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Original Article

The association among ferruginous body, uncoated fibers, asbestos and non-asbestos fibers in lung tissue in terms of length

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Abstract: To demonstrate the correlations between the concentrations of ferruginous body as well as uncoated fiber both of which can be observed with phase-contrast microscope and the concentration of various inorganic fibers including asbestos which requires the observation with TEM or SEM, we measured those indices among Japanese and Korean cases. Though the concentration of ferruginous body in lung tissue is an important index of asbestos exposure, uncoated fibers observed with phase-contrast microscope might be another index especially in such cases with relatively low exposure due to their history of living in a general environment. However, to establish the reliability of uncoated fibers as an index of asbestos exposure, analysis with more cases and from various backgrounds must be carried out.

Key words: Asbestos, Ferruginous body, Uncoated fiber, Lung, Japanese, Korean

Introduction

More and more mesothelioma and lung cancer occur even after the banning of the use of asbestos-containing materials because of the long latency period[1)]. Besides pathological investigation, the asbestos fiber count in the lung tissue is a crucial index of the exposure to asbestos[2)]. To measure the concentration of asbestos fiber, identification of type and counting of each type of asbestos fibers with scanning electron microscope (SEM)[3–5)] or transmission electron microscope (TEM)[6–8)] must be done after preparing the lung tissue by ashing or digesting. Ferruginous body is an inorganic fiber, mainly amphibole asbestos[9, 10)], coated with protein derived from macrophage which attacked the fiber by phagocytosis. Therefore, the concentration of ferruginous body in the lung tissue is assumed to reflect the concentration of asbestos fiber in the lung[8, 11)]. Comparing with the measuring methods using TEM or SEM, counting of ferruginous body in the lung tissue with phase-contrast microscope is less time-consum-

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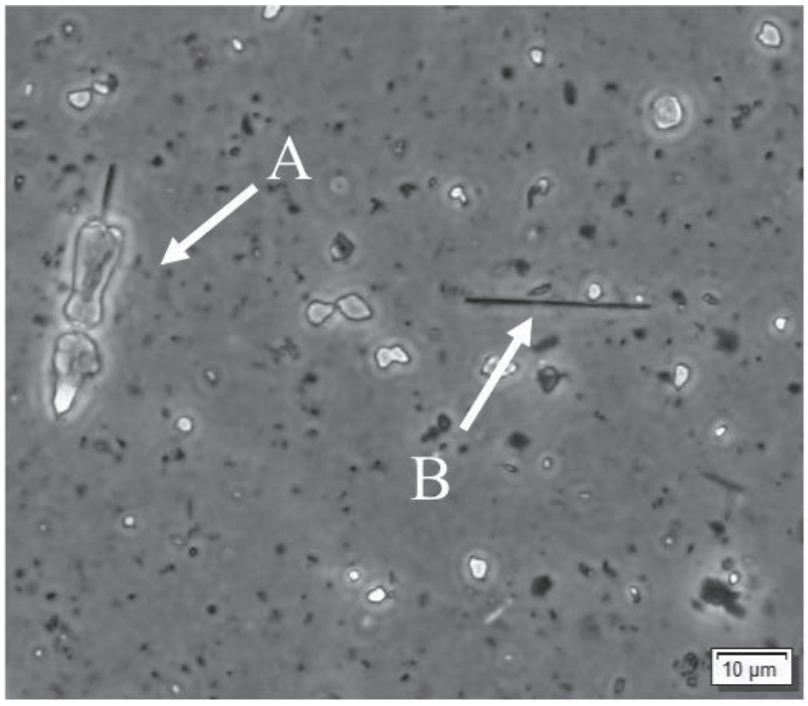

A : ferruginous body
B : uncoated fiber

Fig. 1. Ferruginous body and non-coating fiber

ing and less expensive[12]. Therefore, the concentration of ferruginous body in the lung tissue is frequently measured to evaluate the exposure to asbestos instead of measuring asbestos fibers with TEM or SEM. Though the association between the concentration of ferruginous body and asbestos fibers has been accepted, few papers except Dodson's[13] discussed the association focusing on the types of asbestos. Reliability of concentration of ferruginous body as an index of asbestos exposure remains to be investigated. The aim of the present study was to compare the concentration of fibers detected by phase-contrast microscope and TEM, referring to the association between the concentrations of ferruginous bodies and asbestos fibers, and make a suggestion to evaluate exposure to asbestos.

Materials and Methods

We analyzed ferruginous body in necropsied lung tissues of 18 Japanese cases (12 males, 6 females, mean age: 62.3 years old), and 39 Korean cases (33 males, 6 females, mean age: 38.4 years old) whose concentration of inorganic fibers in the lung were already reported by Lim[14] and Yu[15]. Lung tissue was used from Japanese cases who died of diseases not associated with asbestos, and were necropsied in the period of 1978–1987, in Nagoya, Japan, and surrounding area. Most of the Korean cases were died from accidents, and were necropsied in Seoul and some local cities. The study protocol was approved by the Ethics Committee of Nagoya City Public Health Research Institute.

Measurement of ferruginous body concentrations in the lung

Digestion of lung tissues and counting of ferruginous bodies and non-coated fibers were carried out according to the general method using phase-contrast microscope with small modification of drying of the specimen[11]. In brief, 1–3 g of lung tissues fixed with formalin were segmented and dried with freeze-drier (FDU-2100, EYELA). The dried lung tissues were weighed and digested by the incubation in Clean 99-K200 (Clean Chemical, Osaka, Japan) for 6 hours at the temperature 60°C. The digested tissues were washed 3 times by the addition of distilled water and centrifugation. Purified water was added to the sediment and part of the suspension was diluted with purified water and filtrated on mixed cellulose membrane filter (pore size: 0.8 μm) by suction. The filter was dried and blown by acetone gas using an acetone generator (Quick-FixTM) for fixation and transparency. After sealing, we observed the entire filter with phase-contrast microscope (OLYMPUS BX43) with a magnification of 400 times. We counted the golden yellow-colored fibers coated with iron protein as ferruginous bodies, and fibers without any coating as uncoated fibers (Fig. 1). Because of the diffi-

culty in recognizing both iron protein and fibers, we set minimum observable length of ferruginous bodies as 10 μm, while that of uncoated fibers as 5 μm. Detection limit (DL) of both indices was $0.03 \times 10^3 - 0.19 \times 10^3$/g dry lung. We counted uncoated fibers with an aspect ratio of ≥3 and length of ≥5 μm.

Measurement of inorganic fibers in the lung

We measured inorganic fibers such as asbestos fibers, non-asbestos mineral fibers, as described in our previous reports[6]. In brief, lung tissues were freeze-dried and ashed at low temperature by plasma asher (LTA-2S, Yanagimoto, Kyoto, Japan) for 6 hours. After ashing, 50 ml of distilled water was added and mixed with ultrasound shaker for 5 minutes. Then, filtered with nuclepore filter (pore size: 0.2 μm, Nuclepore Corp, Pleasanton, USA) by suction. The filter was coated with carbon by a suttering device (JEE-4X, JEOL, Akishima, Japan) and put on a nickel grid coated with carbon. Organic materials were washed away by chloroform The sample was put on a TEM grid and 5–50 squares of a grid were observed with a magnification of 10,000 times. We classified a dust with a length/diameter ratio of more than 3 as a fiber. The fibers were classified based on the analysis of the component of elements of the fibers by energy dispersive x-ray analyzer (EDXA, 7000Q, Kevex, Foster, USA). Minimum size of the length and diameter of the fibers were 0.2 μm and 0.02 μm respectively. DL was $0.04 - 0.22 \times 10^6$ fibers/g dry lung tissue. We classified asbestos by comparing samples with standard ones provided by the Japan Asbestos Association.

Statistical analysis

Analysis of all of the samples of both Japanese and Koreans using phase-contrast microscope and TEM was carried out by TS and KS, members of the authors, respectively. The concentrations of ferruginous body, uncoated fibers, inorganic fibers including asbestos were represented as geometrical mean and geometric standardized deviation (GSD). In a case of less than DL, half of DL was used to calculate geometrical mean and SD of each length category. Concentrations of ferruginous body, uncoated fiber, and inorganic fiber in the samples collected in Korea and Japan were compared and Mann-Whitney's *U*-test was used to detect statistical significance. The correlation of those concentrations was tested with Spearman's rank test with exclusion of the concentrations less than DL. Though we also tried the test applying half of DL without excluding the case of less than DL, the results were almost the same, with small differences in the correlation coefficient.

Table 1. Demographic characteristics of subjects

Group	Gender	Age	Occupation	Number
Japanes	Male	60.5 ± 12.7 (38 – 78)	blue-collar worker	7
			white-collar worker	2
			unknown	3
	Female	65.8 ± 14.7 (53 – 80)	housewife	4
			blue-collar worker	1
			unemployed	1
	Total	62.3 ± 11.8 (38 – 80)		18
Korean	Male	37.2 ± 11.4 (14 – 57)	blue-collar worker	13
			white-collar worker	7
			student	2
			unemployed	2
			unknown	9
	Female	45.2 ± 17.3 (30 – 74)	housewife	4
			waitress	1
			unemployed	1
	Total	38.4 ± 12.5 (14 – 74)		39
All		46.0 ± 16.6 (14 – 80)		57

Statflex ver.6 was used for all of the statistical analysis above.

Results

Demographic characteristics of the cases were shown in Table 1. Mean ages of the male and female cases in Japan were 60.5 and 65.8 years old, respectively. Seven male cases were blue-collar workers (5 workers in manufacturing industry, 2 transporters); the rest of the male cases were 2 white-collar workers (a scientist and an office worker) and 3 workers of unknown work history. Female cases include 4 housewives, a blue-collar worker in manufacturing industry and a case without jobs. Mean ages of Korean male and female cases were 37.2 and 45.2 years old, respectively. Korean male cases included 13 blue-collar workers, 7 white-collar workers, 2 students, 2 with unknown history, and the rest were unemployed. Korean female cases were 4 housewives, one waitress and one unemployed. Among 12 male cases whose jobs were identified, 8 cases (7 blue-collar workers and a scientist) were suspected to be occupationally exposed to asbestos. The jobs of Korean cases in Table 1 are those of deceased cases. The detailed occupational histories of the Korean cases could not be obtained.

Ferruginous bodies and amphibole asbestos were not detected in 2 and 3 Japanese cases, respectively. In Korean cases, ferruginous body was not detected in 17 cases and chrysotile and amphibole asbestos were not detected in 16 and 19 cases, respectively. The concentrations of all the

Table 2. Concentrations of ferruginous bodies and uncoated fibers measured with phase-contrast microscopy and inorganic and asbestos fibers with analytical transmission electron microscopy in Japanese and Korean cases.

		phase-contrast microscopy ($\times 10^3$/g dry lung tissue)		analytical transmission electron microscopy ($\times 10^6$ fibers/g dry lung tissue)				
		ferruginous body	uncoated fiber	inorganic fiber	asbestos fiber	chrysotile	amphibole	non-asbestos fiber
Japanese (n=18)	GM	0.59	34.26	42.94	2.38	1.71	0.72	40.56
	range	<0.03–2.78	1.91–109.42	2.42–135.37	0.31–5.78	0.18–5.14	<0.10–2.58	2.10–132.79
	DR (%)	88.9	100.0	100.0	100.0	100.0	83.3	100.0
Korean (n=39)	GM	0.11*	0.72*	10.33*	0.36*	0.23*	0.16*	9.98*
	range	<0.03–0.67	0.09–3.93	0.53–51.50	<0.02–3.81	<0.02–3.67	<0.02–0.89	0.53–51.00
	DR (%)	56.4	100.0	100.0	82.1	59.0	51.3	100.0

GM, geometric mean; DR, detection rate.
Asterisk indicates significant difference at $p<0.01$ comparing Japanese subjects by Mann-Whitney's U-test.

Table 3. The correlation coefficients between concentrations of ferruginous bodies, uncoated fibers detected by phase-contrast microscopy and various fibers measured with analytical transmission electron microscopy stratified by their length.

		Length of inorganic fiber, asbestos fiber, chrysotile, amphibole and non-asbestos fiber							
		<1 μm		≥1 μm		≥5 μm		All	
		n	CC	n	CC	n	CC	n	CC
ferruginous body (n=38)	inorganic fiber	38	0.53**	38	0.40*	38	0.31	38	0.51**
	asbestos fiber	28	0.42*	31	0.57**	18	0.52*	35	0.55**
	chrysotile	26	0.40*	25	0.58**	4	0.00	31	0.52**
	amphibole	12	0.29	22	0.35	14	0.24	24	0.46*
	non-asbestos fiber	38	0.54**	38	0.38*	36	0.30	38	0.50**
uncoated fiber (n=57)	inorganic fiber	57	0.65**	57	0.55**	56	0.39**	57	0.63**
	asbestos fiber	37	0.61**	46	0.68**	24	0.45*	50	0.71**
	chrysotile	33	0.59**	31	0.63**	4	0.56	41	0.72**
	amphibole	15	0.01	32	0.46**	20	0.24	35	0.55**
	non-asbestos fiber	57	0.64**	57	0.51**	54	0.30*	57	0.62**

CC, correlation coefficient.
Single and double asterisk indicate significant correlation between inorganic fibers and ferruginous bodies or non-coating fiber at $p<0.05$ and $p<0.01$ respectively by Spearman's rank test.
*$p<0.05$; **$p<0.01$

indices we measured (ferruginous body, uncoated fiber, two types of asbestos, and non-asbestos fibers) were significantly higher in Japanese than in Korean cases (Table 2). The concentrations of ferruginous body of Korean cases were less than 1,000/g dry lung, while those of 3 out of 18 Japanese cases were more than that level. We detected uncoated fibers and non-asbestos fibers in all the cases. Any kinds of asbestos fibers and chrysotile fibers were found in all of the Japanese cases, while the detection rates of those in Korean cases were 82.1% and 59.0%, respectively. Ferruginous body, and amphibole asbestos in Japanese cases were more than 80%, while those in Korean cases were less than 60% (Table 2).

The correlation of ferruginous body, uncoated fiber and the concentrations of various inorganic fibers were shown in Table 3. The length of asbestos and nonasbestos fibers was classified into those of less than 1 μm and 1 μm or longer. In both length categories, ferruginous body as well as uncoated fiber were significantly correlated with all inorganic fiber, asbestos, chrysotile and nonasbestos. However, amphiboles are not correlated with both of the indices of phase-contrast microscope. Considering that inorganic fibers of more than 5 μm length are observable with phase-contrast microscope, we also analyzed the correlation between inorganic fibers with this length category and various fibers observed with TEM. Significant correlations were detected between the concentrations of ferruginous body and asbestos fibers ($p<0.05$), uncoated

fibers and inorganic fibers ($p<0.01$), uncoated fibers and asbestos fibers ($p<0.05$), uncoated fibers and non-asbestos fibers ($p<0.05$).

Discussion

The more asbestos-related lung cancer occurs, the more important the evaluation of exposure to asbestos would be[16]. The serious problem is that mesothelioma patients are expected to increase for the next 40 years[17]. The measurement of asbestos fibers in lung tissues with analytical TEM is accepted to be the most reliable method for the evaluation of patient exposure to asbestos[8]. On the other hand, counting of asbestos bodies in lung tissue with phase contrast microscope is a more common method for the evaluation. Measurement of asbestos fibers in bronchoalveolar lavage fluid is another evaluation method, though less common[18, 19].

We compared correlation of the concentrations of asbestos bodies and asbestos fibers in the lung tissue obtained by phase-contrast microscope and analytical transmission electron microscope, respectively. We also compared those results among Japanese and Koreans referring to our previous reports[14, 15]. The 57 cases died of accidents and diseases unrelated to asbestos exposure. However, Japanese cases showed significantly higher concentrations than Korean cases in not only asbestos and non-asbestos fibers, but also ferruginous bodies and uncoated fibers in their lung tissues. Compared to those concentrations among the general population reported by Dodson[20] and Monso[21], our results suggested that Koreans have similar levels, and Japanese have higher. But the concentrations of the cases of the present study were substantially lower than those of patients who suffered from asbestos-related diseases.

We found that 3 out of 18 Japanese cases showed concentrations of ferruginous bodies in the lung more than 1,000/g dry lung tissue which is considered to be threshold[10] suggesting occupational exposure to asbestos in Japan. The occupational history was unknown for one case, but the remaining two cases have periods of roofing with slate, or harbor loading and unloading. Their history suggests that at least 2 cases had been occupationally exposed to asbestos fibers even though they did not develop asbestos-related diseases. To compare the concentrations of asbestos fiber, ferruginous bodies and uncoated fibers in lung tissues between the two countries, we should consider that the mean age of the Japanese cases was higher than that of Korean cases for 20 years. However, Japan started the use of asbestos products 20 years earlier than Korea, and imported much larger amounts of asbestos[14]. Moreover, concentrations of airborne asbestos and non-asbestos fibers have been significantly higher in Japan than those in Korea[22]. The concentrations of ferruginous body in the lung tissues of the cases in Japan and Korea seem to reflect the differences of environmental asbestos and non-asbestos fibers in the those two countries.

The concentration of ferruginous body and uncoated fibers were demonstrated to be correlated with that of asbestos and non-asbestos fibers among 57 cases in the present study. However, the concentration of ferruginous body, classified by the length of their length, did not significantly correlate with that of amphibole fibers, the main constituent of ferruginous bodies[23–25], whereas the concentrations of chrysotile fibers, regardless of their length, showed a significant correlation with that of ferruginous bodies. Furthermore, a lower correlation coefficient of amphibole fibers with ferruginous body was demonstrated compared to that of chrysotile fibers. The background of those apparently inconsistent results is our detection of a low level of pulmonary amphibole fibers in a rather small number of cases. On the other hand, the concentrations of amphibole fibers were higher than those of chrysotile fibers[26] and the levels of ferruginous body were much higher in the lung of patients with asbestos-related diseases compared to those in the general population[27]. Therefore, further investigation of the association between ferruginous bodies and asbestos fibers with larger samples including more patients with asbestos-related diseases is necessary. Considering the difference in the risks of asbestos-related disease among asbestos fibers[28], the association of ferruginous body with classified amphibole fibers would also be needed. In the present study, we classified the asbestos fibers based on their length. Asbestos fibers could also be classified by their particle dimension, based on the hypothesis by Stanton *et al.*[29] and WHO[30]. The association of ferruginous body with asbestos fibers based on those classifications with fiber dimension would be informative.

The importance of the count of uncoated fibers in the evaluation of asbestos exposure have not drawn much attention. Uncoated fibers have greater association with asbestos fibers in lung tissue compared with ferruginous body, suggesting that the measurement of uncoated fibers might be more reliable index of asbestos exposure in the case with relatively small exposure to asbestos. We consider that the range of concentrations of the samples in the present study was too small to establish the association between ferruginous bodies and asbestos fibers. Most of the samples of the present study were not occupationally

exposed ones. Therefore, the association found in the present study was limited in the range of exposure of relatively low levels. We should those deviations of our samples take into account. It is accepted that general population is far frequently exposed to chrysotile fibers compared to amphibole fibers and exposure to amphibole fibers is mostly limited to occupational exposure. We could not detect amphibole fibers in some of the samples in the present study. This makes us difficult to draw a conclusion on the association between the concentration of ferruginous bodies and chrysotile or amphibole fibers. Analysis of further samples would establish the association.

In conclusion, though the concentration of ferruginous body in lung tissue is an important index of asbestos exposure, uncoated fibers observed with phase-contrast microscope might be another index, especially in such cases with relatively low exposure due to a history of living in the general environment. In any case, to establish the reliability of uncoated fibers as an index of asbestos exposure, analysis with more cases and from various backgrounds should be carried out.

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