Occupational Exposure to Asbestos and Urinary Bladder Cancer

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By the use of transmission electron microscopy (TEM) and energy dispersion spectrometry the amount (mean value \( \bar{x} = 191 \pm 94 \) fibers/mg of tissue) and the type (chrysotile and tremolite) of asbestos fibers have been determined in tissue samples of four bladder cancer patients affected by pulmonary asbestosis, working in the same plant producing asbestos-cement pipes and boards. Similar measurements were carried out on samples of bladder cancers of eight control patients not professionally exposed to asbestos. Only five of them also revealed chrysotile fibers (\( \bar{x} = 151 \pm 196 \) fibers/mg of tissue). The paucity of the study and control cases and the small quantitative difference between them regarding the presence of infraneoplastic asbestos fibers does not consent us to hypothesize a causal relationship between tumor and occupational exposure. © 1992 Academic Press, Inc.

INTRODUCTION

The excess of pleural and peritoneal mesotheliomas and pulmonary, gastrointestinal, or laringeal tumors in subjects professionally exposed to asbestos fibers (AF) has often been reported in literature; "available data do not support the hypothesis that asbestos is associated with urothelial cancer" (Steineck et al., 1990).

In a study of the mortality in a cohort of insulation workers an increase of deaths for uncommon malignant neoplasms was observed. The authors report: "The overall increase is of some interest, especially in view of the known possibility of AF being disseminated to virtually all organs following inhalation or ingestion" (Selikoff et al., 1979).

Only a few years ago, the presence of chrysotile and amphyboles (180 fibers/mg of dry tissue) was observed inside a necrotic granulomatous lesion of the bladder neck in a patient without evident pleuropulmonary pathology after 17 years of occupational exposure to asbestos (Monseur et al., 1986).

In a recent study on male patients admitted to a hospital in Madrid for bladder cancer, a correlation between the tumor and the occupational exposure to AF was suggested, albeit without sufficient proof to support the findings (Bravo et al., 1988).

In the literature regarding the presence of AF in human urine, affirmative reports in this respect prevale. Indeed, positive observations have been reported in workers occupationally exposed to AF (Wiss, 1953; Finn and Hallenbeck, 1984), as well as in the urine of the inhabitants of Duluth (Minnesota), who drank until
1977 water contaminated by amphiboles in the mean amount of $5 \times 10^7$ fibers/liter (Cook and Olson, 1979). The latter observation, on the other hand, has not been confirmed by research performed on the urine of Everett residents (Puget Sound region, Washington) who drank water contaminated by chrysotile in the amount of $2 \times 10^8$ fibers/liter (Boatman et al., 1983). Finally, Bignon et al. (1980) found attapulgite fibers in the urine of a patient taking a drug containing this fibrous mineral of French extraction.

The suspicion that a contact was possible between the inhaled and/or ingested AF and the vesical mucosa prompted us to report four cases of bladder carcinoma, discovered by chance, in former workers of the same plant in Bari, Italy, where pipes and ondulated laminae in asbestos cement were produced. Totally inadequate from the point of view of primary and secondary systems of prevention at least until 1970, the plant, employing about 160 persons, made use of mixtures of asbestos cement comprising 15–20% of AF. The types of minerals were: chrysotile (60–80%), crocidolite (20%), and amosite (10–20%).

**CASE REPORT**

*Case 1—C.V.* He worked as part of the mechanical maintenance staff in the asbestos–cement plant for 22 years.

At the age of 53, he was diagnosed with pulmonary asbestosis. At the age of 59 he was operated upon for a multiple vegetant neoplasia of the urinary bladder (right hemibladder T3, left parietal wall T1). The histological diagnosis (N.54430) was papillary and infiltrating carcinoma with prevalent squamous pattern and with different degrees of differentiation.

The patient died of neoplastic cachexia at 60 years of age.

*Case 2—L.P.* He worked in different production sections of the asbestos–cement plant for 25 years.

At the age of 56, he was diagnosed with pulmonary asbestosis. At the age of 64 he was operated on for papillary neoplasia of the urinary bladder (T3). The histological diagnosis (N.58396) was urothelial papillary carcinoma with marked dysplastic patterns (grade III). The patient died at the age of 66 due to neoplastic diffusion.

*Case 3—G.M.* The patient was occupationally exposed to AF for 13 years: for 4 years he worked at the production of tubes and laminae in asbestos cement, and afterward as an electric maintenance worker in the same plant.

At the age of 48, he was diagnosed with pulmonary asbestosis. At 53 years of age, following some episodes of terminal hematuria, he was found affected by trigonal and vesicourethral sphincteric papillomas, which were transurethrally resected. The histological diagnosis (N.111527) was papillary neoplasia of the bladder with marked polystratification and dysmorphic and regressive changes of the epithelial elements.

After 9 years the patient is still alive.

*Case 4—C.L.* He worked at the production of asbestos–cement pipes for 35 years.

At 62 years of age, he was diagnosed with pulmonary asbestosis. At the age of 74 he was endoscopically operated because of bladder neoplasia. The histological
diagnosis (N.46915) was urothelial papillary carcinoma with discrete discariosis (grade II) without infiltrating aspects.

He died at the age of 76 due to neoplastic diffusion.

METHODS AND RESULTS

The four cases were collected by one of us (R.M.) to whom the patients and/or their relatives had been referred for the eventual occupational recognition of their diseases, for compensation purposes. In all four patients already affected by pulmonary asbestosis, a bladder urothelial carcinoma was diagnosed.

We would like to know whether there have been similar cases, but have so far not been able to obtain such information.

A tissue burden study on the possible presence of AF was undertaken by selecting one or two paraffin blocks for each case from three different hospital histopathologic laboratories in Bari. The blocks, constituted by only neoplastic urothelial tissue, were already stored for a period ranging from 4 to 10 years preceding our study (Table 1).

The samples were first isolated from the paraffin by immersion in xilene at 60°C in order to collect the mineral component of the neoplastic tissue. The organic component was then oxidated at low temperature in atomic oxygen plasma; the nonorganic component, transferred on thin carbon films and mounted on copper grids, was therefore analyzed by TEM Philips 430, equipped with energy dispersion spectrometer for the X-ray microanalysis Edax 9100 (Figs. 1 and 2).

The same procedure was adopted with the samples of urinary neoplastic tissue of eight control cases; two blocks were used for each of the study cases. The control blocks were selected from the three histopathologic laboratories. These contained similar bladder urothelial tumors of patients who were not affected by pulmonary asbestosis, who had not been occupationally exposed to AF according to anamnestic records, and whose tumors had occurred immediately before or after those of the study cases. The presence of AF in paraffin blocks was quantified without knowing whether these belonged to the study or the control cases. The results are reported in Table 2.

To avoid the risk of possible environmental laboratory contamination we used

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**Table 1**

**AGE AT THE TIME OF THE RESECTION OF BLADDER CARCINOMA, CIGARETTE SMOKING, AMOUNT OF TISSUE AVAILABLE, CONCENTRATION, AND TYPE OF ASBESTOS FIBRILS FOUND INSIDE THE NEOPLASTIC TISSUE OF FOUR PATIENTS AFFECTED BY PULMONARY ASBESTOSIS**

<table>
<thead>
<tr>
<th>Cases</th>
<th>Age (years)</th>
<th>Smoke (cigarettes/day)</th>
<th>Tissue available (g)</th>
<th>F/GOE (ff)</th>
<th>Fibers concentration (ff/mg)</th>
<th>DL (ff/mg)</th>
<th>Type of fibrils</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>0</td>
<td>0.373</td>
<td>0.20</td>
<td>133</td>
<td>30</td>
<td>Chrys.</td>
</tr>
<tr>
<td>2</td>
<td>64</td>
<td>10</td>
<td>0.100</td>
<td>0.20</td>
<td>281</td>
<td>40</td>
<td>Chrys.</td>
</tr>
<tr>
<td>3</td>
<td>53</td>
<td>20</td>
<td>0.090</td>
<td>0.15</td>
<td>89</td>
<td>40</td>
<td>Chrys.</td>
</tr>
<tr>
<td>4</td>
<td>74</td>
<td>5</td>
<td>0.250</td>
<td>0.40</td>
<td>261</td>
<td>35</td>
<td>Chrys. + trem.</td>
</tr>
<tr>
<td>Mean</td>
<td>62.5</td>
<td>11.6</td>
<td>0.203</td>
<td></td>
<td>191</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>8.9</td>
<td>7.6</td>
<td>0.135</td>
<td></td>
<td>94</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note. F/GOE, fiber count per grid opening examined; DL, detection limit (Huang et al., 1988).*
the same method with samples of lung tissues known to be free from asbestos fibers. The fiber concentration obtained in the blank samples was equal to 0 ff/mg with a detection limit equal to 12 ff/mg; this last value was therefore considered the upper limit value of accidental contamination of the samples during the preparation procedures.

Fig. 1. Case 1. Chrysotile fiber of $4 \times 0.1 \, \mu m$ detected by TEM ($\times 30,000$).

Fig. 2. Case 4. Chrysotile fiber of $9 \times 0.07 \, \mu m$ detected by TEM ($\times 21,000$).
TABLE 2

<table>
<thead>
<tr>
<th>Controls</th>
<th>Profession</th>
<th>Age (years)</th>
<th>Smoke (cigarettes/day)</th>
<th>Tissue Available (g)</th>
<th>F/GOE (ff)</th>
<th>Fibers concentration (ff/mg)</th>
<th>DL (ff/mg)</th>
<th>Type of fibrils</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Physician</td>
<td>75</td>
<td>30</td>
<td>0.080</td>
<td>0</td>
<td>0</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Barber</td>
<td>62</td>
<td>10</td>
<td>0.090</td>
<td>0.50</td>
<td>580</td>
<td>40</td>
<td>Chrys.</td>
</tr>
<tr>
<td>3</td>
<td>Carpenter</td>
<td>92</td>
<td>5</td>
<td>0.070</td>
<td>0</td>
<td>0</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Laborer</td>
<td>58</td>
<td>20</td>
<td>0.100</td>
<td>0.30</td>
<td>241</td>
<td>40</td>
<td>Chrys.</td>
</tr>
<tr>
<td>5</td>
<td>Fisherman</td>
<td>60</td>
<td>20</td>
<td>0.070</td>
<td>0.15</td>
<td>115</td>
<td>55</td>
<td>Chrys.</td>
</tr>
<tr>
<td>6</td>
<td>Farmer</td>
<td>67</td>
<td>0</td>
<td>0.320</td>
<td>0.15</td>
<td>75</td>
<td>25</td>
<td>Chrys.</td>
</tr>
<tr>
<td>7</td>
<td>Housekeeper</td>
<td>61</td>
<td>0</td>
<td>0.100</td>
<td>0</td>
<td>0</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Farmer</td>
<td>63</td>
<td>5</td>
<td>0.100</td>
<td>0.40</td>
<td>201</td>
<td>40</td>
<td>Chrys.</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>67.2</td>
<td>15</td>
<td>0.116</td>
<td></td>
<td>151</td>
<td></td>
<td></td>
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<tr>
<td>SD</td>
<td></td>
<td>11.3</td>
<td>10</td>
<td>0.083</td>
<td></td>
<td>196</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. F/GOE, fiber count per grid opening examined; DL, detection limit.

DISCUSSION

By comparing our results as reported in Tables 1 and 2, it is evident that the mean concentration of fibers per milligram of tissue is less in the control cases than in the exposed patients, even though the difference is not statistically significant. Nonetheless it is interesting to note that the three cases totally negative for fibrils were all control cases.

The AF observed in the positive cases (both study and controls) were almost always formed by chrysotile. This material was the most used in the plant where the study cases were employed and it is the type of AF usually found in urban areas (Paoletti et al., 1989). Also in one of the four study cases, a small amount of tremolite fibers was found; this amphybole is a frequent contaminant of chrysotile in natural mines and is commonly found, although sometimes in only very small amounts, in many products containing chrysotile.

The mean length of the AF in the study cases was 8.0 μm with a mean diameter of 0.16 μm. In one case sporadic AF longer than 40 μm were also found. In the positive control cases the mean length was 11.0 μm with a mean diameter of 0.13 μm.

In the technological cycle of the aforementioned plant neither aniline dyes nor any other chemical compound containing known vesicle carcinogens was ever used. Furthermore none of our four patients underwent further exposure to asbestos in extraprofessional activities nor pelvic irradiation.

Our results are rather controversial since they may have been influenced by:

—AF are well-known environment contaminants, especially in large cities like Bari (population 400,000);

—the work-related exposure to AF in our cases has been verified retrospectively, both in study and control cases, acquiring the anamnestic data from the relatives in case of death. Therefore a possible environmental and nonoccupational exposition to AF cannot be ruled out.

It was impossible to verify the presence of contamination by AF in the air of the
laboratory, in the reagents, in the inclusion material, in the water, and in the solvents during the treatment for histologic examination of the neoplastic tissue also because the samples were collected from three different hospitals and were processed at different times several years before our study. Furthermore an investigation conducted in the three laboratories did not reveal the presence of asbestos or asbestos insulation material. In any case, because the possibility of contamination by AF during the procedure cannot be totally excluded, we believe that further study in samples of urothelial carcinomas of asbestos-exposed workers should be carried out on fresh non-paraffin-embedded material.

To our knowledge, our study is the first to show the documented presence of AF inside urothelial tumors among occupationally exposed workers.

The capacity of the AF, either inhaled or ingested, to migrate from the organs communicating with the outside (lungs and digestive apparatus) to the blood and internal organs both in man and in experimental animals has already been ascertained (Auerbach et al., 1980; Carter and Taylor, 1980; Lee et al., 1981; Kobayashi et al., 1987; Huang et al., 1988; Cook, 1984; Kaczenski and Hallenbeck, 1984).

It is still unclear how the AF can reach the urinary bladder. Two hypotheses for the penetration of AF in the vesicle wall have been forwarded in the literature to date: (1) the presence in the urine filtrated by renal glomeruli and (2) vehiculation with blood and lymph (Lee et al., 1981; Boatman et al., 1983). A third hypothesis proposed for pleural and peritoneal tissue is that of translocation, the mechanical migration through the tissue (Suzuki and Kohyama, 1991). In our case this is improbable due to the distance between the bladder and the lung.

Huang et al. (1988) report a higher concentration of AF in all the examined organs (kidneys, liver, spleen, pancreas, stomach, small intestine, colon, and rectum) in subjects already showing a higher pulmonary concentration of the mineral fibers. In this study, other than the lung, the organ richest in mineral concentration was the kidney, in which the amount of AF was about 7% that in the lung. Similar observations have been made with the baboon kidney (Langer, 1974; Patel-Mandlik, 1980).

With regard to length of mineral fibrils, there is evidence in the literature that mineral and plant fibers, with a length >40 μm introduced by ingestion, can be found in the lymphohematic circulatory systems (Volkheimer, 1974; Schreiber, 1974).

The amount of AF found in the urinary neoplastic tissue is not much different from that already reported in identical conditions (Monseur et al., 1986). These values, though, are much lower than those observed by others in the lungs (157 × 10⁶ ff/g) and the kidneys (12.5 × 10⁶ ff/g) of patients affected by asbestosis (Huang et al., 1988). Similar findings have also been reported in pulmonary carcinomas of asbestos-exposed (1141.7 × 10⁶ ff/g) or nonexposed workers (35.8 × 10⁶ ff/g) in the experience of Wagner et al. (1988). We also ignore the possible pathogenetic role of the AF inside the neoplastic vesicle tumor, because our study did not take into consideration the possible presence and concentration of AF in the peritumoral part of the bladder mucosa. Nonetheless it seems useful to study further cases because any exposition to AF of the human tissues is potentially dangerous.
indeed, minimal pathologic exposition in the asbestos carcinogenesis has not been ascertained as yet (Davis and McDonald, 1988).

Finally, the possible role of other known carcinogenic agents in respect to bladder carcinoma, such as cigarette smoking, must be considered in the evaluation of similar cases. Theoretically, the bladder could represent a target of the combined synergetic action of smoke and AF.

In conclusion, this study does not allow the tracing of definitive results; it nonetheless suggests the following possibilities: (1) AF are capable of reaching the bladder by a mechanism that remains to be ascertained (probably via blood and lymph); (2) AF can be observed inside neoplastic urothelial tissue; (3) the small quantitative difference between study and control cases regarding intraneoplastic AF does not establish a definite causal relationship between tumor and occupational exposure to AF.

ACKNOWLEDGMENTS

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REFERENCES


