Determination of Asbestos Bodies in Bronchoalveolar Lavage Fluids in Thailand

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***Objective**: Asbestos bodies (AB), ferroprotein-coated asbestos fiber, may be present in bronchoalveolar lavage fluid (BALF) of asbestos exposed persons. The present study was conducted to evaluate the prevalence and number of asbestos bodies in the BALF of tenable asbestos exposed workers compare to general population in Thailand.*
***Material and Method:** Thirty workers of cement pipe and roof tile factories using chrysotile asbestos and 30 unexposed patients that underwent diagnostic bronchoscopy were included in this study. Determination of asbestos bodies was made by membrane filtration method as described in earlier reports.*
***Results:** The findings were positive in six workers and in one control subject (0.1-3.6 vs. 0.2 AB/ml of BALF, p = 0.449).*
***Conclusion:** AB was identified in workers more often than in pulmonary disease patient. Two of workers had more than 1 AB/ml of BALF.*

***Keywords:** Asbestos, Asbestos bodies, Bronchoalveolar lavage, Lung fluid*

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Asbestos, a group of natural fibrous silicates, has been imported for industrial use in Thailand for over 75 years, mostly for the manufacture of asbestos cement products e.g. asbestos cement sheets, roof tiles, and cement pipe. Exposure to asbestos has long been known to affect human health, especially on people involved in extensively exposed occupation[1,2].

The finding of Asbestos bodies (AB), a ferroprotein-coated asbestos fiber, in lung parenchyma will support the diagnosis of asbestos-related lung disease in suspicious case that has asbestos exposed history[3,4]. Moreover, several studies demonstrated that high number of AB from bronchoalveolar lavage fluid (BALF) in exposed subject was roughly correlated with the concentration of AB in lung parenchyma and related to high incident of asbestos-related disease in this group[13,14,23,24].

However, in Thailand, the case of asbestos-related disease such as asbestosis or asbestos airway disease has been rarely reported[5-8]. Most of mesothelioma cases in case series or case report are infrequently associated with obvious asbestos exposed history. There was a report of finding AB in the lungs in a good number of patients died of various non-asbestos-related diseases[6].

Therefore, the objective of the present study is to determine the AB in BALF in subjects who work in factories with a possibility of asbestos exposure and in general patients as control, with the hope to obtain the results that would be indicative of asbestos exposure and/or the prevalence of AB in BALF in general public.

Material and Method

The study was carried out at the Faculty of Medicine Ramathibodi Hospital, Mahidol University, between December 2012 and November 2013.

Thirty workers from two factories manufacturing asbestos-cement products and 30 patients who underwent routine diagnostic bronchoscopy for pulmonary diseases at the Division of Pulmonary and Critical Care Medicine, Department of Medicine. The informed consent was obtained after describing the study procedure to the subject. Personal

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data, including working and smoking history were collected by questionnaires (Table 1) and chest radiographs were taken in all workers.

Fiberoptic bronchoscopy was performed and BALF obtained in all 60 subjects. Collection of BALF from the right middle lobe bronchus was made after washing with 150 to 200 ml of normal saline solution.

A total leukocyte count was carried out via Neubauer chamber and differential cell count of the smear and Papanicolaou stain of centrifuged sediment of 10 ml of fresh BALF from workers group only. Fresh BALF from each patient, remaining and centrifuged fluid from each worker were mixed with 10 ml of sodium hypochlorite. The digested fluid was filtered through 0.45 micron diameter Millipore filter. The membrane was stained with Prussian blue, then mounted and covered with cover glass and examined under the light microscope. Asbestos bodies were counted and reported as AB/mL of BALF. According to prior study, asbestos bodies were identified by morphology consisting of transparent fibrous core coated with beaded ferro-protein with or without dumbbell-shaped end[3]. The method employed has been the acceptable standard for studying AB in BALF[9,24].

Statistical analysis

All values were expressed as mean, standard deviation (SD) or median, and range for continuous variables, number, and percent for categorical variables. To determine the association of independent variables with positivity of AB, continuous variables were compared using the nonparametric Mann-Whitney U test and χ^2 tests or the Fisher's exact test, in case of small expected frequencies, was used for comparisons of categorical variables.

All statistical tests were 2-sided, and $p<0.05$ was considered statistically significant. All data were analyzed with a statistical software package (SPSS, version 16.0 for windows; SPSS Inc.; Chicago IL).

Results

Basic demographic data and working history

Among 30 worker subjects, 29 were men ranging in age 24 to 66 years with the mean age of 50 (SD ±8.8) years old. Smoking status, exposure data, and chest radiographic results are displayed in Table 1. In the patients group, 10 were male and 20 were female with the mean age of 56 years old. Smoking status, occupation, and indication for bronchoscope are displayed in Table 2.

Table 1. Basic data and working history of the 30 worker subjects

Age (years)	
Range	24-66
Mean ± SD	49.8±8.8
Sex	
Male	29 (96%)
Female	1 (4%)
Smoking status	
Smoker	4 (14%)
Ex-smoker	13 (43%)
Non-smoker	13 (43%)
Working period (years)	
Range	2-41
Mean ± SD	24.0±10.9
CXR findings	
Unremarkable	25 (83%)
Reticulonodular opacity in upper lung field	3 (10%)
Basal lung fibrosis	2 (7%)

CXR = chest X-ray

Table 2. Basic data and occupation of the 30 patients

Age (years)	
Range	18-87
Mean ± SD	56.4±18.2
Sex	
Male	10 (33%)
Female	20 (67%)
Smoking status	
Smoker	5 (17%)
Non-smoker	25 (83%)
Occupation	
Student	2 (7%)
House keeper	12 (40%)
General employee	3 (10%)
Engineer	1 (3%)
Teacher	1 (3%)
Police	1 (3%)
Farmer	3 (10%)
Business owner	3 (10%)
Office worker	2 (7%)
Nurse	1 (3%)
Pharmacologist	1 (3%)
Indication for bronchoscopy	
Lung mass	12 (40%)
Infection	18 (60%)

AB identification in BALF

AB was present in the BALF of six workers, ranging from 0.1 to 3.6 AB/mL of BALF (Fig. 1). All of them had chest radiographic pictures within normal

limits. Among workers with negative BALFs, three of them had reticulonodular opacities in the upper lung fields and two had insignificant looking basal lung fibrosis.

AB was present in one patient, 0.2 AB/mL of BALF. The patient was a retired 83-years-old teacher who had been treated as a case of chronic obstructive pulmonary disease (COPD) cum lung infections, both as tuberculous and non-tuberculous for over two decades (Table 3).

Cellular profile from BALF

The number of inflammatory cell/mL of BALF in patients with pulmonary disease and exposed workers are $3.55x10^5$ ($1-65x10^5$) and $2x10^5$ ($1-21x10^5$), respectively. Profile of inflammatory cell in asbestos positive compare to asbestos negative in exposed workers is not significantly different ($p = 0.86$) (Table 4).

Discussion

The presence of AB in the lung parenchyma or in BALF confirms the exposure to asbestos. A study of AB quantification in BALF was performed in Belgium since 1982 by De Vuyst et al[10] followed by many others, mainly from European countries, USA, and Japan[10-31]. The AB identified varied from 33 to 100% of cases in exposed subjects with mean concentration ranging from 2.38 AB/mL up to over 1,000 AB/mL compared to less than one AB/mL found in general population in most studies[10-31]. According to these findings, there has been postulation that more than one AB per mL present in BALF represents at least 1,000 AB per gram of lung tissue[12,14].

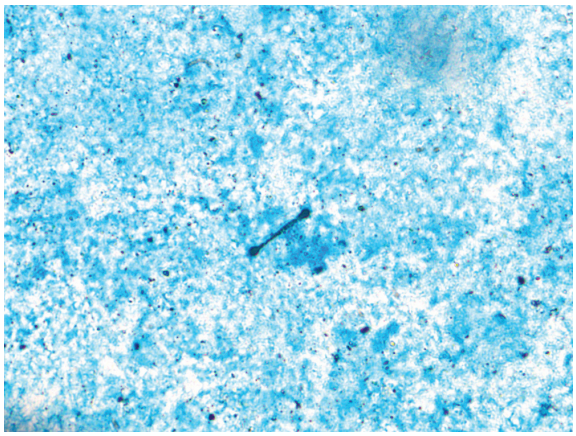

Fig. 1 Asbestos body identified from filtered membrane with Prussian blue stain under light microscope (400x).

In terms of disease correlation in asbestos exposed subject, the mean AB concentration was significantly higher in subjects with asbestosis more than subjects with mesothelioma, benign pleural disease, or in subjects without any disease[12]. However, some patients with asbestos-related diseases might show low AB concentration in BALF or even negative. In addition, even AB in BALF is roughly correlated with AB and asbestos fiber in lung parenchyma. However, negative AB in BALF in some subjects would contrarily contain a high parenchymal burden[13,14,23,24]. Because of the determination of AB from BALF would represent AB formed in the airways, asbestos bodies formed in the lung interstitium cannot be detected. Hence, the finding of asbestos bodies in BALF can merely indicate exposure but not asbestos-related diseases.

In the present study, asbestos body was identified in six of the 30 workers in factories using

Table 3. Asbestos body (AB) prevalence and concentration in exposed workers and patients

	Asbestos exposed workers (n = 30)	General population (n = 30)	*p*-value
AB positive	6/30 (20%)	1/30 (3.3%)	0.103
AB/mL			
Median	0.55	0.2	0.449
Range	0.1-3.6	0.2	

Table 4. Cellular profiles and working periods in exposed workers

	AB positive (n = 6)	AB negative (n = 24)	*p*-value
Cell count (x105), mean ± SD	5.8±2.1	5.5±4.3	0.860
Cell differentiation (%), mean ± SD			
Alveolar macrophage	83.6±4.1	87.5±4.4	0.875
Lymphocyte	9.3±1.3	7.3±3.7	0.220
Neutrophil	7.2±3.9	5.6±4.3	0.752
Working period (years), mean ± SD	28.3±9.5	22.8±11.1	0.242

chrysotile, presenting numbers ranging from 0 to 3.6 AB/mL with median of 0.55 and only two workers had concentration more than one AB/mL while asbestos bodies was found in only one among 30 general population with 0.2 AB/mL concentration. The prevalence of AB in BALF of workers is higher than in control subjects while the median value of positive cases in both groups is not different, which is possibly limited by the few subjects in the study. Furthermore, AB concentration from BALF of workers in the present study was not as high as other studies. This could be affected by several factors. First, the subjects in some studies had already some asbestos-related diseases, which usually contain high levels of asbestos fiber or asbestos bodies in their lungs. The subjects in the present study were apparently healthy without evidence of asbestos-related diseases and they were randomly included into the study with varied job description regarding exposure intensity, which could affect the exposure and accumulation. Second, because of chrysotile, less hazardous asbestos is being the only fiber type used in Thai industries. The chrysotile fibers are easy to fragment and tend to be eliminated from the air passage and the lung parenchyma. They are less likely to form asbestos bodies.

A significant increase of variable leukocytes in AB-positive subjects had been mentioned in a number of reports[30,33-35]. The present study showed negligible significant difference between AB-positive and AB-negative subjects owing to only a few cases showed the number of lymphocyte and neutrophil to be slightly increased.

In conclusion, despite the prevalence of AB in BALF in the worker subjects in the present study was higher than the general population, the difference in the worker group per se was not statistically sound. Therefore, the findings are not yet valuable as indicator of asbestos exposure. Further studies involving more subjects concomitantly with a biomarker study could yield applicable results.

What is already known on this topic?

Asbestos bodies could be present in the bronchoalveolar fluids of persons exposed to asbestos and/or of patients with asbestos-related diseases.

What this study adds?

The findings in the present study merely showed that persons at large in Thailand are vulnerable to inhale asbestos fibers from the environment. The events could not be pinpointed to the source of the asbestos dust owing to the lack of substantial evidence.

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Potential conflicts of interest

None.

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การตรวจหาเทห์ใยหิน (asbestos body) จากสารน้ำล้างหลอดลมถุงลมปอด (bronchoalveolar lavage fluid) ในประเทศไทย

พิมพิณ อินเจริญ, วิบูลย์ บุญสร้างสุข, คทาวุธ สนิทธางกูร, จริยา เลาหวิช, วรชัย ศิริกุลชยานนท์, สมชัย บวรกิตติ

วัตถุประสงค์: *เพื่อตรวจหาอัตราความชุกและปริมาณเทห์ใยหินจากน้ำล้างปอดและหลอดลม ในกลุ่มคนงานที่ทำงานในโรงงานใช้ใยหินเปรียบเทียบกับกลุ่มประชากรทั่วไปที่ไม่มีประวัติสัมผัสใยหิน*

วัสดุและวิธีการ: *ตรวจหาเทห์ใยหินในสารน้ำล้างหลอดลมถุงลมปอดโดยใช้วิธี membrane filtration ในคนงานโรงงานกระเบื้องมุงหลังคาและโรงงานท่อซีเมนต์ที่ใช้ใยหินเป็นส่วนประกอบทั้งหมด 30 ราย เปรียบเทียบกับคนไข้โรคปอดที่ทำการล้างปอดและหลอดลม 30 ราย ที่ไม่มีประวัติสัมผัสใยหิน*

ผลการศึกษา: *พบเทห์ใยหินในกลุ่มคนงาน 6 ราย โดยมีปริมาณเทห์ใยหิน 0.1-3.6 ชิ้นต่อสารน้ำล้าง 1 มิลลิลิตร และพบในกลุ่มคนทั่วไป 1 ราย โดยมีปริมาณ 0.2 ชิ้นต่อสารน้ำล้าง 1 มิลลิลิตร ($p = 0.449$)*

สรุป: *อัตราความชุกและปริมาณเทห์ใยหินในสารน้ำล้างหลอดลมถุงลมปอดในกลุ่มคนงานมีมากกว่า ในกลุ่มประชากรที่ไม่มีประวัติสัมผัสใยหิน อย่างไรก็ตามการเพิ่มจำนวนประชากรที่ตรวจอาจให้ผลที่มีนัยสำคัญทางสถิติชัดเจนขึ้น*