Mucolitic Ambroxol Versus Hypertonic Saline Nebulizer Induction: For Increasing Sputum Volume And Finding Acid-Fast Bacilli

Sunarto Ang[1], Sumardi[2], Erna Kristin[3*]
[1]Post Graduate Study of Tropical Medicine, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta. [2]Division of Pulmonology, Department of Internal Medicine, Faculty of Medicine, Universitas Gadjah Mada/ Dr. Sardjito General Hospital, Yogyakarta. [3]Department of Pharmacology and Toxicology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta.

Corresponding author: erna_kristin@yahoo.co.uk

ABSTRACT

Introduction: Tuberculosis, a major killer disease in the community, was caused by Mycobacterium tuberculosis. According to WHO (2006), pulmonary tuberculosis cases in Indonesia was third ranked in the world. Prevalence of pulmonary tuberculosis in Eastern Indonesia was higher than in Java and Bali, but the findings of positive smear was lowest. AFB discovery will be decreased because of the poor quality and quantity of sputum. The useful of mucolitic ambroxol or hypertonic saline nebulizer induction will be to increase quality and quantity of sputum smear.

Objectives: The aim of this study was to determine and to compare the effectiveness of ambroxol and use a hypertonic saline induction on new suspected pulmonary tuberculosis patients to increase sputum volume and to find AFB.

Methods: 76 new suspected pulmonary tuberculosis patients were divided into 2 groups with double-blind and open-label simple random sampling RCT (Randomized Controlled Clinical Trial-Parallel design) study. The sputum induction using ambroxol or 3% hypertonic saline solution. The primary and secondary outcome were increasing sputum volume and finding AFB by Ziehl-Neelsen staining to calculate the AFB count per 100 fields of view. Non parametric statistical analysis and percentage of success.

Results: All patients can produce sputum. Only one patient ambroxol group can't produce it. The quality and quantity of sputum hypertonic saline induction volume better than ambroxol. AFB finding increase both groups, but no significant difference. AFB finding increase 26.47% (9/34) with ambroxol and 27.78% (10/36) with hypertonic saline induction compared than previous negative smear.

Conclusions: Significant differences increase sputum volume hypertonic saline induction compared than ambroxol. No significant difference AFB finding improvement hypertonic saline induction compared for ambroxol. Finding AFB increase 26.47% with ambroxol and 27.78% with hypertonic saline induction compared previous negative smear.

Keywords: Tuberculosis, Ambroxol, Nebulizer induction, Sputum volume, AFB

INTISARI

Pendahuluan: Penyakit tuberkulosis merupakan pembunuh utama dalam masyarakat, penyakit ini disebabkan oleh Mycobacterium tuberculosis. Menurut WHO (2006) jumlah kasus TB paru di Indonesia berada di peringkat ketiga di dunia. Prevalensi TB paru di Kawasan Timur Indonesia lebih tinggi dibandingkan di Jawa dan Bali, tetapi temuan kasus BTA positif paling rendah. Oleh karena kualitas dan

kuantitas dahak yang kurang baik, sehingga penemuan BTA berkurang. Perlunya upaya penemuan BTA melalui pemberian ambroxol dan nebulizer salin hipertonis untuk meningkatkan kualitas dan kuantitas dahak.

Tujuan: Mengetahui dan membandingkan hasil guna pemberian ambroxol dan nebulizer salin hipertonis pada tersangka penderita TB paru baru untuk meningkatkan volume dahak dan temuan BTA.

Metode: 76 orang tersangka TB paru baru yang dibagi dalam 2 kelompok dengan rancangan penelitian RCT (Randomized Controlled Clinical Trial-Parallel design) secara simple random sampling metode double blind dan open label. Induksi dahak menggunakan ambroxol dan nebulizer salin hipertonis (NaCl 3%). Pemeriksaan meliputi pengukuran volume dahak dan menghitung angka BTA per 100 lapang pandang dengan pewarnaan Ziehl-Neelsen.

Hasil: Adanya peningkatan volume dahak pada kelompok ambroxol (97,37%) dan nebulizer salin hipertonis (100%), volume dahak kelompok nebulizer salin hipertonis lebih baik kualitas dan kuantitas dahak dibandingkan kelompok ambroxol. Adanya peningkatan temuan BTA positif pada kelompok ambroxol dan nebulizer salin hipertonis, tetapi tidak ada perbedaan bermakna kedua kelompok tersebut. Temuan BTA meningkat 26,47% responden kelompok ambroxol dan 27,78% responden kelompok nebulizer salin hipertonis dibandingkan BTA negatif sebelumnya.

Simpulan: Ada perbedaan bermakna berupa peningkatan volume dahak kelompok nebulizer salin hipertonis dibandingkan ambroxol. Tidak ada perbedaan bermakna dalam hal peningkatan temuan BTA kelompok nebulizer salin hipertonis dibandingkan ambroxol. Temuan BTA positif meningkat 26,47% responden pada kelompok ambroxol dan 27,78% responden kelompok nebulizer salin hipertonis dibandingkan BTA negatif sebelumnya.

Kata Kunci: Tuberkulosis, Ambroxol, Nebulizer salin hipertonis, Volume dahak, BTA

INDRODUCTION

Tuberculosis (TB) is a major killer disease in the community, caused by *Mycobacterium tuberculosis*. A third of the world's population had been infected and estimated are 8 million new cases and 3 million deaths patients per year. One of ten patients infected by *Mycobacterium tuberculosis* will become active TB[1,2,3].

The Pulmonary TB cases in Indonesia contributed 10% cases in the world[1]. Based on the survey results to indicate the prevalence and incidence rate differences of TB cases in three regions variation of the estimated incidence of smear positive TB cases in Java and Bali 64/100,000; 210/100,000 at Eastern Indonesia and 160/100,000 in Sumatra. The national prevalence decreased 4% per year and the trend of slower decline in Sumatra and Eastern Indonesia[2].

The prevalence of PTB in Indonesia was 253 cases per 100,000 population per year and the number of new smear positive incidence was 105 cases per 100,000 patients a year, while the death rate due to TB was still high at 38 cases per 100,000 population in a year. TB average second ranked in the outpatient clinic at the general hospital and the first rank in the lung clinic and hospital. In the outpatient unit of the general hospital, new cases of TB accounted for 19% of all new respiratory cases[2,4]. The implementation of tuberculosis eradication program, the tuberculosis diagnosis was established if found AFB in microscopic smear sputum. To find AFB in sputum examination for tuberculosis was not easy[1,5,6,7].

In 2007, the East Kalimantan province has CDR (Case Detection Rate = number of patients finding new smear positive TB) 29.7% and CNR (Case Notification Rate) 62.5%. Samarinda has CNR 68.7% in 2006 and 55,9% in 2007, its to decrease the discovery scope new smear positive patients. The national target was 70%[8]. AFB discovery will be decreased will the poor quality and quantity of sputum. To find AFB in sputum smear microscopic

of suspected tuberculosis was not easy. The problem was the quality and quantity of sputum. The ideal sputum for microscopic examination was mucopurulen about 3-5 ml volume each collection. It takes at least 5,000 AFB per milliliter to be seen microscopically. If less of quality and quantity of sputum, so to find the AFB less also[7,9,10].

The quality and quantity sputum and sputum physicochemical the structure affect the results microscopic examination. Mucopolisaccharides fibers and mucin acid, it can be difficult to catch mycobacterial cells so make it difficult to detect[1,5,11]. Microscopic examination of sputum showed the poor quality sputum smear, so it's need education to health care workers and laboratory personnel. The improving sputum collection need continuous monitoring[12].

Besides sputum physicochemical structure, the acid mucopolisaccharides contains fibers make AFB capture so difficult to be detected. The sputum contained mucin can catch mycobacteria cells and protects them from the decontamination activities[13]. The expectorants can stimulate secretions from the bronchi and tracheal spending, to increase secretions or to break the acid mucopolisaccharides fibers will be become more watery sputum and AFB will be spread evenly[14]. The useful of mucokinetic expectorant (ambroxol) was expected to improve finding positive smear rate higher compared than normal procedure.

Ambroxol as an expectorant mucokinetic helps the secretion of the bronchi branch and increase kinesis lowering the surface tension by stimulating an effective spur surfactant transport of mucus when coughing[14,15]. Ambroxol effects can be used as mucokinetic the respiratory tract[16], especially chronic bronchitis patients[17], which to dilute the sputum patients have trouble removing it[18].

To find a sputum smear examined using sputum induction by inhalation hypertonic saline through a nebulizer, particularly in patients with dry cough or inadequate sputum volume/ scanty (less than 2 milliliters)[19-24]. Sputum induction was an effective method inhalation to obtain adequate quantity of sputum. Because of sputum dilution mucus from steam hypertonic saline can be separated *M. tuberculosis* from the trap[21].

Hypertonic saline and ambroxol were the expectorant mucokinetic type, which beneficial to increase transport of mucus when coughing. Ambroxol to increase coughing because the stimulating effects of surfactant secretion. Hypertonic saline to separate the DNA from the infected mucus of bacteria, as to reduce the mucus viscosity so it can increase sputum volume smear and findings of previous negative patients[15,19,21,22,25].

The aim of this study was to determine and to compare between ambroxol or hypertonic saline nebulizer induction for increase the sputum volume and find AFB on suspects pulmonary tuberculosis patients.

MATERIALS AND METHODS

The study design an open-label and double blind simple random sampling RCTs (randomized controlled Clinical Trial-Parallel design)[26,27]. The independent variables were ambroxol and hypertonic saline nebulizer induction and the dependent variables were sputum volume and AFB findings. Inclusion criteria were: suspecties new pulmonary tuberculosis patients, who either having a dry cough or scanty produce sputum, only produce saliva or quantity sputum less than 2 ml and referral cases that have been examined previously negative smear, have existence of a written consent (informed consent) and 18 to 55 years age old. Exclusion criteria, were: being in treatment with anti-tuberculosis drugs, history of severe respiratory illness, severe illness, hypertension, pregnant or breastfeeding, allergies and contraindications bromhexine or ambroxol and nebulizer induction.

Materials and study tools were ambroxol 30 mg tablet and induction instrument uses ultrasonic nebulizer hypertonic saline (NaCl 3% solution). Sputum volume measurements using a measuring cup and sputum smear stained Ziehl-Neelsen method. The decontaminate nebulizer equipment was washing, decontamination and sterilization (by soaking in 2.4% glutaraldehyde for 10-12 hours at 25ºC temperature).

Ambroxol HCl patients group agree to follow the study (informed consent) will be given the 3 tablets ambroxol HCl 30 mg drug to be taken for 1

day every 8 hours and notified via SMS (Short Message Service).

Nebulizer induction group will be given explanation about hypertonic saline (NaCl 3% solution). A brief description of the procedure was given to the patients. To avoid contamination, patients gargled with normal solution to clear debris from the mouth and oropharynx. All subjects asked to inhale a mist of 3% hypertonic saline solution (sterile) by ultrasonic nebulizer. Inhalation was continued until an adequate sputum sample (minimum 3 ml) or maximum period 30 minutes. The nostrils were closed to prevent nasal inhalation. The inhalation of hypertonic saline was interrupted every 5 minutes, so the patient could expectorate sputum. The implementation of induction nebulizer will be monitoring the patient clinical state and always O, tube and resuscitation equipment.

Ambroxol group performed 3 times sputum collection, including: Sputum S1 was collected the first time visiting. Sputum P was collected the morning of the second day, immediately after waking, before breakfast. And sputum S2 was collected the second day after 6 hours submitted sputum in the morning. The nebulizer induction group 3 times sputum collection also. Sputum S1 was collected first visit and prior to the nebulizer. Sputum P was collected conducted by hypertonic saline nebulizer induction for 5-30 minutes. If the patient could't produce the sputum was be hospitalized overnight at lung ward, was conducted nebulizer 3 times a day. At the following morning (before breakfast) was asked gargle normal saline (NaCl 0.9% solution) to clean detruitus oral cavity. Patients were asked to collect sputum to put in a container. The sputum S2 was collected 6 hours after sputum induction in the morning.

RESULT

1. General description of subjects

129 referral suspected pulmonary TB patients. 89 respondents who fulfilled the inclusion criteria and 40 respondents are excluded because including exclusion criteria. 76 patients agree and 13 patients refused the study. After having history, physical examination and chest x-ray. The patients will be divided into 2 groups according computer program that received ambroxol HCl drug was 38 patients and received hypertonic saline induction was 38 patients. General description of study subjects are presented in the following table:

Table 1. Distribution frequency age group subjects

Age (years)	Ambroxol group (*n*=38)	Nebulizer induction group (*n*=38)
18 – 29	16 (42.1%)	16 (42.1%)
30 - 39	9 (23.7%)	9 (23.7%)
40 - 49	4 (10.5%)	4 (10.5%)
50 - 55	9 (23.7%)	9 (23.7%)
Mean ± SE (years)	35.71 ± 2.041	35.71 ± 2.041

p-*value = 0.401, no significant difference between age of treatment groups*

No significant difference between age of the treatment group.

Table 2. Distribution frequency gender subjects

Sex	Ambroxol group (*n*=38)	Nebulizer induction group (*n*=38)
Male	20 (52.6%)	23 (60.5%)
Women	18 (47.4%)	15 (39.5%)

p-*value = 0.488, no significant gender differences to the treatment group*

No significant gender differences on the treatment group.

2. Sputum volume

The initial sputum volume (S1) was differ from the morning (P). Sputumvolume before giving ambroxol (S1) differ than sputum volume after administration of ambroxol sputum S2. Sputum volume at the morning (P) differ than the sputum S2 sputum. It appears the morning sputum volume was the highest sputum volume produced.

The hypertonic saline nebulizer induction group the initial sputum volume (S1) was different than the morning sputum volume (P). Also sputum volume (S1) was different than 6 hours after sputum

collection time (S2). The morning sputum volume (P) differ than collected after 6 hours post-induction (S2). The highest produce sputum volume at the nebulizer induction time or morning.

A. Comparison of sputum volume between ambroxol and hypertonic saline nebulizer induction group

Table 3. Comparison of sputum volume between ambroxol and hypertonic saline nebulizer induction groups

Collection time	Ambroxol group	Nebulizer induction group	*p* value
S1	1.316 ± 0.1257*	1.763 ± 0.1796**	0.805
P	3.789 ± 0.3308*	8.803 ± 0.7523**	<0.001***
S2	2.605 ± 0.2248*	5.171 ± 0.5868**	<0.001***

Description: S1 = the sputum volume at the initial time sputum collection (before treatment), P = sputum volume at the time of sputum collection time/ morning; S2 = sputum volume at the time of sputum collection 6 hours post-treatment/ morning
n *= 38 respondents, the data in mean ± 2SE (ml)*
**Wilcoxon Signed-Ranks Test significantly different between ambroxol groups if* p*-value <0.05*
***Wilcoxon Signed-Ranks Test was significantly different between nebulizer induction groups if* p *value <0.05*
****Mann-Whitney U-Test significantly different between ambroxol and hypertonic saline nebulizer induction groups if* p*-value<0,001*

Both ambroxol and hypertonic saline nebulizer induction group were significantly different from to increasing sputum volume between initial sputum volume (S1) than morning sputum volume/time (P) or the 6 hours post induction sputum volume (S2), so it was concluded the sputum volume in each group increased significantly.

B. The sputum volume test between ambroxol and hypertonic saline nebulizer induction groups no pair

Initial sputum volume S1 between ambroxol and hypertonic saline nebulizer induction group. The comparison of initial sputum volume (S1) between ambroxol and hypertonic saline nebulizer induction group were no significant difference in initial sputum volume group.

Sputum volume P (morning) between mucolytic ambroxol and induction hypertonic saline nebulizer group. Comparison of morning sputum volume (P) between ambroxol and hypertonic saline nebulizer induction sputum group were significant differences. Sputum volume after treatment (P) showed the sputum volume hypertonic saline nebulizer induction more than ambroxol group (Table 3).

Sputum volume S2 between ambroxol and hypertonic saline nebulizer induction group. It was significant differences sputum volume (S2) between ambroxol and hypertonic saline nebulizer induction group, the sputum volume hypertonic saline nebulizer induction group more than the ambroxol group (Table 3).

C. Comparison of difference increase sputum volume between ambroxol and hypertonic saline nebulizer induction groups

The excess morning sputum volume (P) with initial sputum volume (S1) are significant differences between each other. Also it significant differences between the sputum volume S2 than initial sputum volume (S1). Similarly, sputum volume S2 than morning sputum volume (P). It can be concluded significant differences between sputum volume ambroxol than hypertonic saline nebulizer induction groups, nebulizer induction group was higher than ambroxol group.

Table 4. The difference between sputum volume ambroxol and nebulizer induction groups

The difference	*p*-value
Δ Sputum volume P-S1	0.001*
Δ Sputum volume S2-S1	0.001*
Δ Sputum volume S2-P	0.001*

**Mann-Whitney U Test was significantly different if* p*-value <0.05*

3. AFB Count

Some respondents of study before treatment were found smear findings each group. At initial sputum (S1) either 4 respondents ambroxol group found positive sputum smear and hypertonic saline nebulizer induction group 2 respondents. Finally, the ambroxol group had 34 respondents and 36 respondents nebulizer induction group.

A. Comparative AFB count between ambroxol group and hypertonic saline nebulizer induction groups pairs

Both groups AFB count were increased significantly especially the morning sputum (Table 5).

B. Comparative AFB count ambroxol and hypertonic saline nebulizer induction groups unpaired

No significant difference either initial collecting sputum (S1) was compared than during induction/morning (P) and 6 hours post-treatment/morning sputum (Table 5). Initial sputum (S1), both groups no found AFB (Table 5).

C. Comparison of AFB count increased difference between ambroxol and hypertonic saline nebulizer induction groups

Table 5. Comparative pairs AFB count between ambroxol and hypertonic saline nebulizer induction group

Collecting time	Ambroxol group	Nebulizer induction group	*p*-value
S1	0*	0**	1
P	159.85 ± 78.254	374.17 ± 150.091	0.938
S2	133.59 ± 75.337	248.33 ± 93.420	0.702

Description: S1 = AFB count sputum smear at initial time (before treatment), P = AFB count sputum smear at collection time/ morning; S2 = AFB count sputum smear at the time of taking 6 hours posttreatment/morning

n *= 34 ambroxol group respondents and* n *= 36 hypertonic saline nebulizer induction group respondents, the data in the form of mean ± 2SE (AFB count per 100 fields of view), Mann-Whitney U-Test was significantly different if* p*-value <0.05*

**Wilcoxon Signed-Ranks Test significantly different S1 was compared than P and S2 if* p*-value <0.05*

***Wilcoxon Signed-Ranks Test significantly different S1 was compared than P and S2 if* p*-value <0.05*

The AFB count initial sputum smear (S1) and the morning sputum (P) was no significant difference each other. No significant difference in the number of initial sputum smear (S1) than the 6 hours post treatment sputum smear (S2). Similarly, the number of 6 hours post treatment sputum smear (S2) to the number the morning sputum smear (P). The concluded no significant AFB count difference between ambroxol and hypertonic saline nebulizer induction groups (Table 5).

The AFB count at morning sputum (P) and initial sputum (S1) was no significant difference each other. No significant difference in the excess AFB count smear 6 hours post-treatment/morning sputum (S2) than initial sputum smear (S1). Also it's similarly, the AFB count post-treatment sputum (S2) than the morning sputum (P). It was concluded no significant difference AFB count between ambroxol than hypertonic saline nebulizer induction groups (Table 6).

Table 6. The difference AFB count smear between ambroxol and nebulizer induction Groups

The difference	*p*-value
Δ AFB count P-S1	0.938
Δ AFB count S2-S1	0.702
Δ AFB count S2-P	0.881

**Mann-Whitney U Test was significantly different if p-value <0.05*

4. The percentage of success increased sputum volume

Only one person at ambroxol group can't exclude the sputum after ambroxol administration, it was increased sputum volume 97.37% (37/38) compared to before ambroxol treatment. All patients (100%) at hypertonic saline nebulizer induction group can increase sputum volume compared to before treatment.

5. The percentage success rate in AFB on ambroxol and hypertonic saline nebulizer induction group negative initially sputum (S1).

Percentage success rate to find AFB was different each group. Ambroxol group has 4 positive smear initial sputum respondents. Thirty four respondents whom initial sputum smear (S1) was negative. After ambroxol administration were 9 respondents found both sputum smear positive in the morning (P) and the second sputum (S2). It was increasing 26.47% (9/34) smear positive findings ambroxol group who previous negative smear.

Two respondents hypertonic saline nebulizer induction group whom the initial sputum (S1) found smear positive. Thirty four respondents initial sputum smear (S1) were negative. After nebulizer induction treatment were 10 respondents found both positivesputum smear in the morning (P) and 6 hours after morning sputum (S2). The percentage of success increased to finding positive smear was 27.78% (10/36) respondents hypertonic saline nebulizer induction previous negative smear.

DISCUSSION

1. Comparison of sputum volume

A. Comparison of sputum volume between ambroxol and hypertonic saline nebulizer induction groups pair

The results of sputum collection in the ambroxol group was increased sputum volume after administration of ambroxol compared before drug administration. Because to ambroxol was an expectorants mucokinetic to facilitate or to stimulate the disposal of bronchi and tracheal secretions. The mucolytic physicochemical of secretions change, especially lowering the viscosity to be easily expelled by coughing[14]. Ambroxol has to process acid mucopolisaccharides pharmacokinetics so the fibers will be break down mucus. It was increasing the production of lysosomes and activates hydrolytic enzymes. It will be stimulated serous gland cells causes the viscosity of sputum secretions decrease, so it need process time for metabolism and distribution to the target organ[15,18,28].

Meanwhile the hypertonic saline nebulizer induction group to increase sputum volume significantly, the results of sputum collection was increasing sputum volume induction compared to the sputum volume before treatment. The hypertonic saline nebulizer induction to decrease involvement of mucus in the airways. A hydrated mucus in the airways would be more easily moved by mucociliary and cough increased volume secretion[21,23,24,29]. Hypertonic saline solution cause bronchial irritation and stimulates the secretion of bronchi. After 10-20 minutes nebulizer will be to mobilize fluid production from lower respiratory tract. Repeated coughing helps to move the product into the trachea assisted with the expectorant[19,30].

Both groups increased sputum volume, especially the morning or sputum induction time of the nebulizer. Although it was significant differences each other groups, but comparison between groups initial sputum volume (S1) between the two groups was no significant.

Each group shows that significant differences after administration of ambroxol and after induction to increase the sputum volume product. The high difference significantly in sputum volume was increased in the morning (P) and sputum S2 each groups. The ambroxol group was increasing sputum volume, but the increased sputum volume hypertonic saline nebulizer induction group more than it.

The ambroxol group to increase sputum volume of 97.37% compared to before treatment and the hypertonic saline nebulizer induction group to increase all suspected pulmonary tuberculosis patients (100%) compared to prior induction treatment.

B. Comparison of sputum volume between ambroxol and hypertonic saline nebulizer induction groups no pair

Comparison of initial sputum volume (S1) between ambroxol and hypertonic saline nebulizer induction groups was no significant difference. The initial sputum volume (S1) in both groups did not differ significantly. Preliminary data showed same relatively, because it has been randomized.

Comparison of morning sputum volume (P) between ambroxol and hypertonic saline nebulizer induction group were significant differences. Sputum volume after treatment showed hypertonic saline nebulizer induction group more than ambroxol group (Table 3). The hypertonic saline nebulizer induction will be effect faster and more immediately[19], it was differ the effects of expectorant mucolytic drugs must be through the pharmacokinetic mechanism (absorption, distribution, metabolism and excretion) so requires a time[14]. Ambroxol has the effect of spur secretion of fluid in conditions of moderate to strong stimulated, to maintain viscosity and sputum volume in the medium stimulated, because ambroxol stimulates the secretion of ions Cl^- through Naz / Kz /2Cl^- cotransporter at the basal condition, while the under basal conditions ambroxol will be stop the secretion of Cl^- and $HCO3^-$ and does not to affect to the anions secretion[31].

No significant differences between sputum volume (S2) ambroxol and hypertonic saline nebulizer induction group, which the sputum volume nebulizer induction group more than ambroxol group (Table 3).

The useful of hypertonic saline will be more sputum volume production, because it cause irritation to the mucosal bronchial and to stimulate the secretion of bronchi. The putative mechanism of action to improve mucociliary clearance and or increase osmolarity fluid of respiratory tract, where to increase vascular permeability mucosal bronchi, and submucosal glands produce mucus spur. After 10-20 minutes nebulizer to mobilizes fluid production from lower respiratory tract products and induction effects of nebulizer will be decrease 4 hours later[19,32].

According to Rogers[25] and Rubin[33] that expectorant mucokinetics, like: ambroxol and hypertonic saline to work through the sputum transportation when coughing. The mechanism of action alleged by kinesis mucus and coughing can be removed mucus from lower respiratory tract. *â*2-adrenoceptor agonist will be stimulate increase airflow and ciliary beat, Cl^-/water secretion and mucin, increasing the sputum volume and surfactant reduces mucus adhesions to epithelium caused the sputum airway easy to exit.

The inflammatory process due to bacterial infection occur mucus hypersecretion, cilia dysfunction and changes in the composition of respiratory secretions. It contains inflammatory cells, especially neutrophils. The presence of neutrophils from the release of proinflammatory mediators by epithelial airway damage. It caused the DNA release and filamentous actin (F-actin) and the cytoskeleton of DNA and F-actin copolymerize. Mucin degradation in polymers, the release of DNA and actin network. It causes to increase sputum secretion and to decrease sputum viscosity, so resulting in decreased adhesion to the airway surface epithelium. It was caused sputum become unsticky to adhesion the respiratory tract surface and to cough cause sputum easily out include the AFB contained[25,34].

2. Comparison of AFB finding

Ambroxol, as an expectorant to increase volume and thin sputum, was making it easier for spending sputum from branches of the terminal bronchial. The sputum had contained acid mucopolisaccharides fibers and mucin, could complicate capture mycobacteria cells making it difficult to detect[1,5,11]. Mucolytic expectorant will be describe the bonding acid mucopolisaccharides fibers so that sputum becomes more dilute and reduced viscosity. To provide sputum release for mycobacteria which it trapped by acid mucopolisaccharides and mucus that are easier to find[14,15]. After ambroxol treatment led smear easy to find, was reflected significant differences in the AFB number of smear before giving ambroxol compared after administration of ambroxol primarily on sputum smear rates in the morning or at any rate of sputum smear (S2). Because it possible coincided with the melting of the lesions or tubercles next day, especially at the morning[2,7,9]. Mucolytic has the pharmacokinetic effects, are significant differences in the increase in sputum volume. It is will be to increase sputum volume but did not always correlate with increased AFB count of smear. The peak of mucolytic ambroxol levels (*Tmax*) <1.6 hours in plasma despite having a long half-life of 10 hours, the effect will be decrease pharmacokinetic through oxidative metabolism was triggered by cytochrome P450 CYP3A4 so mucolytic effect to decline followed a decrease of decomposition of acid mucopolisaccharides also followed by a decrease mucus bronchi biodegradable fibers that led to the discovery of smear rates declined as well[14,25].

Ambroxol effects through coughing effectiveness will spur surfactants secretion. The mechanism of action through the ability mucokinetic, mucociliary activity, stimulate the production of surfactants, anti-inflammatory and antioxidative action alleged role in the ability to stimulate the secretion of bronchi[15].

Comparative between the initial sputum smear (S1) is different from the time of the induction rate of sputum smear hypertonic saline nebulizer in the morning (P). The mechanism of mucolytic was to decrease involvement mucus in the airways. The mechanism of nebulizer induction in osmolarity through the saline solution on epithelium airway will be inhaled and followed by the increase in water containing airway mucus. A hydrated mucus in the airways increased volume secretion would be more easily moved by mucociliary and cough[21,23,24,29,35]. Hypertonic saline can also to separate the DNA from the infected mucin in the mucus, through a reduction in mucus viscosity. Hypertonic solution cause bronchial irritation and stimulates secretion of bronchi. After 10-20 minutes nebulizer occurs that mobilizes fluid product from lower respiratory tract. Repeated coughing can helps to move the product into the trachea, including AFB that trapped in mucopolisaccharides acids that can be helped with expectorant, so the discovery of a larger AFB smear[19,30]. Nebulizer induction was an effective method as the initiation to discovery of suspects pulmonary tuberculosis who can't remove sputum or previous negative smear examination results. The chest x-ray on suspected pulmonary tuberculosis patients who have cavitation and infiltrates will be to increase 19.9% the findings AFB sputum through the hypertonic saline induction[36].

The hypertonic saline nebulizer induction group smear rates prior to the nebulizer (S1) is different from the AFB count smear after induction (NaCl 3% solution) during or initial morning (P). While AFB count prior to the nebulizer (S1) was similar collected after 6 hours AFB sputum collection time/ morning S2. Similarly, the AFB count smear during induction nebulizer or morning (P) was not different from the AFB count smear was collected after 6 hours post-nebulizer induction using hypertonic saline S2.

This study shows the AFB findings in the initial sputum (S1) was not different than the AFB count smear after 6 hours nebulizer (S2). Similarly, AFB count findings at the time of induction nebulizer (P) than AFB count after 6 hours nebulizer (S2). The majority of respondents had performed induction nebulizer only one time with a nebulizer induction will be to increasing AFB count smear sputum, although there were no significant differences in

the AFB count smear rates after nebulizer 6 hours (S2). After 10-20 minutes fluid production will be mobilizing product of lower respiratory tract. Repeated coughing will be helps moving the product into the trachea[30,37].

It has relationship between to find leukocytes and neutrophils increased with the AFB discovery smear in pulmonary tuberculosis patients diagnosed after bronchoscopy are accompanied fever[41]. The AFB count smear nebulizer or morning (P) was not different from AFB count smear was collected after 6 hours postnebulizer induction use hypertonic saline (S2). Similarly, the AFB count smear prior to the nebulizer (S1) was similar collected after 6 hours AFB count sputum collection time/morning S2.

The mechanism action nebulizer induction made solution will become droplets. Smaller droplets will be deposited into the peripheral lung. Therefore hypertonic saline fluids used for deposition through a process of osmosis to interstitial fluid to the lower respiratory tract. Hypertonic saline solution will cause irritation to spur secretion of the bronchi. After 10-20 minutes nebulizer bronchi secretion will be mobilize material from lower respiratory tract, including AFB. Repeated coughing will help transfer the material into the trachea functions as an expectorant[42]. Some studies have reported that repeated sputum induction after initial induction between 8-24 hours would cause an increase neutrophils count in the second sample, so it isn't recommended sputum induction with a nebulizer repeated[38,39]. The 48 hours interval time between nebulizer induction will be to provide a significant number of normal cells in individuals, so many studyers to advise a minimum time limit for repeat nebulizer induced sputum isn't more than 2 days to avoid the effects of "carry-over" of induction[40]. Sputum induction results to contain a high concentration of liquid phase components such as eosinophil cationic proteins (ECP), mucin glycoprotein and albumin than in BAL. It was indicates the existence from respiratory secretions than space alveolar[43].

The sputum smear, which found bacteria, is a mucin protein-rich complex medium and a DNA degradation product. The nebulizer induction produce sputum contains many mediators compared than bronchoalveolar lavage. Finding AFB smear rates between the two groups are significant differences. The ambroxol group to find smear rate more survive either the morning sputum (P) and post-administration drugs, because ambroxol has half-life long enough and high volume distribution[15].

Comparative sputum volume between ambroxol and hypertonic saline nebulizer induction groups no pair Comparison of initial sputum volume (S1) between ambroxol and hypertonic saline nebulizer induction groups were no significant difference, because it was randomized.

Comparison of morning sputum volume (P) between ambroxol and hypertonic saline nebulizer induction groups were significant differences. The sputum volume hypertonic saline nebulizer induction group more than the ambroxol group (Table 5).

Because hypertonic saline nebulizer induction effect was faster and more immediately[19], was different the effects of expectorant mucolytic drugs that through the pharmacokinetic mechanism (absorption, distribution, metabolism and excretion) so requires a certain time[14]. Surface ambroxol molecules and its metabolites were not found in electron-deficient regions, so many compounds can't react with glutathione and DNA nucleobase. The existence of electron-rich region on the surface of the lower respiratory tract and induction effects will be decline after 4 hours[19,32].

The comparison AFB count between ambroxol and hypertonic saline nebulizer induction nebulizer groups

The AFB count sputum smear S1 ambroxol and hypertonic saline nebulizer induction groups. The statistical test of AFB count initial sputum/before treatment (S1) ambroxol and hypertonic saline nebulizer induction groups are no significant difference (p-value = 0.639), it were relatively equal smear both groups because it was randomized. The AFB finding on sputum smear before treatment (S1) because more careful examination and appropriate procedures for sputum examination in the hospital compared to previous primary care. According to

Sakundarno12 the quality of sputum was necessary to find the AFB of smear, so training needed to obtain high quality sputum for laboratory examination. If poor quality and quantity of sputum, to find AFB also lacking. According to ATS9, Siddiqi et al.10 and WHO7, the quality of sputum and microscopic preparations, either increase the sensitivity up to 80% compared than smear culture method.

The AFB finding morning sputum (P) smear between ambroxol and hypertonic saline nebulizer induction groups did not differ significantly. Although was a significant increase in sputum volume at hypertonic saline nebulizer induction compared than ambroxol group, but this was not followed always increase the AFB findings smear. The mechanism of bronchial mucosal stimulation to irritation can increase sputum volume but not always increase the AFB count findings, because the AFB need acid mucopolisaccharides decomposition. The previous study (Williams *et al*[45]. Brown *et al*[46]; Uskul *et al*[47]) was showed the induced sputum, gastric washings and bronchoalveolar lavage did not differ findings smear rates despite increased sputum volume induction but hypertonic saline nebulizer induction cheaper and more effective than other procedures.

The AFB findings post-treatment sputum smear (S2) in both groups was not significant, although there were significant differences in sputum volume between each groups. It was to increase sputum volume hypertonic saline nebulizer induction group compared than ambroxol group. It can be explained that the nebulizer induction to increase sputum volume, but wasn't always followed by increasing AFB findings. Because it has other processes can influence AFB findings, such as acid mucopolisaccharides bond decomposition.

The ambroxol and nebulizer induction groups had significant differences to AFB finding initial sputum compared than the morning sputum/during sputum induction and 6 hours after morning sputum. Statistical analysis showed that the data was no significant difference increased BTA findings both ambroxol and nebulizer induction groups. Unpaired statistical analysis showed no significant difference either groups.

3. The percentage of success increased sputum volume

Ambroxol group was increasing sputum volume 97.37% compared to before giving drugs, while the hypertonic saline nebulizer induction group to increase entirely (100%) to all suspected pulmonary tuberculosis patients sputum volume increase compared to before hypertonic saline nebulizer induction. It was not different to other study. According to Gupta and Garg19 the success of 97% from the quality of less than 2 ml previous sputum.

4. The percentage of success increased AFB count

Ambroxol group has to increase AFB findings to smear of 26.47% compared to prior treatment, while hypertonic saline nebulizer induction group will be increase smear findings of 27.78% compared to before induction previous negative smear. It was not different previous studies at 19-42%, like: 19% (Anderson *et al*[48].); 25% (Parry *et al*[23].; Merrick *et al*[49].); 33.9% (Li *et al*[20].); 34% (Conde *et al*[50].); 42% (Hartung *et al*[51].); 38% (Gupta and Garg[19]) and 19.9% (Garcia *et al*[36].). Mechanism of action of saline as mucolytic to reduction involving the mucus respiratory tract. The fluid osmolarity will be dilution during hypertonic saline inhalation. Because the hydrated mucus respiratory will be facilitate movement by mucociliary clearance was accompanied by increased sputum volume. Hypertonic saline solution will be separating DNA mucus from the infected mucus smear, causes to decrease viscosity mucus[25].

Statistical analysis showed significant differences in AFB smear findings in each group like increased sputum volume, where the nebulizer induced group more than ambroxol. Improving AFB finding, especially the sputum collection in the morning, either ambroxol or nebulizer induction.

CONCLUSION

The expectorant ambroxol or saline induction hypertonic nebulizer will be to increase sputum volume and AFB count findings on suspected pulmonary tuberculosis patients. It was significant differences between the provision to increase

sputum volume between ambroxol and hypertonic saline nebulizer induction, where nebulizer induction more than ambroxol. No significant difference to increase AFB findings expectorant between ambroxol and hypertonic saline nebulizer induction. 97.37% ambroxol patients group and all patients (100%) hypertonic saline nebulizer induction group can increase quality and quantity sputum compare previous treatment. 26.47% patients ambroxol group were found positive smear and 27.78% patients hypertonic saline nebulizer induction group compared than previous negative smear.

SUGGESTION

The suspects pulmonary tuberculosis patients, who can't remove sputum or less than 2 milliliters advise to take ambroxol first, if failed to proceed hypertonic saline nebulizer induction. The continued study development with a higher standard smear findings (ie. culture smear).

ACKNOWLEDGEMENTS

The success of this study on supported fund and study facilities by the East Kalimantan provincial government. We thank Doni Irfandi Alfian, MD., Emil Bachtiar Moerad, MD., Mrs. Antasari and Mr. Yatman to assist the implementation of study; Sjarif Ismail, MD.,M.Kes, Zulaela, Dipl.Med.Stat,M.Si and Dewi Ismimasitoh (CE&BU staff FM GMU), which provide inputs in the data study processing, Mrs. Zaimah and all microbiology laboratory staff at A. Wahab Sjahranie General Hospital. The author to thank all who helped and involved in this study.

Ethical approval**:** The study protocol was approved by the ethical review committee of the Faculty of Medicine, Universitas Gadjah Mada, Indonesia.

REFERENCES

1. World Health Organization. The Diagnostic Pipeline, Tuberculosis Diagnosis And Drug Sensitivity Testing. Stop TB Department. Geneva: World Health Organization, 2006; 4-5.
2. MOH-ROI. Nasional Guideline for Tuberculosis Treatment. Jakarta: Ministry of Health Republic of Indonesia, 2007.
3. World Health Organization. The Global Burden of TB, Global Tuberculosis Control A Short Update To The 2009 Report. Stop TB Department. Geneva: World Health Organization, 2009;4-6.
4. World Health Organization. Indonesia. Global Tuberculosis Control, WHO Report. New Delhi: World Health Organization, 2008;113-6.
5. World Health Organization. Bacteriological Results, Treatment of Tuberculosis Guidelines. Stop TB Department. Geneva: World Health Organization, 2010; 25-7.
6. Indonesian Pulmonologist Association. Diagnosis. Tuberculosis Guidelines Diagnosis & Treatment in Indonesia. Jakarta, 2002.
7. World Health Organization. Tuberculosis, Pulmonary–diagnosis. Toman's Tuberculosis Case Detection, Treatment, and Monitoring–Questions and Answers. Geneva: World Health Organization, 2004.
8. MOH-ROI. East Kalimantan Province Health Profile 2008. Available from: URL:http://www.depkes.go.id
9. American Thoracic Society. Diagnostic Standards and Classification of Tuberculosis in Adults and Children. Am J Respir Crit Care Med, 2000;161:1376-95
10. Siddiqi K, Lambert M-L, Walley J. Clinical diagnosis of smear-negative pulmonary tuberculosis in low-income countries: the current evidence. Lancet, 2003;3:288.
11. Pfyffer GE. Mycobacterium: General Characteristics, Laboratory Detection and Staining Procedures. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, editors. Manual of Clinical Microbiology, Volume 1, 9th ed. Washington: ASM Press, 2007:543 72.
12. Sakundarno M, Nurjazuli N, Jati SP, Sariningdyah R, Purwadi S, Alisjahbana B, van der Werf MJ. Insufficient quality of sputum submitted for tuberculosis diagnosis and associated factors, in Klaten district, Indonesia. BMC Pulmonary Medicine, 2009;9(16): 1-11.
13. Nolte FS, Metchcock B. Mycobacterium. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, editors. Manual of Clinical

Microbiology, 6th ed. Washington, D.C.: ASM Press, 1995:400-37.

14. Mutschler E. Arzneimittelwirkungen, 5 vollig Neubearbeitete und Erweiterte Auflage. [Translate] Widianto MB, Ranti AS. Dinamika Obat Buku Ajar Farmakologi dan Toksikologi. 5thed. Bandung: ITB Press, 1999.
15. Malerba M, Ragnoli B. 2008. Ambroxol in The 21st Century: Pharmacological and Clinical Update. Expert Opin Drug Metab Toxicol 2008;4(8):1119-29.
16. Seligman. Mucolytics. In: Kacmareck, Stoller, editors. Current Respiratory Care. Philadelphia: B.C. Decker, 1988:41-5.
17. Guyatt GH, Townsend M, Kazim F, Newhouse MT. A controlled trial of ambroxol in chronic bronchitis. Chest, 1987;92:618-20.
18. Disse B. Clinical evaluation of new therapies for treatment of mucus hypersecretion in respiratory diseases. Ingelheim: Novartis Found Symp, 2002;248:254-82.
19. Gupta KB, Garg S. Use Of Sputum Induction For Establishing Diagnosis In Suspected Pulmonary Tuberculosis. Indian J Tuberc, 2005;52:143-6.
20. Li LM, Bai LQ, Yang HL, Xiao CF, Tang RY, Chen YF, Chen SM, Liu SS, Zhang SN, Ou YH, Niu TIM. 1999. Sputum induction to improve the diagnostic yield in patients with suspected pulmonary tuberculosis. Int J Tuberc Lung Dis, 1999;3(12):1137–9.
21. Kawada H, Suzuki N, Takeda Y, Takahata M, Suzuki T, Kobayashi N, Kudo K, Kabe J. 1996. The usefulness of induced sputum in the diagnosis of pulmonary tuberculosis. Kekkaku, 1996;71(11):603-6.
22. Chang KC, Leung CC, Yew WW, Tam CM. Supervised and induced sputum among patients with smear-negative pulmonary tuberculosis. Eur Respir J, 2008;31(5):1085–90.
23. Parry CM, Kamoto O, Hurries AD, Wirima JJ, Nyirenda CM, Nyangulu DS, Hart CA. The use of sputum induction for establishing a diagnosis in patients with suspected pulmonary tuberculosis in Malawi. Tuberc Lung Dis, 1995;76:72-6
24. Yue WY, Cohen SS. Sputum Induction by Newer Inhalation Methods in Patients with Pulmonary Tuberculosis. Chest, 1967;51:614-20.
25. Rogers DF. Mucoactive Agents for Airway Mucus Hypersecretory Diseases. Respiratory Care, 2007;52(9):1176-97.
26. Harun SR, Putra ST, Wiharta AS, Chair I. Uji klinis. In: Sastroasmoro S, Ismael S (Ed.): *Dasar-Dasar Metodologi Penelitian Klinis*. Jakarta: Sagung Seto, 2006; 144-64.
27. Cummings SR, Grady D, Hulley SB. Designing a Randomized Blinded Trial. In: Hulley SB, Cummings SR, Browner WS, Grady DG, Newman TB, editors. Designing Clinical Study, 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2007:147-61.
28. Sharma MK. Role of Mucokinetics in Respiratory Medicine, 2008: Available from: URL:http://www.cdc.gov
29. Economidou F, Samara KD, Antoniou KM, Siafakas NM. Induced Sputum in Interstitial Lung Diseases: Novel Insights in the Diagnosis, Evaluation and Study. Respiration, 2009;77:351–8.
30. Fireman E. Induced Sputum as a Diagnostic Tactic in Pulmonary Diseases.Pulmonology Update IMAJ, 2003;5:524-7.
31. Hasegawa I, Niisato N, Iwasaki Y, Marunaka Y. Ambroxol-induced modification of ion transport in human airway Calu-3 epithelia. Biochem Biophys Res Commun, 2006;343:475-82.
32. Sobiecka M, Kus J, Demkow U, Filewska M, Jozwik A, Rohrenschef PR, Wynimko JC. Induced Sputum In Patients With Interstitial Lung Disease: A Non- Invasive Surrogate For Certain Parameters In Bronchoalveolar Lavage Fluid. J Physiol Pharmacol, 2008;59(6 Suppl):645-57.
33. Rubin BK. Mucolytics, Expectorants and Mucokinetic Medications. Respiratory Care, 2007;52(7):859-64.
34. Rubin BK. The Role of Mucus in Cough Study. Lung, 2009;188(Suppl 1):S69–S72.

35. Rogers DF. Airway Clearance: Physiology, Pharmacology, Techniques, and Practice. The 39th Respiratory Care Journal Conference. Cancu´n, 2007 April 21–23.
36. Garcia SB, Perin C, da Silveira MM, Vergani G, Menna-Barreto SS, Dalcin PTR. 2009. Bacteriological analysis of induced sputum for the diagnosis of pulmonary tuberculosis in the clinical practice of a general tertiary hospital. Am J Respir Crit Care Med, 2009;179:A2600.
37. Gupta KB, Garg S. Sputum induction - A useful tool in diagnosis of respiratory diseases. Lung India, 2006;23:82-6.
38. Nightingale JA, Rogers DF, Barnes PJ. Effect of repeated sputum induction on cell counts in normal volunteers. Thorax, 1998;53:87-90.
39. Holz O, Richter K, Jörres RA, Speckin P, Mücke M, Magnussen H. Changes in sputum composition between two inductions performed on consecutive days. Thorax, 1998;53:83-86.
40. Purokivi M, Randell J, Hirvonen M-R, Tukiainen H. Reproducibility of measurements of exhaled NO, and cell count and cytokine concentrations in induced sputum. Eur Respir J, 2000;16:242-6.
41. Um SW, Choi CM, Lee CT, Kim YW, Han SK, Shim YS, Yoo CG. Prospective Analysis of Clinical Characteristics and Risk Factors of Post-bronchoscopy Fever. Chest, 2004; 125(3):945-52.
42. Zahrani KA, Jahdali HA, Poirier L, Rene 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.
43. Alexis NE, Hu SC, Zemen KAT, Bennett WD. 2001. Induced sputum derives from the central airways. Am J Respir Crit Care Med, 2001;164: 1964–70.
44. Huq F. Molecular modelling analysis of the metabolism of ambroxol. J. Pharmacol Toxicol, 2008;3(2):147-52.
45. Williams TM, Wells AU, Harrison AC, Lindstrom S, Cameron RJ, Forkin E. Induced sputum and bronchoscopy in the diagnosis of pulmonary tuberculosis. Thorax, 2002; 57:1010-14.
46. 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.
47. Uskul BT, Turker HT, Kant A, Partal M. Comparison of Bronchoscopic Washing and Gastric Lavage in the Diagnosis of Smear-Negative Pulmonary Tuberculosis. *The Southern Medical Association*, 2009;102(2):154-8.
48. Andersen C, Inhaber N, Menzies D. Comparison of sputum induction with fiberoptic bronchoscopy in the diagnoses of tuberculosis. Am J Respir Crit Care Med, 1995;152:1570-4.
49. Merrick ST, Sepkowitz KA, Walsh J, Damson L, McKinley P, Jacobs JL. Comparison of induced versus expectorated sputum for diagnosis of pulmonary tuberculosis by acid-fast smear. Am J Infect Control, 1997;25:463-6.
50. Conde MB, Soares SLM, Mello FCQ, Rezende VM, Almeida LL, Reingold AL, Daley CL, Kritski AL. Comparison of Sputum Induction with Fiberoptic Bronchoscopy in the Diagnosis of Tuberculosis: Experience at an Acquired Immune Deficiency Syndrome Refence Center in Rio de Janeiro, Brazil. Am J Respir Crit Care Med, 2000;162:2238–40.
51. Hartung IK, 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.