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Improved Method for Analysis of Airborne Asbestos Fibers Using Phase Contrast Microscopy and FTIR Spectrometry

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Background: In recent years, some studies have tried to improve Phase Contrast Microscopy (PCM) for counting asbestos fibers. Due to the lack of a universally accepted alternative method, this study aimed to improve PCM for better counting of asbestos fibers.

Materials and Methods: Confirmed asbestos standards were applied using a dust generator for sampling. Sampling from the dust generator was carried out according to the Occupational Safety and Health Administration (OSHA) ID-160 method and 95 samples with diverse densities were prepared to be counted using conventional and modern PCM. All samples were counted single blindly by a co-author of this study and the obtained data were analyzed by paired t-test, correlation coefficient and Bland-Altman analysis. Duplicate samples were prepared for qualitative analysis by Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM) and X ray.

Results: Asbestos densities on filters were in the range of less than 100 to 600 fibers/mm^2. Statistically, significant differences were observed for the count density of the 95 samples counted by the two phase contrast microscopes ($P<0.001$). Nikon microscope demonstrated higher counts compared to conventional microscope and had a lower coefficient of variation. Samples were analyzed qualitatively using FT-IR and SEM, and the presence of asbestos fibers was confirmed.

Conclusion: The improved PCM and FT-IR methods presented in this study demonstrated more precise and accurate determination of personal exposure to airborne asbestos fibers and subsequent risk assessment.

Key words: Phase contrast microscopy, Asbestos fibers, FT-IR spectroscopy, Scanning electron microscopy

INTRODUCTION

Asbestos is the term used for six groups of inorganic silicon compounds, which can be found in the form of inorganic fibers in nature (1). Asbestos has unique physical and chemical properties such as insolubility in water, resistance to heat and fire, and resistance to chemical substances and it is widely used in many manufacturing and industrial processes (2).

According to epidemiological reports, carcinogenicity of asbestos fibers has been established for human beings (3, 4). Generally, exposure to all types of asbestos causes life-threatening conditions such as lung cancer, malignant mesothelioma and asbestosis (5-9). Although the World Health Organization (WHO) and the International Labor Organization (ILO) emphasize on abandoning the use of asbestos, Iran and some other developing countries continue to use it for industrial purposes (10). According to the WHO, about 125 million individuals in the world are still exposed to asbestos through their occupations (11). According to a report from the ILO, there are about 100,000

mortalities annually due to occupational exposure to asbestos (12, 13).

PCM has been used for counting fibers since early 1970s and it is considered an inexpensive, simple technique. This method has been used for epidemiological studies and risk assessment of occupational exposures. However, there are few limitations associated with its use; for example, PCM cannot distinguish between asbestos and non-asbestos materials and cannot detect fibers with diameters less than 0.2μ. Also, it must be emphasized that the coefficient of variation reported by OSHA's compliance inspections to be 12% for 395 replicate samples and fiber densities ≥100 f/mm² (14) has been regarded unfavorable by some analysts (15).

Due to the toxicity of this substance and inefficacy of conventional PCM for accurate counting of asbestos fibers, a number of new alternative methods such as SEM (16), Transmission Electron Microscopy (TEM) (17), and Polarized Light Microscopy (PLM) (18) have been suggested for determination of the airborne asbestos fibers. However, electron microscopes can detect smaller fibers and they are not reliable for fiber counting. SEM has the drawbacks of very small field of view of the filter due to extreme magnification as well as high cost and lack of accessibility (19, 20). Due to the cost issues and other challenges of alternative methods, there have been attempts to improve the efficacy of conventional PCM by equipping it with a high resolution camera and an appropriate software program (21, 22). Due to the fact that advanced methods may not be easily accessible in Iran and other developing countries, this study attempted to improve the conventional PCM for counting asbestos fibers using a modern phase contrast microscope equipped with a 12-megapixel camera and 21-inch monitor. Also, in order to overcome the inability of phase contrast method (OSHA ID-160) for positive recognition of asbestos fibers, FT-IR was used in this study.

MATERIALS AND METHODS

Quantitative analysis

Quality of asbestos obtained from the Iranian universities was confirmed by X ray diffraction (XRD) and SEM analysis. Confirmed asbestos was fed into a closed dust generator for production of airborne asbestos. A dust generator system in this study was fabricated from borosilicate glass. A 2-mm deep groove was machined in the top the glass mating with the rim of the main glass chamber to form a closed system. A filter holder sampler was mounted onto a small opening on the top of the chamber and compressed air was blown from the bottom of the chamber (23). Sampling of airborne asbestos from closed dust generator was done according to OSHA ID160 method (14). The conductive filter holder consisted of a 25-mm diameter 3-piece cassette. This cassette had a 50-mm long, electrical conductive extension cowl mounted on a 0.8 μ pore size mixed cellulose ester (MCE) filter (SKC No. 225-1939) and was attached to a calibrated personal sampler pump (SKC-Model Eighty Four, PA 15330, USA). Sampling for the airborne asbestos was performed with variable flow of 0.5 to 1.75 l/m for a period of 30 minutes to collect asbestos fibers with diverse densities. A total number of 95 samples with 6 different sampling flows were obtained from the dust generator. All filters were placed on prewashed 70 mm × 25 mm slides and were subsequently cleared using an acetone vaporizer device. All samples were prepared for analysis by the conventional phase contrast microscope equipped with Walton- Beckett graticule type G-22 (Leitz Model 22E, Germany) and modern microscope (Nikon Model TE2000-U) attached to a 12-megapixel camera and 21-inch monitor with ×500 and ×400 magnifications, respectively. Samples were coded by a third party and given to the co-author for counting. It must be emphasized that the co-author was blinded to identity of samples. Daily, 4-5 samples were chosen randomly and each sample was analyzed five times by both conventional (Leitz) and modern phase contrast microscope (Nikon). Fibers at least 5μ in length and length to width ratio of 3 or greater were counted (14, 24).

Methods for qualitative analysis

Three standard asbestos samples obtained from Iranian universities, were analyzed qualitatively by XRD and SEM. XRD was done using Philips analytical X-ray diffractometer type PW3710 equipped with a graphite monochromator. Radiation was produced by a copper target at 40 kV and 30 mA. The instrument was configured with 1° divergence and 0.1 mm receiving slits. The diffracted beam data were obtained by scanning the 2θ diffracted beams in the range 3–80^{o} using PC-APD. XRD analysis is based on measurement of X ray at certain angle of diffraction and the instrument scans the diffraction at a constant speed (4 degrees/minute in this study). Raw materials were also analyzed by SEM (model WEGA/TESCAN, Czech Republic) and energy-dispersive X-ray analysis (EDXA) was used for elemental percent estimation and the results were compared with reference data (16).

FT-IR has never been used for determination of the quality of airborne asbestos fibers before and therefore the library of FT-IR spectrometer did not contain information for identification of any asbestos materials. Thus, 16 samples from a confirmed XRD and SEM chrysotile asbestos, in the range of 1 to 10 mg were weighed and mixed with 200 mg of potassium bromide powder to form a homogenous powder. The mixtures were pressed with 19 MPa pressure for 5 minutes and the transparent tablet was analyzed by FT-IR according to a method for bulk asbestos analysis in the soil (25).. Prepared samples were scanned by FT-IR spectrometer in the range of 4000 to 400 cm^{-1} with resolution of 4 cm^{-1}. Spectrum of samples obtained in the present study was uploaded into the library of FT-IR spectrometer for analysis of chrysotile asbestos samples for detection of chrysotile asbestos in unknown samples. Each spectrum obtained for unknown samples was compared with the spectrum of standard chrysotile asbestos. A standard infrared spectrum represents a fingerprint of asbestos with absorption peaks corresponding to the frequencies of vibrations between the bonds of the atoms constituting the material (26).

Bulk raw materials from three shoe brake manufacturers and wastage from auto brake repairs were also obtained for FT-IR and SEM analysis. Two personal samples from workers involved in auto brake repairs were also taken according to the OSHAID:160 method (14). Filters were removed lightly from holder and placed in dish covered with 200 mg of potassium bromide powder and after burning filter at 500° C in an oven, mixtures were pressed into tablets.

In addition, duplicate bulk samples were analyzed by XRD and SEM for confirmation of FT-IR results. Data of SEM can be compared to the standard reference composition of chrysotile. Chrysotile contains Si and Mg. Relative elemental concentrations can be estimated from the peak values in the energy-dispersive spectrometry (EDS) analysis of the raw material (19).

Statistical analysis of the data obtained from 95 samples along with data obtained from OSHA's mathematical model for calculation of coefficient of variation as a function of fiber densities (14), was conducted by SPSS version 16 using paired t-test, Pearson's correlation coefficient and Bland and Altman method. Normality of data was examined by Kolmogorov–Smirnov test. Quantitative values were reported as mean ± standard deviation (M±SD).

RESULTS

Results of the quantitative analysis

Ninety-five samples were obtained from the dust generator fed Chrysotile asbestos. The mean fiber density of the 95 samples counted by Leitz and Nikon microscopes was in the range of 9.6 - 551.8 f/mm^2 and 13.8 - 588.5 f/mm^2, respectively. All data obtained in this study exhibited normal distribution. Paired t-test showed a significant difference in the fiber density counted by the two microscopes ($P<0.001$) with a higher number of fibers counted by Nikon microscope. The Bland and Altman method of analysis was used for assessing the agreement between the results obtained from Leitz and Nikon microscopes. As shown in Figure 1, the differences of mean

densities were mostly within two standard deviation units of the mean (Mean ± SD). Only six out of 95 samples were out of this range (i.e., outliers).

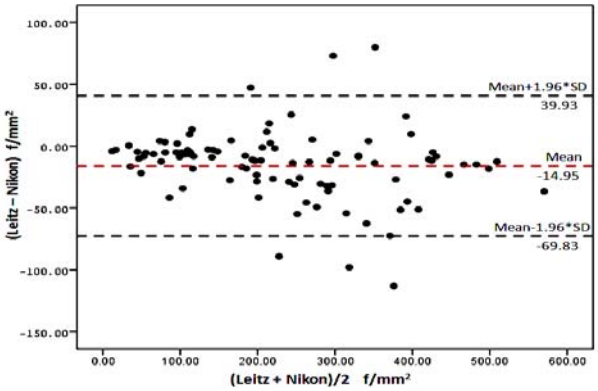

Figure 1. Bland and Altman plot compares the mean fiber densities measured by Leitz and Nikon microscopes.

The overall mean of the coefficient of variation was computed for each of the four categories of fiber densities within the range of >0-600 f/mm² (Table 1). Compared to Leitz microscope, Nikon microscope had a lower coefficient of variation. The Mean ± SD of the coefficient of variation counted by the Leitz microscope was 0.12 ± 0.065, and 0.10 ± 0.047 by Nikon microscope.

Table 1. Comparison of the coefficient of variation of Leitz and Nikon microscopes for the four fiber densities

Fiber density range (f/mm²)	Coefficient of variation	
	Leitz	Nikon
>0-100	0.19	0.15
100-200	0.10	0.08
200-300	0.10	0.08
>300	0.11	0.09

Coefficient of variations of asbestos versus fiber count of samples in the range of 0 to 600 f/mm² by either Leitz or Nikon microscopes was plotted along with comparable data calculated from the mathematical model presented by OSHA's compliance inspections, and demonstrated similar descending trends of the coefficient of variation for higher fiber densities (Figure 2). Pearson's correlation coefficient showed a significant correlation (r^2 = 0.962) between the mean densities (Figure 3).

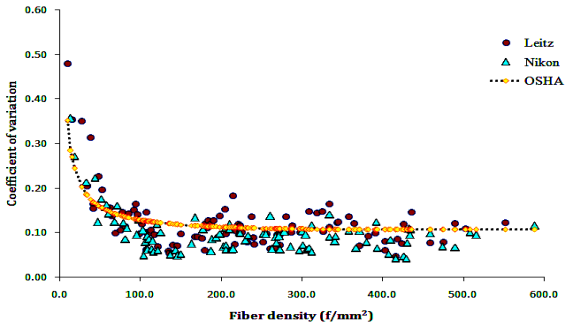

Figure 2. Coefficient of variations of fiber densities for Leitz and Nikon microscopes along with OSHA's data

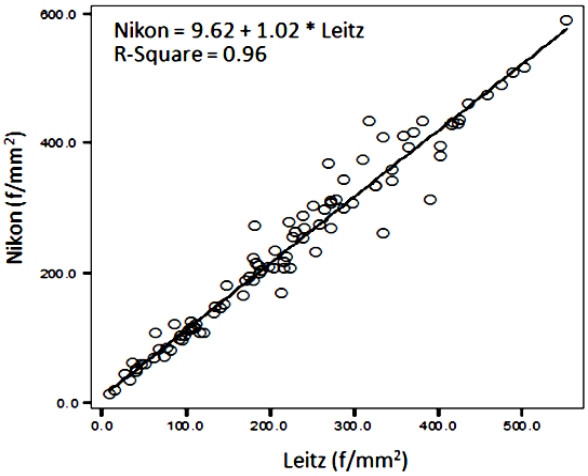

Figure 3. Correlation of fiber densities (f/mm2) counted by Leitz and Nikon microscopes

Results of qualitative analysis

After FT-IR spectrometry and comparison of spectra with standard spectra (25), it was found that just two of the three standard samples obtained from Iranian universities (A and B) were pure chrysotile asbestos. These results were also confirmed by SEM. An example of an EDS spectrum of chrysotile of sample A is shown in Figure 4, where the magnesium to silica (Mg/Si) ratio is 1.39.

Figure 5 shows matching of the two FTIR spectra of standard chrysotile for A and B samples. The main FTIR absorption peaks for the two standard chrysotiles obtained

from Iranian universities A and B were also identical (Table 2).

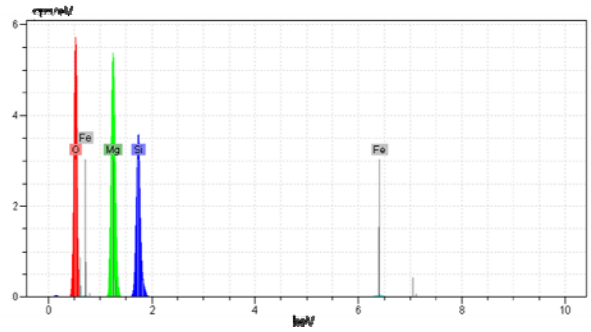

Figure 4. EDS spectrum of standard chrysotile for sample A

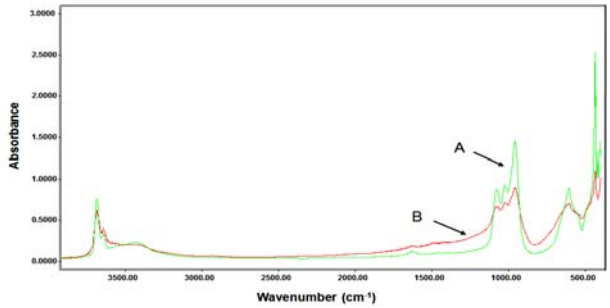

Figure 5. FTIR absorption spectra for the standard chrysotile from the (A) and (B) universities

Table 2. Absorption peaks for pure chrysotile asbestos (samples A and B)

Chrysotile	Vibration of functional groups (wave number cm^{-1})		
	OH stretching vibration	Si-O stretching vibration	Cation-oxygen vibration
A	3688, 3645	1080, 1024, 956	605, 435, 404
B	3687, 3644	1079, 1024, 956	605, 433, 403

Qualitative analysis of chrysotile asbestos is achieved through observation of IR absorption of functional groups at various wave numbers mentioned in Table 2. Figure 6 shows the absorption spectra of five samples. Number 1 shows standard asbestos (A) spectra which, its quality was also confirmed by XRD to be chrysotile asbestos. The spectra of sample numbers 2, 4 and 5 were raw materials of manufacturing brake shoes samples were not recognized as chrysotile asbestos which the results were also confirmed by SEM. Sample number 3 (wastage of auto brake repairs) was recognized as chrysotile asbestos which was confirmed by SEM.

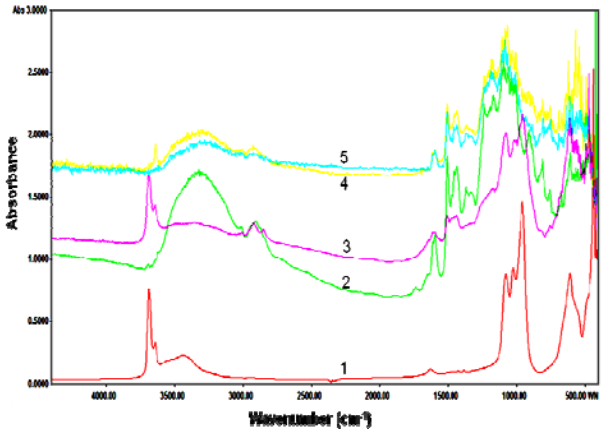

Figure 6. Comparing the standard spectrum of chrysotile asbestos (No.1: sample A) with 3 spectra of samples from brake shoes manufacturers (No. 2, 4 and 5) and No. 3: auto brake repairs.

DISCUSSION

In this study, 95 samples were counted five times by Leitz Model 22 E (conventional microscope) and Nikon Model TE2000-U (modern microscope). The results of this study for fiber counts by either microscopes showed the same trend of reduction in coefficient of variations for higher fiber densities in the range of 0-100 f/mm² and relatively constant coefficient of variation as the fiber densities went beyond 100 f/mm² reported by OSHA's compliance inspections (14).

According to the Bland-Altman analysis, the strong correlation of fiber densities with coefficient of variation demonstrated that both microscopes with different magnifications (Leitz ×500 and Nikon ×400) verified the asbestos counting performance of Nikon microscope. However, despite the lower magnification of Nikon ×400 compared to Leitz ×500, significantly higher fiber densities of asbestos were observed for samples with Nikon microscope.

Statistical analysis of data obtained from Nikon microscope demonstrated better coefficient of variations for all fiber densities compared to Leitz microscope; which is in accord with the data calculated by a mathematical model presented by OSHA's compliance inspections.

While in studies by Mao et al, and Kakooei et al, using microscopes equipped with a camera and a display monitor for counting of chrysotile fibers, produced inferior results compared to the conventional microscopes (22, 27). Kakooei's unfavorable result may be due to the use of low resolution of the camera (1.3 megapixel).

Favorable coefficient of variations obtained by Nikon microscope confirms previous observations of more fiber densities seen with Nikon, demonstrating its inherent capabilities for superior counting of asbestos fibers. The coefficient of variations obtained for a larger sample size by OSHA's compliance inspections (N=395) was inferior compared to the coefficient of variations observed by Nikon microscope in our study (N=95). This comparison definitely exhibits the superior innate capability of Nikon microscope for counting asbestos fibers.

Superior determination of asbestos densities by lower magnification of the modern microscope (Nikon) could be due to: (1) the easier counting of asbestos fibers under the microscope's fields and the image displayed on a 21-inch computer monitor and (2) the high resolution (12 megapixels) camera capturing better images of counting fields in Nikon compared to the image observed through the eyepiece in the conventional Leitz microscope. The higher count of fibers suggests recognition of thinner fibers using a monitor attached to a 12-megapixel camera.

Based on the results, modern phase contrast microscope equipped with a high-resolution camera and appropriate monitor, had a definitely improved efficacy compared to the conventional PCM presented before for quantitative analysis of airborne fibers. It is assumed that PCM method can be further upgraded using a higher resolution camera and monitor.

According to the American Conference of Governmental Industrial Hygienists and the United States Environmental Protection Agency, risk assessment of occupational and environmental exposure is based on number of airborne asbestos fibers per unit volume of air (28, 29). Due to the shortcomings of detection of asbestos fibers by PCM, the application of FT-IR spectroscopy, electron microscopes (SEM and TEM) (16, 17) and XRD (30) was previously recommended. Considering the similarity of the qualitative results of XRD, SEM and FT-IR in this study, the applicability of FT-IR spectrometry for airborne chrysotile asbestos was demonstrated for the first time.

Overall, this study demonstrated higher precision in quantitative analysis of airborne asbestos fibers, which could be a significant improvement for ongoing methods presented by OSHA or the National Institute for Occupational Safety and Health (NIOSH) organizations. The shortcomings of OSHA or NIOSH methods in positive recognition of chrysotile asbestos fibers in either bulk material or airborne samples, were overcome by FT-IR spectrometry analysis through its applicability for recognition of asbestos materials. Therefore, considering the improved efficacy of PCM and FT-IR analysis presented in this study, they may be used for determination of personal exposure and subsequent risk assessment.

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