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MINERAL FIBRES, FIBROSIS, AND ASBESTOS BODIES IN LUNG TISSUE FROM DECEASED ASBESTOS-CEMENT WORKERS

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Samples of lung tissue taken from deceased asbestos-cement workers, with (N = 7) and without (N = 69) mesothelioma, and from individually matched controls (N = 96) were examined. The number of asbestos bodies and the grade of fibrosis were determined with light microscopy, and the type, size, and number of fibres with transmission electron microscopy and energy dispersive X-ray spectrometry. The asbestos content was, as expected, higher among the exposed workers than among the controls. Chrysotile was the major fibre type in terms of fibre count. Conversion to fibre mass reversed the pattern to a predominance of amphibole fibres, and accentuated the differences between exposed workers and controls. Duration of exposure and the number of asbestos bodies displayed significant associations with all the singular amphiboles (amosite, crocidolite, tremolite, and anthophyllite), but not with chrysotile. Fibrosis was related to the tremolite content, but not to chrysotile, both among exposed workers and controls. The workers with mesothelioma had higher levels of crocidolite and tremolite than the other asbestos-cement workers.

Asbestos exposure can be verified and quantified microscopically in lung tissue by counting asbestos bodies (1), or by determining the actual fibre content (2). However, several important aspects of the interpretation of the lung burden still remain to be settled. For example the chrysotile ore usually contains small amounts of amphiboles (tremolite and anthophyllite). Evidence from some studies of lung tissue from chrysotile workers (3, 4), and populations endemically exposed to tremolite (5), suggests that tremolite accumulates in lung tissue and might exert a stronger fibrogenic effect than chrysotile. This has important implications for risk assessment. Moreover, several studies indicate a stronger mesothelioma-provoking effect from amphibole than from chrysotile asbestos, but it is still unclear to what extent the chrysotile ore, and specifically the chrysotile proper, might cause mesothelioma.

In a cohort study of asbestos-cement workers with mainly chrysotile exposure, we found an increased risk of pleural mesothelioma, lung cancer, gastrointestinal cancer, and non-malignant respiratory disease, with surprisingly steep dose-response relationships more similar to the ones usually observed in the chrysotile-textile than in the asbestos-cement industry (which uses almost only chrysotile; 6).

In two other studies of workers from the same plant, the dose-response relationships were analysed between estimated asbestos exposure, fibrosis, and asbestos bodies on the one hand (1), and actual mineral fibre content and its relation to pathological changes, especially fibrosis and mesothelioma, on the other. These data are summarized here.

SUBJECTS AND METHODS

Plant and exposure

The plant manufactured asbestos-cement products from 1907 to 1977. Chrysotile was the major asbestos type (>95%). Until 1966 small amounts of crocidolite (usually <1%), and until 1956 small amounts of amosite (about 1% 1953–1956), were used. All asbestos was milled before mixing. The chrysotile qualities were of the commercial classes 3–7, with a predominance of short fibres after 1952, when wet instead of dry milling was introduced. Average exposure for different jobs has been calculated from data on dust measurements and technical changes (7). The only workers continuously exposed to average levels above 2 f/ml were millers, mixers, and workers performing polishing and sawing operations.

Lung specimens

Samples of lung tissue (formalin-fixed and paraffin embedded) from 89 deceased asbestos-cement workers were examined histologically together with those from 89 controls without known asbestos exposure, matched individually by sex, age (±5 years), year and place of death. For 20 of the exposed workers, tissue for analysis of the mineral fibre content was not available. Lung tissue from another seven asbestos-cement workers with pleural mesothelioma was, however, added in the study of the fibre content, together with seven individually matched controls. The analysis of the mineral fibre content thus finally included 76 asbestos-cement workers and 96 controls. Information on smoking habits was collected through a postal inquiry sent to the relatives.

Analysis

The tissue specimens were digested chemically. The number of fibres per gramme of dry weight, fibre length and diameter were determined using transmission electron microscopy (TEM). Fibre type, as well as asbestos type, was determined by Energy Dispersive X-ray Spectrometry. The analysis was performed »blindly« with regard to asbestos workers and controls. The mass estimates for the different asbestos types were calculated from fibre length, diameter, shape, and specific gravity (8). Asbestos bodies, per 25 mm of unstained section, were counted in the light microscope and grouped into four levels (0, 1–14, 15–75, 76+); the degree of fibrosis was classified (0–4), according to *Hinson and co-workers* (9).

RESULTS

Fibre content

The exposed persons with mesothelioma had a significantly higher number of total asbestos fibres than the ones without mesothelioma, who in turn, had higher levels than the controls. Chrysotile was the major fibre type. The differences were most pronounced for the amphibole fibres, and among these, especially for crocidolite, but were evident also for tremolite and anthophyllite. As regards amosite, there was no significant difference between workers with and without mesothelioma; the workers, however, had higher levels than the controls (Figure 1).

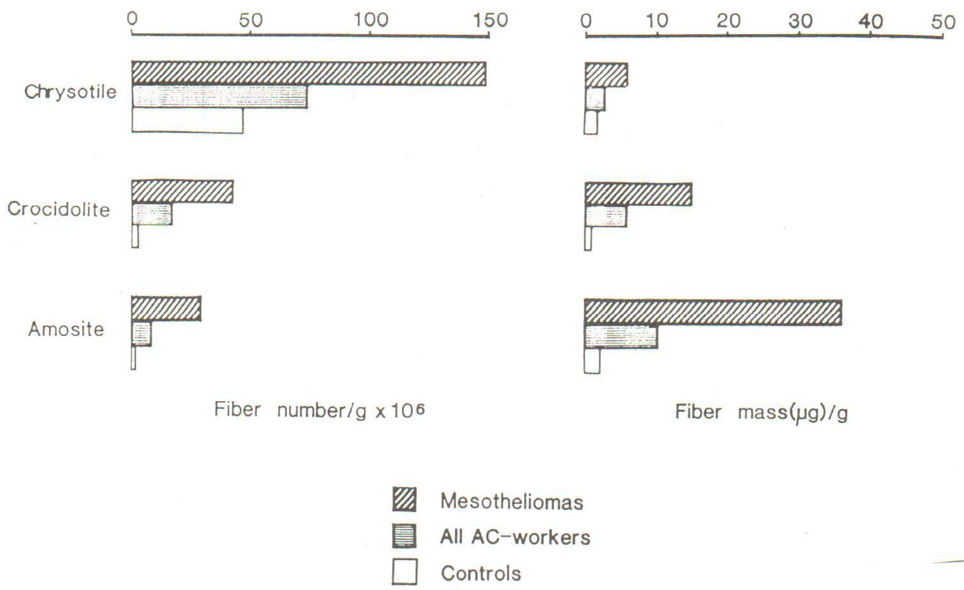

Figure 1 *The number and mass of asbestos fibres per gramme of dry weight (geometric means) in lung tissue for all asbestos-cement (AC) workers (N = 76), AC workers with mesothelioma (N = 7), and controls (N = 96)*

The chrysotile fibres extracted from the tissue were generally finer and shorter than the other asbestos types, and their mass per unit number was low, when compared to the amphibole types (10–11). The conversion of mineral content from fibre number to mass provided a quite different pattern, with a dominance of amphibole fibres and

Table 1

Percentage of fibres, for each type of asbestos in lung tissue, which have a length >6 μm and a diameter <0.25 μm for U.S. chrysotile textile workers (N=45) and controls (N=34) and for Swedish asbestos-cement (AC) workers (N=76), and controls (N=89; 10)

	Swedish		U. S.	
	AC workers	Controls	CT workers	Controls
Chrysotile	1.4	2.2	7.7	0.2
Tremolite	0.9	2.9	2.8	0
Crocidolite	4.2	0	43	0
Amosite	4.2	0	20	0
Anthophyllite	3.8	0	0	0

accentuated differences in the lung burden between mesothelioma cases, all asbestos-cement workers, and controls (Figure 1).

When fibre size was considered, the percentage of chrysotile fibres longer than 6 μm and thinner than 0.25 μm did not differ between the groups (Table 1), whereas the amphibole fibres (except tremolite) tended to be longer in the occupationally exposed group.

In the 76 asbestos-cement workers, there was a significant relationship between duration of employment and the sum of amphibole fibres, as well as for each of all the amphiboles tested separately. For the major fibre type, chrysotile, there was a non-significant tendency. Using the fibre mass the correlations were strengthened.

Histopathology

Only 12 out of 89 controls had asbestos bodies (all of the lowest score). In contrast, 57 of the exposed workers had asbestos bodies, 21 with score 2–3. The occurrence and score of asbestos bodies were strongly related to the estimated cumulative dose. Over half (69%) of the workers with an estimated exposure above 40 fibre years/ml had an asbestos bodies score of 2–3, against only two per cent of the workers with a cumulative dose below 10 fibre-years/ml.

Among the 89 controls, 56 had the diagnosis of pulmonary fibrosis, but only six had severe fibrosis (grades 3–4). By contrast, 68 of the exposed workers had fibrosis, including 16 with severe fibrosis. Age was significantly associated with fibrosis among the exposed workers, but not among the controls.

There was an association, among the 67 controls with known smoking habits, between tobacco consumption and grade of fibrosis. Such an association was not evident among the exposed workers. When exposed workers with (N=81) and without known smoking habits were compared, the fibrosis grade did not differ significantly. The same result was found for the controls.

In a paired analysis, comparing the degree of fibrosis in the exposed worker with that in the matched control, fibrosis was more pronounced among the exposed workers. An association with cumulative asbestos dose was suggested, but did not reach significance. Duration of exposure did not show a significant association.

Among the exposed workers, there was a strong association between asbestos body score and grade of fibrosis. A similar relationship was also found among the controls.

Mineral fibre content and histopathology

There was a relationship between the level of asbestos bodies and the fibre counts/mass among the 69 exposed persons without mesothelioma, whose tissue samples were also examined histologically. This was statistically significant for the sum of, and for all the singular, amphiboles including tremolite, but no such correlation was found for chrysotile, or for non-asbestos fibres. Neither were any significant correlations found among the controls. The results were similar using mass estimates and fibre counts.

The relationship between mineral content and fibrosis in exposed workers and controls was studied by logistic regression, adjusting for age and smoking habits. Among the asbestos-cement workers as well as the non- or light- smoking controls, there was an association between the presence of fibrosis and the tremolite content. No relationship with fibrosis was found for the chrysotile or anthophyllite levels, in either of the two groups. The association between fibrosis and the amosite and crocidolite content was weaker than the one found for tremolite.

CONCLUSIONS

The asbestos fibre levels were, as expected, higher among exposed workers than among controls. When fibre number was considered, chrysotile was the major fibre type, which is in accordance with the asbestos consumption in the factory.

The chrysotile fibres were, however, finer and shorter than those of the amphiboles, and the amphibole fibres among the controls tended to be shorter and finer than among the exposed workers. Therefore, when fibre mass was considered instead, in the exposed group, the amphibole burden was greater than the chrysotile one, while in the controls, the concentration ratio was about unity. Thus the differences between the exposed and non-exposed group were accentuated. The mesothelioma cases had higher levels of crocidolite and tremolite as compared to the workers without mesothelioma.

Strong correlations were found between duration of employment and both commercial (amosite and crocidolite) and non-commercial (tremolite and anthophyllite) amphibole levels, whereas no such relation was evident for chrysotile.

Among the exposed workers, the number of asbestos bodies was strongly associated with estimated exposure, and with the levels of all the singular amphiboles in lung tissue, but not with chrysotile or non-asbestos fibre levels. A score of 15 asbestos bodies or more per 25 μm unstained section was found only in the exposed group, and mainly at exposure levels above 10 fibre-years/ml. Thus counting of asbestos bodies in the light microscope can be useful to indicate significant occupational exposure in the absence of other exposure data.

Severe fibrosis was more common among the asbestos-cement workers than among the controls. An association with cumulative asbestos dose was suggested. Surprisingly, no association was found between the chrysotile content and fibrosis among the asbestos-cement workers. This does not, however, prove that chrysotile lacks a fibrogenic effect, as the pathological process might have been initiated before the fibres were cleared. On the contrary, a significant correlation was established with the amount of tremolite. This may be due to the fact that this fibre type is far more persistent in lung tissue and has thus not disappeared. Its persistence may also make it particularly fibrogenic. In fact, tremolite fibres seem to be more fibrogenic than the commercial amphiboles, whereas no fibrogenic effect was established for anthophyllite.

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Sažetak

MINERALNA VLAKNA, FIBROZA I AZBESTNA TJELEŠCA U PLUĆNOM TKIVU RADNIKA KOJI SU BILI IZLOŽENI AZBESTNOM CEMENTU

Ispitivani su uzorci plućnog tkiva umrlih radnika koji su bili izloženi azbestnom cementu, s mezoteliomom, i bez njega, i njihovim odgovarajućim kontrolama. Broj azbestnih tjelešaca i stupanj fibroze određeni su svjetlosnim mikroskopom, a tip, veličina i broj vlakana elektronskom mikroskopijom i rendgenskom spektrometrijom. Sadržaj azbesta bio je veći kod izloženih radnika negoli u kontrola. Krizotilna vlakna bila su najbrojnija među formiranim vlaknima. Nađeno je da je duljina izloženosti i broj azbestnih tjelešaca u korelaciji sa svim pojedinačnim amfolitima (amozit, krocidolit, tremolit i antofilit), ali ne i s krizotilom. Nalaz fibroze bio je u korelaciji s tremolitom, za razliku od krizolita, i to u eksponiranih i kontrolnih radnika. Uzorci plućnog tkiva radnika s mezoteliomom sadržavali su više krocidolita i tremolita od drugih radnika koji su bili izloženi azbestnom cementu.

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