Original Article

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Dignostic Utility of HBME1 to Differentiate Between Reactive Mesothelial Cells and Adenocarcinoma Cells in Body Fluids

Charusheela Gore, Karanpatel, Rupali Bavikar* and Ashily

Dept. of Pathology, Dr. D. Y. Patil Medical College, Hospital & Research Centre & DPU, Pune, Maharashtra

ABSTRACT

Background: Reactive mesothelial cells show overlapping features with malignant cells.To differentiate between the two Immunocytochemistry (ICC) can be used.HBME1 is the antigen present on mesothelial cells.We have tried to evaluate the efficacy of HBME1 to differentiate between reactive mesothelial cells and adenocarcinoma cells.

Methods: Total 60 cases with clinical dilemma or suspicious on conventional cytology were selected .Cell block was prepared and staining with monoclonal antibody HBME1 was done.Staining pattern was classified as cytoplasmic ,membranous or combined.

Result: Out of 42 ,28 cases showed negative staining ,8 cases showed cytoplasmic positivity and 4 cases showed combined positivity.All the benign cases showed membranous potivity.

Conclusion: The staining pattern of HBME1 can be used to differentiate between reactive mesothelial cells and adenocarcinoma cells.

Keywords: *HBME1, Reactive Mesothelial Cells, Adenocarcinoma*

Introduction

Serous cavities in the body are lined by mesothelial cells .Any pathology like infection, trauma, or neoplasia in serous cavities leads to hypertrophy and proliferation of mesothelial cells. These reactive mesothelial cells show variable morphological spectrum .They may show features overlapping with malignant cells often leading to false positive reports of serous fluids.

Immunocytochemstry (ICC) is preferred method to differentiate between reactive mesothelial cells and adenocarcinoma. [1,2] ICC done on cell block and cytospin is cost effective and shows better results as compared to flow cytometry. [3,4] There are many mesothelial markers including Calretinin ,thrombomodulin,cytokratin 5/6, and HMBE1.[5,6] HMBE 1 is monoclonal antibody which reacts with surface antigen on mesothelial cells. [5,6]

This study is done to evaluate the diagnostic utility of HBME1 to distinguish between mesothelial cells from adenocarcinoma.

Material and Methods

This is prospective study from July 2010 to June 2012 done in department of pathology in DY Patil Medical college Pune.Total sixty cases of pleural or peritoneal effusions were selected for study.

Inclusion criteria was ,cases with diagnostic dilemma clinically or on conventional cytology, malignant pleural effusions ,and few benign pleural effusions were also included. The diagnosis of each case was confirmed by computer tomography, bronchoscopy, surgical exicision and histhopathological examination.

The specimen (pleural or peritoneal fluid) was cytocentrifuged at 800 rpm for 3 minuts. The cell button was taken on labeled and paraffin coated slides .The slides were immunostained using two step polymer (ENVISION ™) method. Primary antibody used was Monoclonal anti mesothelial antibody clone HBME 1 (Dako) .The DAB (3,3 Diaamininobenzine) was used as chromogen and nuclei were counter stained with Mayer's Haematoxylene. The staining pattern was classified as membranous(thin and thick) ,cytoplasmic and combined (membranous +cytoplasmic).

Results

Out of 60 cases 50% were males and 50% were females. The incidence of malignant effusion was higher (80%) in females as compared to males (60%).Total 42 cases were malignant effusions .In malignant effusions ,36 (85.8%) cases were that adenocarcinoma followed by 2 cases sqaumous cell carcinoma and 2 cases of Ewing's sarcoma (Table 1).In adenocarcinoma 16 out of 36 were from alimentary tract followed by lung(8) and ovary. (8). (Table 2)

The staining pattern of HBME1 in benign cases was membranous in 12 cases and combined in two cases (table3)(Figure1) .Out of 42 malignant cases, 28 cases

showed negative staining for HBME1(Figure 2) ,8 cases showed cytoplasmic positivity(Figure 3),4 cases showed combined(Figure4) and only2 cases showed membranous positivity.(table 4)

Staining pattern in adenocarcinoma was cytoplasmic and combined except ovarian malignancy .2 cases of ovarian malignancy showed membranous positivity.(Table V)

Discussion

The reactive mesothelial cells may show changes like nucleomegaly,irregular nuclear membrane ,coarse chromatin and conspicuous nucleoli ,difficult to differentiate from malignant cells. The reactive mesothelial cells ,with degenerative intracytoplasmic vacuoles may be misinterpreted as adenocarcinoma cells with mucin

Table I : Subtyping of Malignant Lesions

Subtype of malignant lesions	No. of Cases
Adenocarcinoma	36
Squamous cell carcinoma	2
Ewings sarcoma	2
Signet ring lymphoma	1
Non-Hodgkin's Lymphoma	1

Table II : Classification of Adenocarcinoma By Specific Origin (N=36)

Type of adenocarcinoma by specific origin	No. of cases
Ovary	8
Lung	8
Breast	4
Alimentary Tract	16

Table III: HBME-1 Staining in Benign or Reactive Lesions.

Negative	Positive			Total No. of cases
	Membranous	Cytoplasmic	Combined	
4	12	0	2	18

Table IV: HBME-1 Staining in Malignant Lesions

Negative	Positive			Total No. of cases
	Membranous	Cytoplasmic	Combined	
28	2	8	4	42

Table V – Staining pattern in specific type of adenocarcinoma

	Negative	Membranous	Cytoplasmic	Combined	Total
Ovary	2	2	2	2	8
Lung	6	0	2	0	8
Breast	4	0	0	0	4
Alimentary canal	10	0	4	2	16

TABLE VI -Predictive Value,Sensitivity and Specificity of HBME1 in differentiating reactive mesothelial cells and adenocarcinoma cells.

	Calculated Value	95% C.I
Positive Predictive Value(PPV)	50%	24% - 76%
Negative Predictive Value(NPV)	87.5%	60.4% - 97.8%
Sensitivity	77.8%	40.2% - 96.1%
Specificity	66.7%	43.1% – 84.5%

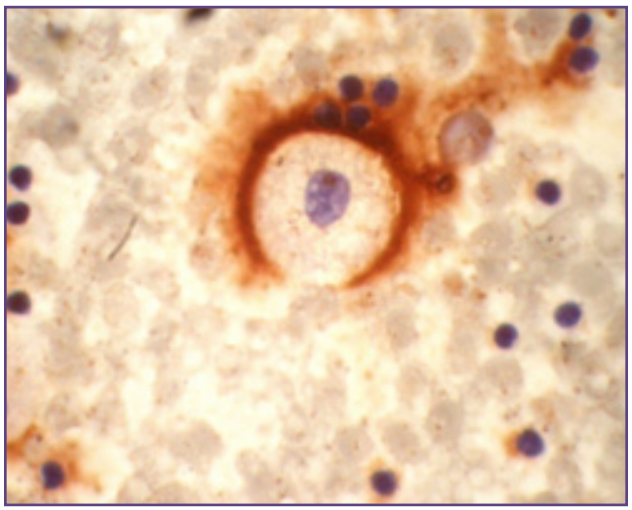

Fig. 1: Membranous staining seen in reactive mesothelial cells.(HBME1,ICC X400).

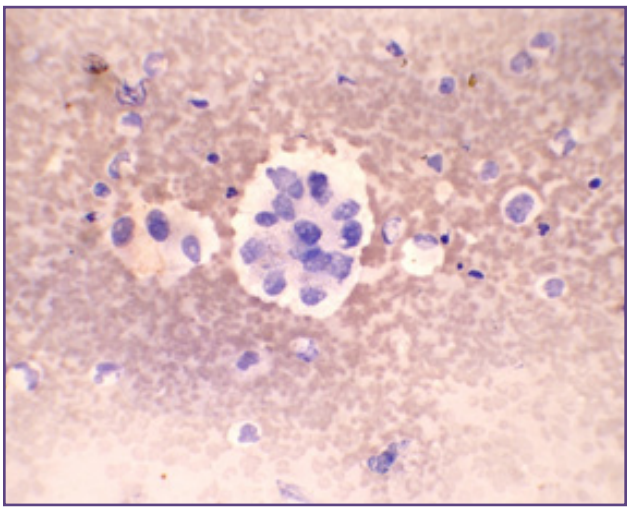

Fig. 2: Negative staining in adenocarcinoma cells. (HBME1,ICC X400).

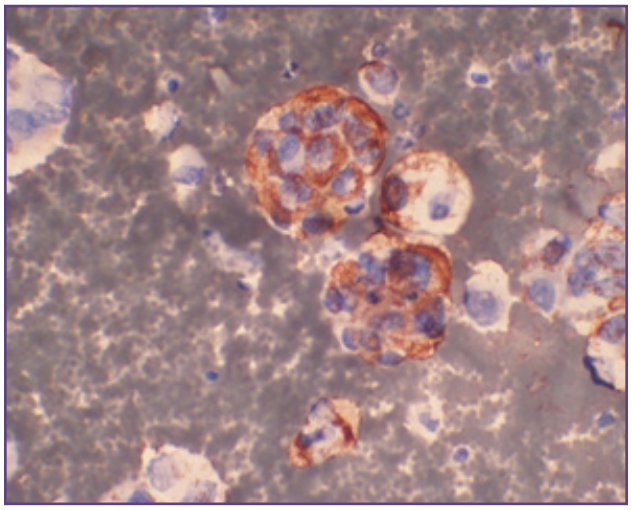

Fig. 3: Cytoplasmic staining seen in metastatic adenocarcinoma Cells..(HBME1,ICC X400).

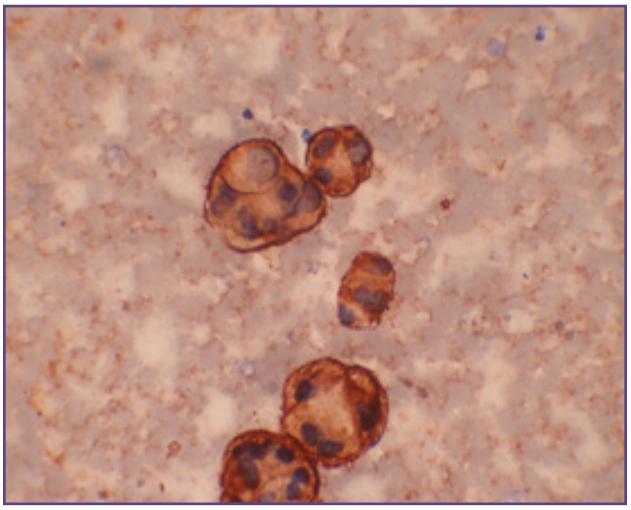

Fig. 4: Combined staining seen in metastatic adenocarcinoma Cells..(HBME1,ICC X400).

vacuole. Adenocarcinoma being the most common malignancy in serous cavities,the differntiation between two is mandatory.HBME1 is monoclonal antibody which reacts with unknown antigen presents on mesothelial cells.Immunocytochemistry with HBME1 can be used to differentiate between reactive mesothelial cells and malignant cells, the reactive mesothelial cells show membranous pattern of staining while adenocarcinoma cells show cytoplasmic staining.[7-9]

In our study out of 18 benign cases ,14 (77.8%) were positive for HBME1 while 4 (22%)cases were negative .Out 14 positive cases 12 cases showed membranous positivity and 2 cases showed cytoplasmic positivity .These findings are in correlation to Ascoli et al [10] study which in which predominant pattern of staining in reactive mesothelial cells was membranous while few cells showed cytoplasmic positivity..Study conducted by Rehmani et al.[11] showed 100 % membranous positivity in reactive mesothelial cells while none of the cells showed combined positivity. These findings suggest predominant staining pattern for benign mesothelial cells is membranous.

Out of 42 malignant cases 28 (66.7%) were negative for HBME1 while 14 (33.3%) cases were positive .In Ascoli et al [10] study 24% malignant cases showed positive staining with HBME1 .

The most common malignancy was adenocarcinoma, total 36 cases,22(61.1%) cases were negative for HBME1 staining while 14 (38.9%)cases showed positivity. Out

of positive cases 8(57.1%) cases showed cytoplasmic positivity and 4 (28.6%)cases showed membranous positivity. Only 2 cases (14.3%)cases showed membranous positivity ,both were ovarian adenocarcinoma.

Thus in adenocarcinoma predominant staining pattern was cytoplasmic followed by combined .Ovarian tumor cells showed membranous positivity. In Ascoli et al [10] ovarian adenocarcinoma (83%) showed membranous pattern while other carcinomas showed cytoplasmic immunoreactivity.

Positive membranous staining in Ovarian adenocarcinoma can be explained on the basis of origin of tumor. Ovarian adenocarcinoma originate from germline epithelium of ovary which is origin of mesothelial cells.[12] Utility of HBME1 is limited to differentiate single scattered cells from ovarian adenocarcinoma and reactive mesothelial cells.

Positive predictive value and negative predictive value was 50% and 87.5% respectively .[TableVI] The sensitivity and specificity of immunomarker was 77.8% and 66.7%respectively. According to Politi et al [13] the sensitivity and specificity for HBME-1 was 98% and 71% respectively.

Conclusion

Staining pattern with HBME1 is useful in differentiating reactive mesothelial cells and adenocarcinoma cells .In case of ovarian adenocarcinoma the utility of HBME1 is limited due to similar immunoreactivity as reactive mesothelial cells .

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***Corresponding author:**
Dr Rupali Bavikar, C 505, Siciliaa, BT Kawade Road, Pune 01, India
Phone: +91 8149367249
Email: rupalibavikar@yahoo.co.uk

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