Clinicopathological Study on Malignant Pleural Mesotheliomas

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Abstract

We investigated the clinicopathological features of cases with malignant pleural mesothelioma (MPM), which had been operated on at our institutes. There were nine cases in which pleuropneumonectomy had been performed for MPM (1 epithelioid, 3 biphasic, 5 sarcomatoid) between 1995 and 2003. The age of the patients was from 41 to 69 years old, and all were men. Four cases had occupational exposure to asbestos. Five cases were with stage II and four with stage III. The level of hyaluronic acid in pleural effusion was high in most of cases. Tumor cells in epithelioid mesothelioma and in biphasic mesothelioma were positive for PAS staining and Alcian Blue staining, but tumor cells in sarcomatoid mesothelioma were positive for them in only one case. We evaluated the results of immunohistochemical staining according to the histological elements. All epithelial elements (EE) reacted for cytokeratin AE1/AE3, EMA, and HBME1, however, some EE reacted for vimentin and calretinin. On the other hand, all the sarcomatous elements (SE) were positive for vimentin and calretin, however, some SE were positive for cytokeratin AE1/AE3 and none were positive for EMA and HBME1. EE and SE were positive for D2-40 in some cases. Five cases were alive and four died after the surgery, and two-years survival was 44%. Immunohistochemical panels, especially calretin, were helpful in the diagnosis of MPM. Although the prognosis for MPM is poor, there are some cases whose prognoses improve with surgical removal of the tumor.

Patients with malignant pleural mesothelioma (MPM) are rarely diagnosed at an early stage. MPMs do not respond to chemotherapy or radiotherapy, and they relapse even when they are removed surgically at an early stage. The clinical entity of MPM has now been well recognized by doctors who are not specialists in respiratory diseases, and patients with MPM at early stage are now referred to special hospitals, where extrapleural pneumonectomy (EPP) is performed for MPM.

It is thought that development of MPM is due to exposure to asbestos fibers, but some patients with MPM are not exposed to them. It is not fully recognized what is the earliest event in the development of MPM and how it progresses. The diagnosis of MPM depends on the histological findings of the biopsy specimens, but evaluation of the small biopsy is not easy. The aim of this study was to elucidate the early clinical findings and early microscopic changes of MPM that were removed surgically and confirmed to be MPM by histological examination.

Materials and Methods

Thirteen cases with MPM who underwent EPP between 1995 and 2004 were investigated (Table 1). Age and sex of the patients, exposure to asbestos fibers, stage of MPM, initial signs or symptoms, level of hyalulonic acid in pleural effusion, and preoperative diagnosis were evaluated. The adjuvant therapy after the surgery and the prognosis of the patients were also evaluated.

The surgical materials were fixed in formalin and embedded in paraffin, and 4 micron meter sections were cut and stained with Hematoxylin and Eosin staining. A tumor that was composed of epithelioid components was designated as epithelioid subtype, that of sarcomatoid components as sarcomatoid subtype. A tumor that was composed of combined epithelioid and sarcomatoid components with each comprising at least 10 percent of the tumor was designated as biphasic subtype (WHO 1999). Immunohistochemical staining was performed using the following antibodies: cytokeratin AE1/AE3 (monoclonal, prediluted), cytokeratin high molecular weight (HMW) 34βE12 (monoclonal, prediluted), epithelial membrane antigen (EMA) (monoclonal, 1:800), vimentin (monoclonal, 1:125), desmin (monoclonal, 1:200), smooth muscle actin (SMA) (monoclonal, 1:1600), HBME1 (monoclonal, 1:800), WT1 (monoclonal, 1:1000), carcinoembryonic antigen (CEA) (monoclonal, 1:500), BerEP4 (monoclonal, prediluted) (DAKO), CAM5.2 (monoclonal, prediluted, Becton Dickinson), CD34 (monoclonal, 1:10, Immunotech), D2-40 (monoclonal, prediluted, Signet), calretinin (polyclonal, prediluted, Zymed). MAX-PO(M) and MAX-PO(R) (Nichirei) were used for monoclonal and polyclonal antibodies, respectively. Catalyzed signal amplification system (DAKO) was used for WT1. The results of staining were evaluated for epithelioid and sarcomatoid components, separately.

Results

The age of the patients ranged from 41 to 69 and all were male. Six of the patients were exposed to asbestos fibers. Two were with Stage I, five with Stage II, five with Stage III, and one with Stage IV mesothelioma (Table 1).

Ten of the patients visited the clinician because of chest pain (6 cases), cough (3 cases), and fever (1 case). Three cases were asymptomatic. Pleural effusions were detected with chest X-ray at annual check-up in two of them, and with X-ray taken at the hospital where cholecystectectomy was undertaken as a therapy for cholecystolithiasis in one case.

Levels of hyalulonic acid in pleural effusions were measured in ten cases. They were all high, ranging from 28,300-2,660,000 ng/ml.

Cytological examination of pleural effusions was performed in nine cases. Atypical cells were detected in pleural effusions in two cases, and existence of malignant tumor was strongly suspected in seven cases. Percutaneous biopsy of the tumor was performed in two cases. In one case, MPM was suspected, but the biopsy sample was not diagnostic in another case. Biopsy under thoracoscopy was preformed in 12 cases and biopsy with small incision was performed in one case. Twelve of them were diagnosed as MPM. One was diagnosed as malignant tumor, but not as MPM.

Lungs removed with surgery were covered with thick pleura. In earlier cases, there was a pleural

cavity between parietal and visceral pleura, but in advanced cases they were fused. In cut sections, the thickening pleura were white and covered the whole lung. The tumor existed at the pleura and invaded the lung, diaphragm, and chest wall in some cases. In earlier cases (cases 12 and 13), mesothelioma cells proliferated both on the parietal and visceral pleura, but there was no invasion of the lung, diaphragm, or chest wall. The tumor cells were in one layer in one area, but papillary proliferation was observed in another area. The mesothelioma lesions were discontinuous on the pleura, and the pleura were covered with normal mesothelial cells in some areas.

There were four cases with epithelioid MPM, five with sarcomatoid MPM, and four with biphasic MPM. Periodic acid staining and alcian blue staining were positive in all the epithelioid and biphasic MPMs, but positive in one case of sarcomatoid MPM. Immunostaining for cytokeratin AE1/AE3, CAM5.2, cytokeratin HMW 34βE12, HBME1 was positive in all the epithelioid components. Immunostaining for EMA, calretinin, D2-40, WT1 was positive in all but one of the epithelioid components. Epithelioid components that were negative for these were in biphasic MPM. A small number of tumor cells was positive for CEA in one case and for BerEP4 in two cases. Desmin was positive in two cases and smooth muscle actin was positive in one case, but intensity of the staining was weak. Vimentin, which is a marker for non-epithelial cells, was positive in most tumor cells in two cases, and in a few tumor cells in two cases. On the contrary, vimentin was positive in all of the sarcomatoid components. Some sarcomatoid components were positive for cytokeratin AE1/AE3, CAM5.2, EMA, HBME1, calretinin, WT1, D2-40, but none of the sarcomatoid component was positive for cytokeratin HMW 34βE12, CEA, BerEP4.

Seeding of the mesothelioma cells on the tract of the thoracoscopy was observed in three cases (Case 8, 10, and 13), but the tract was free of mesothelioma cells in one case (case 6).

Hyperthermic intrathoracic chemotherapy after the EPP was performed in five cases. Radiotherapy was performed at the local recurrence in another two cases. Five are alive now and seven dead, and the 2-year survival was 31%. Types of relapse were local recurrence in all cases, and there was lumbar metastasis in one case. Four of 5 patients who underwent hyperthermic intrathoracic chemotherapy are alive now.

Discussion

It is rare for us to see the initial changes of MPM, and it is not elucidated how MPM develops and progresses. It is reported that early disease manifests as multiple discrete pleural nodules, predominantly on the parietal pleura (Adams VI et al. 1986). Boutin et al. reported that nonspecific inflammatory lesions, pachypleuritis, nodules smaller than 5mm, and a combination of pachypleuritis and small nodules were observed in the patients with early stage malignant mesothelioma, and that the visceral pleura was invaded later (Boutin C et al. 1993). However, thoracoscopy did not disclose any nodules in case 12 of our study, although mesothelioma cells proliferated both on parietal and visceral surfaces and papillary proliferation was observed in thicker areas of surface growth. Mesothelioma cells had invaded the visceral pleura, but not invaded the pulmonary parenchyma. An abrupt change from an area of flat normal mesothelial cells to a region of proliferation of mesothelioma cells was observed. This case showed, contrary to the report of Boutin et al., that mesothelioma cells proliferate both on parietal and visceral surfaces before the small nodules are observed with thoracosocpy.

Malignant seeding along the tract of cytology or biopsy needles, chest tubes, thoracoscopy trocars and surgical incisions is a common complication of diagnostic and therapeutic procedures in patients with MPM (Boutin C et al, 1995). Mesothelioma cells invaded along the tract of thoracoscopy trocars in cases 8 and 10, but not in case 6.

Numerous immunohistochemical markers are used in the diagnosis of MPM. It is reported that cytokeratin AE1/AE3, CAM5.2, cytokeratin HMW 34βE12, vimentin, HBME1, calretinin, and WT1 are expressed frequently in MPM, but CEA and BerEP4 are not. CD34 is positive for localized fibrous tumor, but negative for MPM. D2-40 is an antibody that detects lymphatic endothelial cells; however, it is also expressed in mesothelial cells, myoepithelial cells of breast, and basal cells of prostate. The following tumors were reactive: gastriointestinal stromal tumor, meothelioma, seminoma, and pleomorphic adenoma (Kaserling E 2004). In our study, D2-40 was expressed in the epithelioid component of MPM, and in some sarcomatoid components of MPM. CEA and BerEP4 were expressed in some epithelioid mesotheliomas, but the percentage of positive cells was very small. Vimentin was positive for sarcomatoid components of MPM, and SMA and desmin were positive in some sarcomatoid components. There are no specific markers for MPM, and a combination of histological findings and a panel of immunohistochemical markers enables a correct diagnosis.

Epithelioid mesothelioma must be distinguished from benign reactive mesothelial hyperplasia, and sarcomatoid mesothelioma from fibrous thickening of pleura. Cytological atypia of epithelioid mesothelioma is a useful feature, but reactive mesothelial cells have a wide range of nuclear variability. However, papillary structures in pleural tissue may be a finding highly suggestive of malignancy. Cellularity of tumor cells in sarcomatoid mesothelioma is higher than that in reactive fibroblastic pleural lesions, but neoplastic cells in desmoplastic mesothelioma are widely separated by a thick band of hyalinized collagen, and the differentiation may be difficult.

Cury et al. reported that strong diffuse linear staining for EMA is a good marker of MPM, and nuclear staining for p53 is suggestive of epithelioid mesothelioma (Cury PM et al. 1999). However, in our study, the epithelioid component of one of the biphasic mesotheliomas did not react with EMA, and none of the sarcomatoid components reacted with EMA. Kitazume et al. reported that MPMs constantly express E-cadherin, whereas reactive mesothelial cells lacked its expression, and E-cadherin would be a useful marker in the differential diagnosis between the two (Kitazume H et al. 2000).

Prognosis of MPM is poor and it was reported that the median survival was only 9 months (Ruffie P et al. 1989). Boutin et al. reported that a median survival was 32.7 months in the patients with stage IA in which only the parietal or diaphragmatic pleura was involved, and 7 months in the patients with stage IB in which the visceral pleura was invaded (Boutin C et al. 1993). Chemotherapy is found to have an average partial response rate of 20% (Sterman DH et al. 1999). Radiotherapy has been ineffective in prolonging survival in mesothelioma patients. Radiotherapy with curative intent would irradiate large volumes of the thorax and is limited by unacceptable pulmonary toxicity. Complications of radiotherapy for MPM include nausea and vomiting, radiation hepatitis, esophagitis, myelitis, myocarditis, and pneumotitis. However, early local radiotherapy in preventing malignant seeding after invasive diagnostic procedures in patients with MPM is effective (Boutin C et al. 1995).

EPP is a radical procedure that includes en block removal of the parietal pleura, ipsilateral lung, pericardium, and the ipsilateral hemidiaphragm. Complications of EPP are supraventricular arrthymia, pneumonia, bronchopleural fistulae, and some patients may die due to respiratory failure, myocardial infarction, and pulmonary embolism. Median survival after EPP ranges from 9 to 19 months, and 2-year survival is 33% (Ruth S et al. 2003). However, prolonged survival is expected after EPP in cases with extremely early cases such as cases 12 and 13 in our study. Multimodality therapy, including surgery, radiotherapy or photodynamic therapy for residual local disease, and systemic chemotherapy targeting distant spread, appear to be the most successful for management of potentially respectable disease (Parker C, Neville E 2003). Five cases were treated with hyperthermic intrathoracic chemotherapy after EPP in our study, and 4 cases are alive now. However, more data are needed for the evaluation of the efficacy of this therapy.

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Table 1. Summary of patients with malignant pleural mesothelioma who underwent extrapleural pneumonectomy.

Cases	Subtype	Sex	Age	Exposure to asbestos	Hyaluronic acid (ng/ml)	Stage	Prognosis
1	Biphasic	Male	40	Unknown	33,000	III	Unknown
2	Epithelioid	Male	53	Absent	270,000	II	Dead
3	Biphasic	Male	58	Absent	54,800	IV	Dead
4	Epithelioid	Male	62	Absent	455,000	II	Alive
5	Epithelioid	Male	64	Absent	NM	III	Dead
6	Sarcomatoid	Male	48	Present	380,000	III	Alive
7	Sarcomatoid	Male	51	Present	35,700	II	Dead
8	Sarcomatoid	Male	41	Absent	28,300	III	Alive
9	Biphasic	Male	58	Present	NM	III	Dead
10	Sarcomatoid	Male	59	Absent	NM	II	Dead
11	Sarcomatoid	Male	69	Present	34,000	II	Alive
12	Epithelioid	Male	61	Present	2,660,000	I	Alive
13	Biphasic	Male	48	Present	94,200	I	Dead

NM: not measured.

Table 2. Results of immunohistochemical staining for malignant pleural mesothelioma（Positive cases/Total cases）

	Epithelioid component (%)	Sarcomatoid component (%)
CK AE1/AE3	7/7 (100)	6/8 (75)
CAM 5.2	7/7 (100)	4/8 (50)
CK HMW 34betaE12	7/7 (100)	0/8 (0)
EMA	6/7 (85.7)	0/8 (0)
vimentin	4/7 (57.1)	8/8 (100)
desmin	2/7 (28.6)	4/8 (50)
SMA	1/7 (14.3)	6/8 (75)
HBME1	7/7 (100)	0/8 (0)
calretinin	6/7 (85.7)	4/8 (50)
WT1	6/7 (85.7)	7/8 (87.5)
D2-40	6/7 (85.7)	4/8 (50)
CEA	1/7 (14.3)	0/8 (0)
BerEP4	2/7 (28.6)	0/8 (0)
CD34	0/7 (0)	0/8 (0)

CK: cytokeratin, HMW: high molecular weight, EMA: epithelial membrane antigen, SMA: smooth muscle antigen, CEA: carcinoembrionic antigen.

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