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ARTICLE

Specificity of MOC-31 and HBME-1 Immunohistochemistry in the Differential Diagnosis of Adenocarcinoma and Malignant Mesothelioma: a Study on Environmental Malignant Mesothelioma Cases from Turkish Villages

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Histological diagnosis of malignant mesothelioma (MM) and differentiation from adenocarcinoma is often difficult. A number of clinical, radiologic, histologic and histochemical criteria have been used as diagnostic aids, but most cases cannot be readily classified on the basis of these characteristics. In recent years, a panel of immunohistochemical antibodies have been increasingly applied for the differential diagnosis of these two tumors. MOC-31 has been recently used as specific for adenocarcinomas while reacting with a minimal number of benign and malignant mesothelial proliferations, and HBME-1 has also been presented as a mesothelial cell marker. In this study, we aimed to show the importance of these two antibodies among the environmental MM cases from Southeastern Turkey. Fifty five cases of MM and twenty adenocarcinomas were included in this study. Histochemical (PAS, PAS-D, mucicarmine) and immunohistochemical (Keratin, EMA,CEA, MOC-31, HBME-1) stains have been performed on each case. Keratin was positive in all cases. EMA stained 50 of 55 MM and all the adenocarcinoma cases. According to our results, dPAS, mucicarmen, CEA and MOC-31 positivity was statistically significant in the diagnosis of adenocarcinoma whereas HBME-1 was demonstrable in most MM cases (52/55) and 11 adenocarcinoma cases. – This study confirmed that in the diagnostic distinction between MM and adenocarcinoma, immunohistochemistry is an important diagnostic tool, however, a panel of antibodies must be used rather than any single antibody. HBME-1 should be included in this panel; MOC-31 can be used where CEA is not available or to doublecheck the reactivity of this antibody. (Pathology Oncology Research Vol 8, No 3, 188–193)

Keywords: Environmental malignant mesothelioma, adenocarcinoma, MOC-31, HBME-1, Turkey

Introduction

Malignant mesothelioma (MM) is a primary tumor arising in serous membranes, most frequently in the pleura. The histopathologic differentiation of MM from adenocarcinoma is often difficult. Clinical, radiologic, histologic and histochemical studies can be helpful but they do not always provide a definitive solution for the diagnostic dilemma. An immunohistologic approach is valuable for the differential diagnosis of MM and adenocarcinoma invading the pleural surface.[1] MOC-31 has recently been used as a spesific marker for adenocarcinoma while reacting with a minimal number of benign and malignant mesothelial proliferations.[2] HBME-1 is a novel monoclonal antibody produced using a suspension of whole human MM cells as an immunogen; it recognizes an antigen, still unknown, shared by the normal mesothelium, bronchial and endocervical epithelia and cartilage, and expressed in many instances by their malignant counterparts.[3]

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A new series of environmental malignant mesothelioma has been reported from Southern Anatolia more recently.[4] Although no significant differences were noted in the histopathologic features of these cases and the occupational malignant mesotheliomas, in this report, we aimed to compare their immunohistochemical features by using HBME-1 and MOC-31 antibodies as well as other immunohistochemical stains such as keratin, EMA and CEA.

Materials and Methods

Fifty five cases of MM, ten cases of pulmonary adenocarcinoma and ten cases of metastatic adenocarcinoma from other sites retrieved from the files of pathology departments of Çukurova University Hospital, two other major hospitals of Adana city and a private pathology laboratory belongs to one of the authors were included in this study.

Tissue sections, 5 µm thick, were cut and stained with haematoxylin and eosin for morphological assesment. Representative tumor blocks were selected for histochemical and immunohistochemical analyses. Sections were stained with periodic acid schiff reagent (PAS) with and without diastase pretreatment, and mucicarmine.

Immunohistochemistry was performed using an avidin-biotin-peroxidase technique with antibodies to keratin (AE1/AE3, monoclonal mouse antibody, Dako-dilution 1:50), EMA (monoclonal mouse antibody, Dako-dilution 1:80), CEA (monoclonal mouse antibody, Novacastra-dilution 1:50), MOC-31 (monoclonal mouse antibody, Biogenex-dilution 1:20), and HBME-1 (monoclonal mouse antibody, Dako-dilution 1:50). Tissue sections, 5µm thick, were deparaffinized and rehydrated through a series of graded alcohols. The sections were incubated with 0.1% trypsin for 30 min. (Trypsin digestion was not performed for MOC-31 and HBME-1) Endogenous peroxidase activity was blocked by a 15-minute incubation in 3% hydrogen peroxide-methanol solution and washed in phosphate-buffered saline. Sections were incubated for 60-90 min with MoAbs, washed and then incubated 30 minutes with biotinylated horse anti-mouse IgG immunglobulin (Dako, K0675). After washing, the sections were incubated for 30 minutes with strept avidin peroxidase reagent and washed again. The immunperoxidase was visualized with AEC (3 amino 9 ethyl carbazole) (Biogenex, HK1295K). Sections were counterstained with Mayer's hematoxylin and 0.3% ammonia water and then coverslipped.

Differences of staining ratio between MM and adenocarcinoma groups were tested by the chi-square and the exact Fisher chi-square test. A p value of < 0.05 was taken to indicate a significant difference.

Results

The slides were evaluated by the two pathologists (DG, EHZ). The diagnoses of all cases were based on hematoxylin-eosin stained sections, PAS-D and mucicarmine stains and immunohistochemical panel (keratin, EMA, CEA, MOC-31, HBME-1). Clinical and radiological data were also evaluated. In all MM cases, the patients presented with pleural effusion and diffuse pleural thickening either bilaterally or localized into one hemithorax radiologically. The involvement of the pleura was diffuse without a parenchymal lesion reminiscent of a lung primary. Most of these cases had a history of living in white stucco painted houses during their childhood and early adult life. In adenocarcinoma cases, pleura was not or focally involved by a parenchymal lesion of the lung. In ten of these cases there was a previously diagnosed extrapulmanary primary tumor.

Histologically, the MM cases were classified as epithelial (40 cases), sarcomatous (7 cases), and biphasic (8 cases). One of the sarcomatous cases was desmoplastic. Epithelial and biphasic MM cases showed tubulopapillary

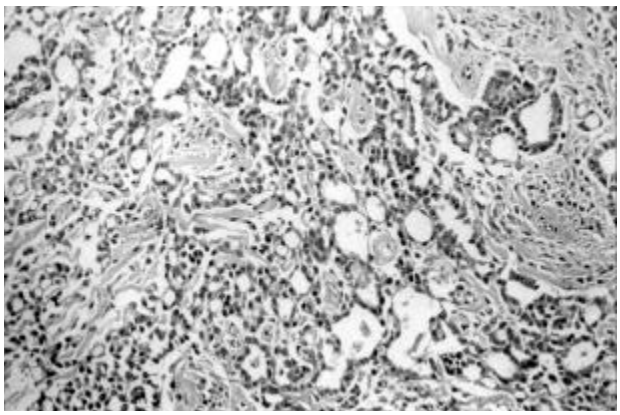

Figure 1. Epithelial MM with a tubular growth pattern (HE; 100X)

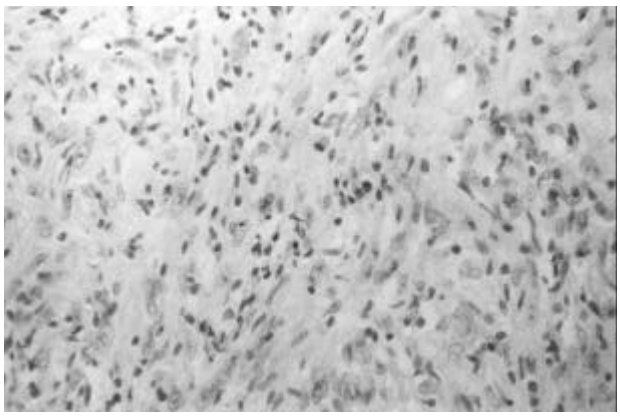

Figure 2. Sarcomatous MM with a few inflammatory cells (HE; 200X)

Table 1. **Histochemical and immunohistochemical results**

	MM		*Pulmonary adenocarcinoma*		*Metastatic adenocarcinoma*	
	Number	*% (+)*	*Number*	*% (+)*	*Number*	*% (+)*
Keratin	55/55	100	10/10	100	10/10	100
EMA	50/55	90.9	10/10	100	10/10	100
CEA	3/55	5.4	10/10	100	8/10	80
MOC-31	5/55	9	7/10	70	7/10	70
HBME-1	52/55	94.5	7/10	70	4/10	40
PAS-D	0/55	0	10/10	100	10/10	100
Mucicarmine	3/55	5.4	10/10	100	9/10	90

tubular, papillary, and solid growth patterns or a combination pattern with one of the above patterns. (*Figures 1,2*)

The histochemical and immunohistochemical results are shown in *Table 1*. Histochemically, none of the MM cases had PAS positive cytoplasmic vacuoles after diastase digestion, but PAS-D stain was positive in all adenocarcinomas. Mucicarmen stained 19 of 20 adenocarcinomas and 3 MM cases. Keratin was positive in all cases. EMA stained 50 of 55 MM (90.9%) and all the adenocarcinomas. In the epithelial component of MM, thick membranous staining was prominent whereas the staining pattern in adenocarcinomas was predominantly cytoplasmic (*Figures 3,4*). EMA was negative in 4 of 6 sarcomatous MM and 1 desmoplastic MM. CEA was positive in 18 of 20 adenocarcinomas (90%) and stained weakly 3 MM cases (5.4%).

MOC-31 stained 14 of the adenocarcinomas (70%) as well as 5 of 55 MM (9%). In adenocarcinomas, both cytoplasmic and thin membranous staining was noted (*Figure 5*). In addition, non-neoplastic mesothelial cells stained with MOC-31 in 3 MM cases.

HBME-1 was positive in 52 of 55 MM. Thick and strong membranous staining was prominent in epithelial MMs and in the epithelial component of biphasic MMs (*Figure 6*). In sarcomatous and desmoplastic MM, cytoplasmic staining was observed. HBME-1 stained 11 of 20 adenocarcinomas. In addition, HBME-1 was positive in normal bronchial epithelium.

Statistically, the positive staining of HBME-1 in MM and positivity of CEA, MOC-31, PAS-D and mucicarmine stains in adenocarcinomas were significant. The results of the statistical analysis are shown in *Table 2*.

Discussion

Adenocarcinoma and mesothelial cell proliferation are the most important lesions in the differential diagnosis of epithelial MM. Histochemical and immunohistochemical panels were used to make the distinction between MM and these lesions of which prognosis and therapy are quite different.

Histochemical stains for neutral mucin (periodic acid Schiff after diastase digestion, PAS-D) in adenocarcinoma and for acid mucins (alcian blue with and without hyaluronidase pretreatment, AB ± H) in mesotheliomas have been considered diagnostic but approximately 5% of epithelial MM shows positive cytoplasmic staining with mucicarmine due to cross reaction of hyalurinic acid and attenuate after pretreatment with hyaluronidase[5]. In this

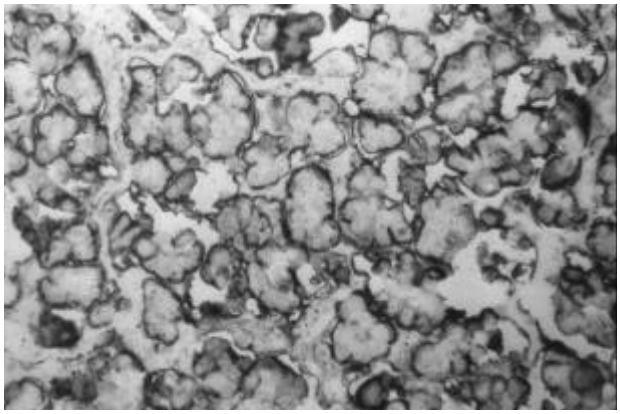

Figure 3. EMA immunostain showing a thick membranous staining in epithelial MM.

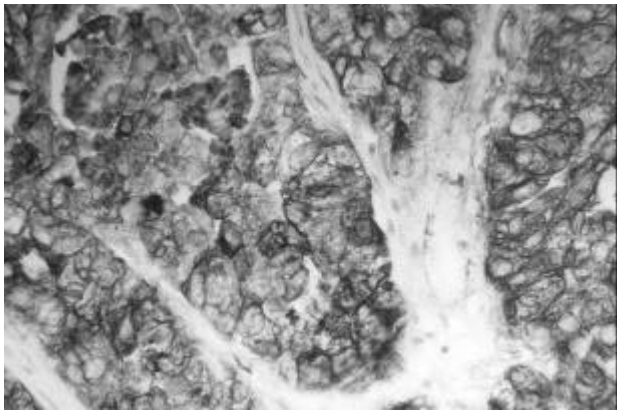

Figure 4. EMA immunostain showing diffuse cytoplasmic staining in a metastatic adenocarcinoma.

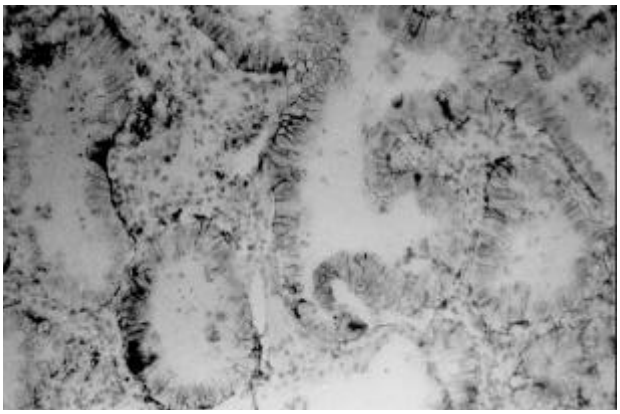

Figure 5. Immunoreactivity for MOC-31 in a pulmonary adenocarcinoma. Both cytoplasmic and thin membranous staining is prominent.

study, PAS-D stain was negative in all MM cases and positive in all adenocarcinomas. Mucicarmen stained 3 of 55 MMs and a positive cytoplasmic staining was noted in all but one of the adenocarcinomas. PAS-D stain was one of the most important stains in the panel of MM.

The panel of monoclonal antibodies such as keratin, CEA, Leu-M1, B72.3, Ber Ep4 have been increasingly used.[6-10] Keratin reacting with both epithelial MM and adenocarcinoma, has been used as a control of immunoreactivity in these tumors. More importantly, it is useful in the distinction of sarcomatous MM and sarcomas.[11]

Leu-M1, B72.3 and Ber Ep4 are expressed much more often in adenocarcinomas than MMs (43-94% υ 0-6% and 86-90% υ 0-20% and 64-87% υ 1-20%, respectively).[1,6,12,13]

The EMA reactivity in mesotheliomas was often markedly concentrated on the cell membrane whereas the staining pattern in adenocarcinomas was predominantly cytoplasmic.[14] In our cases, the epithelial cells of MM showed thick membranous staining.

CEA is detectable in the majority of adenocarcinomas 65-95%), but only in rare cases of malignant mesothelioma.[6,15-17] Depending on the method used and chosen antibody, CEA is found positive in 1-11% of MM cases.[18] Dejmek et al[14] showed that reactivity to polyclonal CEA was seen in all but one of the adenocarcinomas in a series of 20 cases and in 20 of 103 MMs, mainly in the epithelial cells. The tested monoclonal antibody labeled 70% of the adenocarcinoma but none of the 103 MM. Shebani et al[1] showed that polyclonal CEA was positive in 2 of 28 MM and in 48 of 50 adenocarcinomas (96%) although monoclonal CEA was negative in all MM cases and in 14 adenocarcinomas. In our study, monoclonal CEA antibody was focally positive in 3 of 55 MM and was negative in two cases of adenocarcinomas. Monoclonal antibody to CEA, showed a higher reactivity among our adenocarcinoma cases. The positivity among pulmonary adenocarcinomas was 100% whereas 2 cases metastatic from other sites did not react with the antibody. CEA is the most important antibody of the MM panel.

MOC-31 is an antibody that was recently reported to be useful in distinguishing adenocarcinoma from mesothelioma. This monoclonal antibody is directed against a 41-kDa, membrane based glycoprotein of unknown function that has been rarely detected on hyperplastic and neoplastic mesothelial cells.[2,19] One series showed anti MOC-31

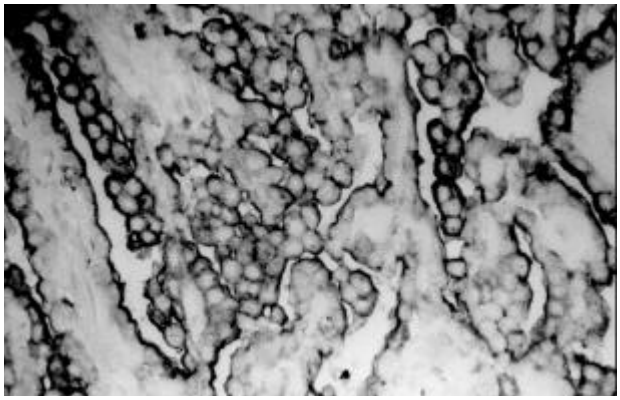

Figure 6. HBME-1 immunostain showing thick and strong membranous staining in epithelial MM.

Table 2. **The results of the chi-square and the exact Fisher chi-square test in MM and adenocarcinoma groups**

	MM		*Adenocarcinoma*			
	Number	*% (+)*	*Number*	*% (+)*	*Test value **	*P – value*
Keratin	55/55	100	20/20	100	–	0.316
EMA	50/55	90.9	20/20	100	–	0.316
CEA	3/55	5.4	18/20	90	52.00	0.000
MOC-31	5/55	9	14/20	70	28.77	0.000
HBME-1	52/55	94.5	11/20	55	–	0.000
PAS-D	0/55	0	20/20	100	75.00	0.000
Mucicarmine	3/55	5.4	19/20	95	56.73	0.000

*****The results of which test value were given tested by chi-square test, others were by Fisher chi-square test.

immunoreactivity in 43 of 44 adenocarcinomas and 1 of 43 mesotheliomas.[20] Other series reported that all 23 adenocarcinomas strongly expressed the marker, whereas only one of the 23 mesotheliomas showed weak reactivity.[2] In another large series, MOC-31 reactivity was obtained in 2 of 38 MMs, all of 40 pulmonary adenocarcinomas, 45 of 55 non-pulmonary adenocarcinomas, all of 6 small-cell carcinomas, 15 of 19 bronchial carcinoids and 10 of 15 transitional cell carcinomas respectively.[21] In our study, MOC-31 stained 14 of 20 adenocarcinomas (70%), as well as 5 of 55 MM cases (9%). The staining pattern in adenocarcinomas was both cytoplasmic and thin membranous. In MM, this Ab showed weak and focal reactivity. Additionally, non-neoplastic mesothelial cells were stained with MOC-31 in 3 cases of MM.

HBME-1 is a mouse monoclonal antibody raised against a suspension of human mesothelioma cells from patients with epithelial type malignant mesothelioma. HBME-1, has been shown to react with normal mesothelium and epithelial malignant mesothelioma.[22]

Bateman et al[13] reported anti HBME-1 positivity in all 17 MM and in 10 of 14 adenocarcinoma cases in their series. A comparative study in a series of 42 MM cases has revealed positive immunstaining for thrombomodulin, OV 632, and HBME-1 in 22, 27, and 36 cases respectively. In this study, among 32 adenocarcinoma cases, 2 were stained with thrombomodulin, 20 with OV 632, and 23 with HBME-1. Regarding to their results, the investigators suggested that thrombomodulin was more specific than HBME-1 and OV 632).[22] A review of the literature showed several comparative studies among anti-mesothelial antibodies such as thrombomodulin, calretinin, AMAD-2, HBME-1 and more recently cytokeratin 5/6 evaluating the low specificity of HBME-1.[13,23-26] However, these reports, including ours, confirm the useful diagnostic role of negative staining with HBME-1 in making a diagnosis other than MM.

In our study, HBME-1 was positive in 52 of 55 MM and 11 of 20 adenocarcinomas (55%) with a lower staining rate among adenocarcinomas. However, we noted the difference in the staining pattern of these tumors. The thick and strong staining features were prominent in the epithelial cells of MM, whereas adenocarcinoma cells showed weak and cytoplasmic staining.

Our findings did not reveal any significant difference between immunohistochemical features of our cases and occupational MM cases reported in the literature. This study confirms the importance of an immunohistochemical panel in the diagnosis of MM and the important role of CEA in this panel. Histochemical stains, especially PAS-D should be used in collaboration with the immunohistochemical stains. In our experience, HBME-1 should also be included in this panel, MOC-31 can be used where CEA is not available or to doublecheck the reactivity of this antibody.

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