Environmental Malignant Mesothelioma in Southern Anatolia: A Study of Fifty Cases

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Malignant mesothelioma is a highly aggressive tumor of the serous membranes, which in humans results from exposure to asbestos and asbestiform fibers. Although occupational malignant mesothelioma is still the most common form of this lesion, naturally contaminated soil can play an important role in the development of environmental malignant mesothelioma in some parts of the world. Fifty cases of malignant mesothelioma (MM) from southern Turkey with no occupational history of asbestos exposure were reviewed regarding pathologic and clinical features. A case of hyaline fibrous plaque of the pleura was also included in this series. Histologically the cases were classified as epithelial (36 cases); sarcomatous (7 cases); and biphasic (7 cases). One of the sarcomatous cases was desmoplastic. Ultrastructural examination of the tumor tissue in three cases revealed long-surface microvilli in epithelial cells. Intersititial cells of the lung in one case showed electron-dense asbestos fibers in the cytoplasm. Mineralogical analyses of the lung tissue in three cases of MM and the case of pleural plaque showed high amounts of asbestos fibers most consistent with tremolite and actinolite. The clinical and pathologic features of our cases support that the environmental inhalation of asbestos is still a major health problem in some parts of Turkey. *Key words*: actinolite, asbestos, EDXA, environmental malignant mesothelioma, southeastern Anatolia, tremolite, Turkey. *Environ Health Perspect* 108:1047–1050 (2000). [Online 11 October 2000]

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Environmental exposure to asbestos and asbestiform fibers is not only a major cause of malignant mesothelioma (MM) of the serous membranes, but also leads to a variety of other benign changes in the lung such as fibrous pleuritis, pleural hyaline plaques, and diffuse intersititial fibrosis as well. Although some authors have reported some series of pulmonary pathologic conditions, including malignant mesothelioma, due to environmental exposure to asbestos fibers from different parts of the world (including Italy, Greece, and Cyprus), Turkey became well known for its "mesothelioma villages," Tuzkoy, Karain, and Sarihidir, located in central Anatolia near Nevsehir (Figure 1) (*1–4*). In central Anatolia, a natural fibrous zeolite, erionite has been documented as the cause of pleural malignancy; however, some studies of tremolite- and chrysotile-associated malignant mesothelioma series have also been reported (Figure 1) in parts of Turkey (*5–7*). Review of these references revealed mostly clinical, epidemiologic, and radiologic studies, with an additional mineralogic analysis performed in some of them.

Adana is the fourth largest city in Turkey, located in southern Anatolia, close to the East Mediterranean Sea. Çukurova University Hospital, in Adana, is the largest health consultation center in southeastern Anatolia. A retrospective study on pleural malignancies, in collaboration with other hospitals in the city, revealed 50 malignant mesothelioma cases reported in the last 10 years, from small villages around Adana and the neighboring cities Diyarbakir, Sanliurfa, Malatya, and Adiyaman (Figure 1).

In this study, we focused on the pathologic features of malignant mesothelioma cases in our region, their geographical distribution, and the types of mineral fibers as potential causative agents.

Materials and Methods

We reviewed the files of pathology departments of Çukurova University Hospital, Adana SSK Hospital, Adana State Hospital, and a private pathology laboratory for cases of malignancies of the visceral membranes. Some recently diagnosed malignant mesothelioma cases in Çukurova University as well as a case of fibrous pleuritis with hyaline plaque were also included in the study. The diagnosis of malignant mesothelioma was confirmed by reviewing hematoxylin–eosin (HE)-stained sections. Histochemical stains including periodic acid–Schiff (PAS) with and without diastase predigestion and mucicarmine, and immunohistochemical studies using antibodies against broad spectrum keratin (1:50; DAKO Corporation, Carpinteria, CA, USA), epithelial membrane antigen (EMA, 1:80; DAKO A/S, Glostrup, Denmark), and carcinoembrionic antigen (CEA, 1:50; Novocastra Laboratories, Newcastle upon Tyne, UK) were also performed. Ultrastructural examination of tumors was available in three cases and of lung tissue in one. After being immersed in Karnovsky's fixative, tissue samples for transmission electron microscopy (TEM) were rinsed in 1% osmium tetraoxide, dehydrated by graded series of ethanol, and finally embedded in epon. We examined uranyl acetate–lead citrate-stained 50-nm sections using a Zeiss 900 EM transmission electron microscope (Zeiss, Oberkochen, Germany). We obtained clinical information regarding the age, sex, history, and radiologic features from medical records. Available follow-up information was obtained by contacting the patients and their families.

We analyzed tissue fibers on paraffin-embedded lung tissue specimens in the case of fibrous pleuritis and in three cases of malignant mesothelioma using a JEOL JSM-6400 scanning electron microscope (SEM; Jeol USA, Peagody, MA, USA) equipped with an energy dispersive spectrometer at Duke University (Durham, NC, USA). Lung tissue portions were recovered from paraffin blocks, deparaffinized in xylene, and rehydrated to 95% ethanol. The sodium hypochlorite technique was used on the tissue samples, which were processed for digestion and the residues collected on 0.4-µm pore size Nuclepore filters (Costar Corp., Cambridge, MA, USA). The filters were then cut in half, with one half mounted on a glass slide for asbestos body quantification by light microscopy, and the other on a carbon disc with colloidal graphite, sputter-coated with gold, and examined by SEM and energy dispersive X-ray analysis (EDXA). We identified fiber numbers and types as previously described (*8–10*).

Results

Table 1 shows the age, sex, and histologic subtype distribution of our cases. The most common clinical symptoms were dyspnea and chest pain. All the patients presented with pleural effusion, and the radiologic data

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revealed diffuse pleural thickening either bilaterally or localized to one hemithorax. All the patients had lived in small villages of the region for at least 10 years, none had an occupational history of asbestos exposure, and most of them had lived in white stucco-painted houses during their childhood and early adult life. All the female patients were homemakers; the male patients had the following occupations: farmer, 19; government worker, 11; engineer, 2; driver, 1; teacher, 1; technician, 1; medical secretary, 1. In two cases, the tumor invaded both the pleura and the pericardium.

Review of the HE-stained slides demonstrated fibrous plaques as an additional feature in five cases. Tubulopapillary, tubular, and papillary growth patterns were observed in 15, 10, and 4 epithelial cases, respectively. In some cases, an *in situ* component was prominent (Figure 2). Other cases showed either pure solid growth pattern or a combination of two or more of the above patterns. Only two cases (one epithelial and one biphasic) revealed an adenoid pattern (Figure 3). Sarcomatous cases showed the features of a high-grade tumor with pleomorphism and high mitotic rate in all cases, excluding the desmoplastic case (Figure 4). Focal or diffuse myxoid degeneration and areas of hyalinization were common features.

Ultrastructurally, epithelial cells connected each other with well-developed desmosome-type cell junctions. Moderate numbers of long surface microvilli and deposits of intracytoplasmic glycogen were also observed. Some of the poorly differentiated cells lacked microvilli and showed nonspecific ultrastructural features. Spindle cells did not show well-formed cellular junctions, mostly resembling fibroblasts with distended rough endoplasmic reticulum. No surface microvilli could be identified in these cells. Electron microscopic examination of the lung tissue in one case revealed electron-dense fibers, consistent with asbestos in the cytoplasm of the interstitial cells (Figure 5). Histochemical stains showed negative staining for PAS with diastase predigestion (dPAS), and in a few epithelial cases, mucicarmine stained focal areas.

Immunohistochemistry demonstrated positive staining for keratin in all cases. EMA was diffusely positive in all epithelial cases and epithelial components of all biphasic cases and showed a thick, membranous staining pattern. Fibrous MM cases showed focal positive or negative staining for EMA. The desmoplastic MM case was negative for EMA. None of the cases was immunoreactive for CEA.

Mineralogic analyses of lung tissue from three MM cases and one pleural fibrous plaque case are summarized in Table 2. All cases showed high levels of tissue fiber burden (Figure 6). The morphology and elemental composition of these fibers were mostly consistent with tremolite (Figure 7) and actinolite (Figure 8). In the case of pleural plaque, two additional fibers indicative of erionite were also identified with an elemental composition including silicon, aluminum, sodium, and also a peak for calcium or potassium. No erionite fibers were identified in the lung tissues of our three MM cases.

Follow-up information was available in 10 cases excluding the case of fibrous plaque. Eight patients died of disease 4–14 months after the diagnosis. Two patients (cases #11 and 22) are alive 30 and 12 months after diagnosis. One of these patients (case #22) had nodular invasion of the chest wall on physical examination.

Discussion

As shown in Figure 1, series of environmental malignant mesothelioma and other mineral-fiber–related conditions in Turkey and

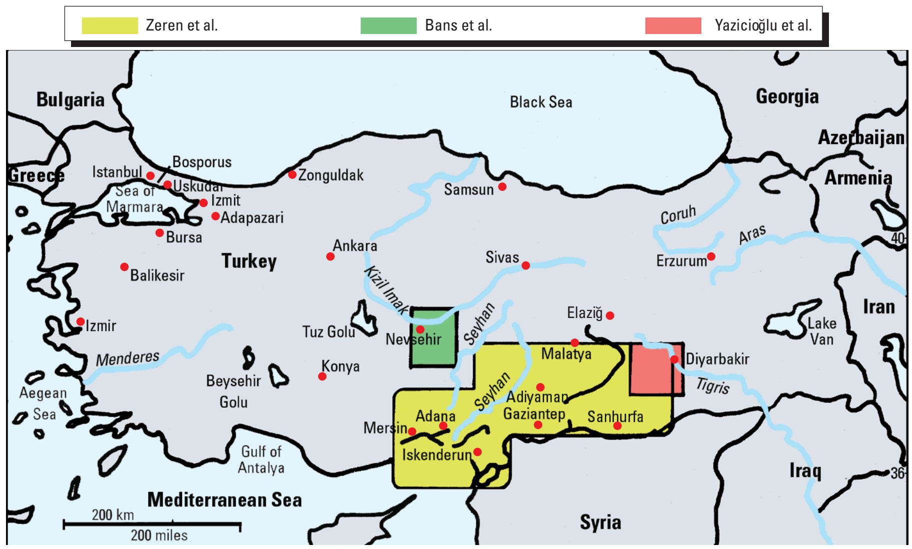

Figure 1. Map of Turkey showing the main areas of reported environmental malignant mesothelioma cases. Baris et al. (*4*) reported erionite as the causative agent, whereas tremolite asbestos was analyzed in some of the cases reported by Yazicioglu et al. (*5*).

Table 1. Age, sex, and histopathologic subtype distributions of 50 patients with malignant mesothelioma.

Age groups	Sex	Case no.	Epithelial	Sarcomatous	Biphasic	Desmoplastic
21–30	M	3	2	1	—	—
	F	2	1	1	—	—
31–40[a]	F	2	1	—	1	—
41–50	M	9	8	—	1	—
	F	4	2	—	2	—
51–60	M	8	7	1	—	—
	F	1	–	1	—	—
61–70	M	12	8	1	2	1
	F	5	4	—	1	—
≥ 70[b]	M	4	3	1	—	—
Total	M	36	28	4	3	1
	F	14	8	2	4	—
	M and F	50	36	6	7	1

[a]There was no male case in this age group. [b]There was no female case in this age group.

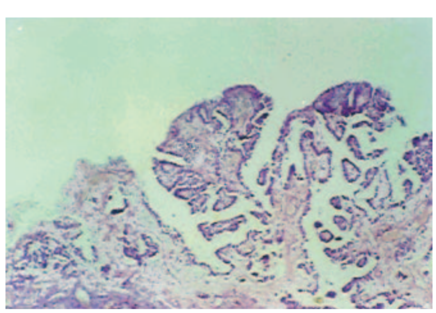

Figure 2. Epithelial with papillary pattern and *in situ* component (H&E; 40×).

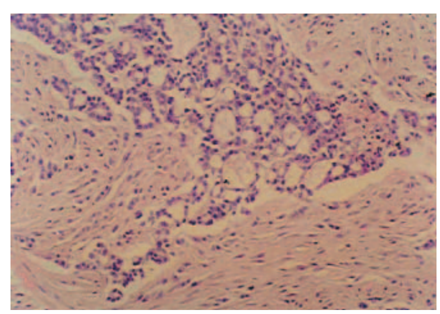

Figure 3. Biphasic MM with adenoid cystic pattern of the epithelial component (H&E; 100×).

among Turkish immigrants living in some other countries have been well documented (*4–7,11–18*). In Central Anatolian villages, mineralogic analyses performed on lung tissue samples as well as street samples revealed large amounts of zeolite (erionite) fibers, whereas in Karain village, these were mixed with small amounts of tremolite and chrysotile fibers. Baris et al. (*4,6*) examined all the environmental sources in these villages and identified the largest asbestos mines and deposits in other parts of Turkey. These authors identified erionite as an etiologic factor in this highly malignant tumor. In other studies from Diyarbakir, tremolite has been reported as the causative agent of malignant mesothelioma and lung carcinoma (*5*). Some cases in our series were from Diyarbakir, Sanliurfa, and Adiyaman, as well as neighboring villages. The fiber contents have been analyzed in three of our cases from this region and the type of asbestos fibers were mostly tremolite and actinolite, in concordance with previous studies (*5*). In a more recent study, Dumortier et al. (*11*) reported a series of bronchoalveolar lavage fluids from 65 individuals environmentally exposed to asbestos and asbestoslike fibers from different parts of Turkey. The main analyzed fiber types were tremolite and chrysotile. In contrast, the most common asbestos fiber in a series of occupational malignant mesothelioma cases reported from the United States was amosite (*10*). Our fourth analyzed case of fibrous plaque also revealed amphibole fibers consistent with actinolite and tremolite. A small amount of erionite in this case was also noted. It is of interest that the erionite fibers found were of low aspect ratio (length to diameter) compared to the tremolite/actinolite fibers from the same case. We also received some cases from the Hatay (Iskenderun and neighboring villages) region, which is known to have mineral deposits; however, detailed studies from this region do not exist in the literature. Our analyzed case of pleural plaque was from a small village near Adana, although the patient had a history of living in several different places throughout the country. In the absence of a history of occupational exposure, the finding of actinolite, erionite, and tremolite fibers within the lung tissue of this patient suggests environmental sources of exposure in some other parts of Anatolia. Further epidemiologic studies with thorough sampling of environmental sources such as water supply, soil, house paint, and street dust could help to clarify the morbidity and mortality in the population due to mineral fiber inhalation.

We had difficulty obtaining the patient follow-ups due to inadequate recording of the cases. Some patients were admitted to the health centers in more densely populated cities such as Ankara and Istanbul, and subsequently lost to follow-up. In a few cases (i.e., case # 22), we were fortunate to be informed about the patient when he returned to his home town for the continuation of treatment.

Microscopic data from our 50 cases revealed the most common features of MM, as described elsewhere (*19*). Most of our cases demonstrated epithelial features. Among epithelial cases, the most significant pattern was a tubulopapillary growth pattern of well-differentiated tumor cells either diffusely or focally, which gives the characteristic appearance of this neoplasm. No predilection of particular histologic subtypes to certain geographical areas was observed. Although Adams et al. (*20*) reported a better survival for epithelial MM, no prognostic significance of these subtypes was noted in our series. In spite of adjuvant chemotherapy, survival time ranged between 4 and 30 months.

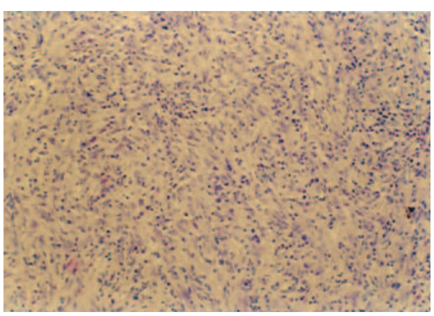

Figure 4. H&E section (100×) from case no. 41 showing MM composed of spindle cells. A few inflammatory cells are also present.

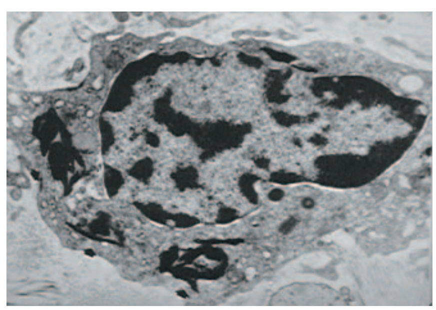

Figure 5. Ultrastructural examination of the lung revealed electron-dense fibers in the cytoplasm of intersititial macrophages (arrows). Uranyl acetate–lead citrate stain; 20,000×.

Table 2. Mineral fiber analyses in four cases.

Case no.	Age/sex	Asbestos/g (LM)[a]	Asbestos (SEM)[b] All fibers	Uncoated	Actinolite	Tremolite
MM1	60/M	36	1,300	34,200	6,840	23,500
MM2	68/M	1,400	4,490	450,000	162,000	292,000
MM3	65/M	8,160	11,400	289,000	286,000	< 16,000
PFP	52/M	21,900	107,000	477,000	435,000	21,800

Abbreviations: PFP, pleural fibrous plaque.
[a]Asbestos bodies per gram of wet lung tissue as determined by light microscopy; normal range is 0–20 asbestos bodies/g. [b]Asbestos bodies per gram of wet lung as determined by scanning electron microscopy; normal range is < 440 asbestos bodies/g; for uncoated fibers normal is < 440–13,000; for actinolite and tremolite, normal is < 440–2,500. All uncoated fibers are > 5 µm in length.

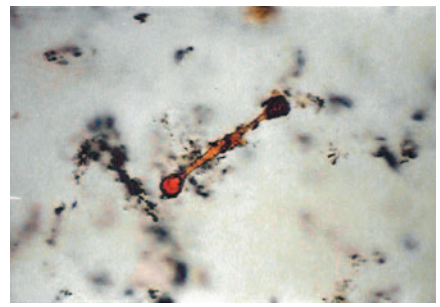

Figure 6. Examination of lung tissue revealed ferriginous bodies (500×).

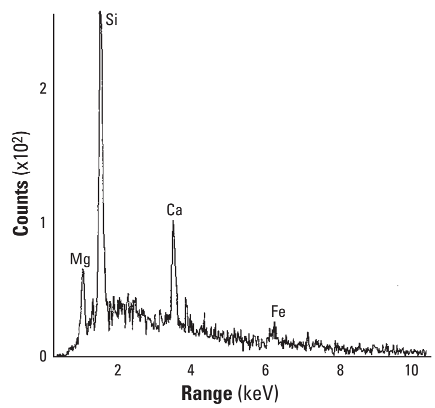

Figure 7. Energy dispersive spectra of case MM2 shows a large peak for silicon and two intermediate peaks for calcium and magnesium. This elemental composition is consistent with tremolite.

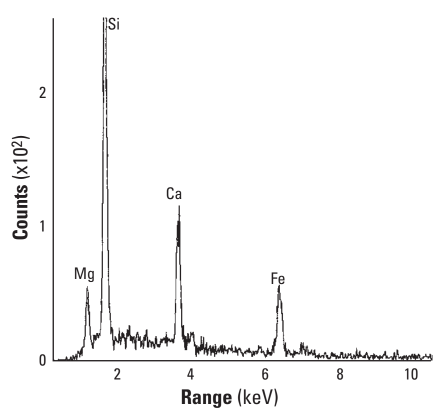

Figure 8. Seven examined uncoated fibers out of 20 showed the elemental composition of silicon, magnesium, calcium, and iron in a proportion indicative of actinolite.

In five cases, we observed fibrous plaques invaded by atypical mesothelial cells. Hyalinizing fibrosis was noted in 10 cases. The pleural fibrosing effects of the mineral fibers are apparent in our series, as is the finding of interstitial fibrosis in available lung specimens of five cases.

This study describes a rather large series of cases of malignant mesothelioma, a neoplasm which is rare in the general population, in a particular area of southern Anatolia. Mineralogic analyses implicate tremolite/actinolite, not erionite, in this region, probably from environmental sources. Future studies should include analyses of potential environmental sources of these fibers with the purpose of preventing further exposures and eventual eradication or at least reduction of such mineral-fiber–induced diseases.

References and Notes

1. Magnani C, Leporati M. Mortality from lung cancer and population risk attributable to asbestos in an asbestos cement manufacturing town in Italy. Occup Environ Med 55:111–114 (1998).
2. Constantopoulos SH, Goudevenos JA, Saratzis N, Langer AM, Selikoff IJ, Moutsopoulos HM. Metsovo lung: pleural calcification and restrictive lung function in northwestern Greece. Environmental exposure to mineral fiber as etiology. Environ Res 38:319–331 (1985).
3. McConnichie K, Simonato L, Mavrides P, Christofides P, Pooley FD, Wagner JC. Mesothelioma in Cyprus: the role of tremolite. Thorax 42:342–347 (1987).
4. Baris I, Simonato L, Artvinli M, Pooley F, Saracci R, Skidmore J, Wagner C. Epidemiological and environmental evidence of the health effects of exposure to erionite fibres: a four year study in the Cappadocian region of Turkey. Int J Cancer 39:10–17 (1987).
5. Yazicioglu S, Ilcayto R, Balci K, Sayli BS, Yorulmaz B. Pleural calcification, pleural mesotheliomas, and bronchial cancers caused by tremolite dust. Thorax 35:564–569 (1980).
6. Baris IY. Asbestos and erionite related chest diseases. Ankara:Semih Ofset Matbaacilik Ltd. Co., 1987.
7. Baris YI, Bilir N, Artvinli M, Sahin AA, Kalyoncu F, Sebastien P. An epidemiological study in an Anatolian village environmentally exposed to tremolite asbestos. Br J Ind Med 45:838–840 (1988).
8. Roggli VL, Pratt PC, Brody AR. Asbestos content of lung tissue in asbestos associated diseases: a study of 110 cases. Br J Ind Med 43:18–28 (1986).
9. Roggli VL, Pratt PC, Brody AR. Analysis of tissue mineral content. In:Pathology of asbestos-associated diseases. Boston, MA:Little Brown, 1992;299–345.
10. Roggli VL, Pratt PC, Brody AR. Asbestos fiber type in malignant mesothelioma: an analytical scanning electron microscopic study in 94 cases. Am J Ind Med 23:605–614 (1993).
11. Dumortier P, Çöplü L, de Maertelaer V, Emri S, Baris I, De Vuyst P. Assessment of environmental asbestos exposure in Turkey by bronchoalveolar lavage. Am J Respir Crit Care Med 158:1815–1824 (1998).
12. Rohl AN, Langer AM, Moncure G, Selikoff IJ, Fischbein A. Endemic pleural disease associated with exposure to mixed fibrous dust in Turkey. Science 216:518–520 (1982).
13. Baris YI, Artvinli M, Sahin AA. Environmental mesothelioma in Turkey. Ann NY Acad Sci 330:423–432 (1979).
14. Sebastien P, Gaudichet A, Bignon J, Baris YI. Zeolite bodies in human lungs from Turkey. Lab Invest 44:420–425 (1981).
15. Baris Y. The clinical and radiological aspects of 185 cases of malignant pleural mesothelioma. IARC Sci Publ 30:937–947 (1980).
16. Selcuk ZT, Coplu L, Emri S, Kalyoncu AF, Sahin AA, Baris YI. Malignant mesothelioma due to environmental mineral fiber exposure in Turkey. Analysis of 135 cases. Chest 102:790–796 (1992).
17. Ozesmi M, Hillerdal G, Svane B, Widstrom O. Prospective clinical and radiologic study of zeolite-exposed Turkish immigrants in Sweden. Respiration 57:325–328 (1990).
18. Larrouy C, Tandjaoui-Lambiotte H, Mellat M, Fabre C, Defrejacques C, Adotti F, Piquet J. Environmental interstitial pneumonia caused by asbestos. Study of a Turkish family exposed to tremolite. Rev Pneumol Clin 46:78–82 (1990).
19. Battifora H, McCaughey WTE. Tumors of the Serosal Membranes. Washington, DC:Armed Forces Institute of Pathology, 1994.
20. Adams VI, Unni KK, Muhm JR, Jett JR, Ilstrup DM, Bernatz PE. Diffuse malignant mesothelioma of pleura: Diagnosis and survival in 92 cases. Cancer 58:1540–1551 (1986).