, or oxygenation between the two groups. Neither palpitations nor arrhythmia were documented during and after the period of nebulization. However, nebulized 3% NaCl significantly produced more

Table 1. Baseline characteristics of patients in the nebulized 3% sodium chloride solution and racemic salbutamol solution groups.

Characteristic	3% Sodium chloride ($n = 53$) *n* (%)	Salbutamol ($n = 59$) *n* (%)	*p* value
Age (years) (mean ± standard deviation)	49.7 ± 15	49.6 ± 14	0.732
Male sex	25 (47.2)	33 (55.9)	0.354
Underlying diseases			0.997
Diabetes mellitus	5 (9.4)	6 (10.2)	
HIV	4 (7.5)	6 (10.2)	
Steroid use	3 (5.7)	3 (5.1)	
Others	7 (13.2)	7 (11.9)	

Table 2. Patient chest film: location and pattern abnormality.

Characteristic	3% Sodium chloride ($n = 53$) *n* (%)	Salbutamol ($n = 59$) *n* (%)	*p* value
Abnormal chest X-ray location			0.993
Upper lobe	31 (58.5)	34 (57.6)	
Middle lobe	8 (15.1)	10 (16.9)	
Lower lobe	5 (9.4)	5 (8.5)	
Multilobar	9 (17.0)	10 (16.9)	
Chest X-ray pattern			0.995
Cavity	9 (17)	10 (16.9)	
Patchy	8 (15.1)	8 (13.6)	
Reticulonodular	20 (37.7)	21 (35.6)	
Nodular	5 (9.4)	6 (10.2)	
Reticular	11 (20.8)	14 (23.7)	

adverse events compared with the salbutamol solution, such as chest tightness (15% *versus* 5%) and symptomatic bronchospasm requiring rescue bronchodilator (11.3% *versus* 0%, $p = 0.02$) (Table 4). Six cases with symptomatic bronchospasm in the 3% NaCl group were treated with nebulized salbutamol solution (1–2 doses/case). Symptoms were relieved and the patients were discharged within 2–4 h following treatment.

Discussion

We found that SI with nebulized racemic salbutamol solution offered equal benefits to nebulized 3% NaCl solution in the diagnostic yield for smear-negative pulmonary TB and it did not incur any ADRs.

It was known that SI with nebulized hypertonic saline could facilitate sputum expectoration and increase the diagnostic yield of pulmonary TB, especially in the case of negative AFB staining with self-sputum collection [Paggiaro *et al.* 2002; Hong Kong Chest Survice/Tuberculosis Research Center Madras/British Medical Research Council, 1979; Narain *et al.* 1971; Hensler *et al.* 1961]. However, hypertonic saline itself can produce chest tightness and bronchospasm requiring rescue bronchodilator, and even death [Saetta *et al.* 1995]. A few studies showed that beta2-agonists could also facilitate sputum expectoration [Ansari *et al.* 2013; Bennett, 2002; Yazdani *et al.* 2002; Mortensen *et al.* 1991]. In the present study, racemic salbutamol (S-isomer salbutamol) was used for SI and the results showed that the nebulizer solution of racemic salbutamol was as effective as hypertonic saline in increasing both sputum quantity and diagnostic yield of TB without any adverse events. The nebulized form of salbutamol was preferred to an oral form because of the more rapid onset of action and fewer side effects [National Asthma Education and Prevention Program, 2007]. Our study showed that nebulized S-isomer salbutamol could increase the diagnostic yield of

Table 3. Diagnostic yield of sputum induction with nebulized 3% sodium chloride solution *versus* nebulized racemic salbutamol solution in suspected smear-negative pulmonary tuberculosis cases.

Characteristic	3% Sodium chloride ($n = 53$) *n* (%)	Salbutamol ($n = 59$) *n* (%)	*p* value
Acid-fact bacilli positive	7 (13.2)	9 (15.3)	0.757
Culture positive	9 (17.0)	13 (22.0)	0.502
Acid-fact bacilli or culture positive	9 (17.0)	13 (22.0)	0.502

Table 4. Sputum induction-related adverse events.

Characteristic	3% Sodium chloride ($n = 53$) *n* (%)	Salbutamol ($n = 59$) *n* (%)	*p* value
Overall adverse events	18 (34.0)	6 (10.2)	0.02
Bronchospasm	6 (11.3)	0	
Cough	4 (7.5)	3 (5.1)	
Chest tightness	8 (15.1)	3 (5.1)	
No event	35 (66.0)	53 (89.8)	

TB similarly to nebulized R-isomer salbutamol as reported in the study by Ansari and colleagues [Ansari *et al.* 2013]. However, the percentage of positive AFB smear within the first spot-induced sputum by nebulized S-isomer salbutamol was lower than the R-isomer salbutamol (15% *versus* 60%, respectively), because all patients who had positive AFB smears on 3 consecutive days of self-expectorated sputum were initially excluded from our study. Therefore, these results could not be directly compared.

Our study has several strengths. It was a prospective, randomized comparative trial, and moreover, the sample size was calculated; the diagnostic gold standard for active TB was applied; SI was used only after the failure of diagnosis by self-expectorated sputum as suggested by Geldenhuys and colleagues (real-life practice) [Geldenhuys *et al*. 2012, 2014]; SI adverse events were also reported. However, there were limitations to our study. First, an observational bias could occur in our single-blind experimental design; however, all laboratory technicians were blinded to the SI arm. Second, a crossover design was not applied in the study because of concern about the impact of carryover effects and washout periods between each SI. Third, sample size calculation was based on a high estimated diagnostic yield of salbutamol; this might have meant that the calculated sample size was inadequate to demonstrate a small difference in diagnostic yield between the two groups. Fourth, ultrasonic nebulization, which could increase the amount of sputum, was not used because it was costly and not available in Thailand. However, the study showed that the TB diagnostic yield could also be increased by the use of a compressor-type nebulizer. Fifth, this was a tertiary hospital-based study that was confined to the more complicated participants and might explain why it had a relatively high number of adverse events with hypertonic saline. Therefore, SI by salbutamol was potentially vulnerable in the tertiary hospital compared with the primary healthcare setting. Lastly, there were no data on cumulative diagnostic yield of repeated SI and also patients with asthma or COPD were not recruited to this study due to ethical issues. As patients with asthma or COPD were at risk of developing symptomatic bronchospasm, they could not be randomized to receive SI with hypertonic saline. Therefore, further studies are needed to confirm the efficacy of repeated SI with nebulized racemic salbutamol solution, and also to validate the safety profile of nebulized racemic salbutamol for SI in patients with asthma and COPD.

Conclusion

SI by nebulized racemic salbutamol solution offers equal benefits to 3% NaCl solution in increasing both sputum quantity and diagnostic yield in smear-negative patients suspected

of having pulmonary TB. SI using nebulized racemic salbutamol does not produce bronchospasm and chest tightness occurs less frequently, therefore, it should be considered as a good alternative noninvasive method for the diagnosis of pulmonary TB when hypertonic saline is unavailable or contraindicated.

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Declaration of Conflicting Interests

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