UTILITY OF HBME1 IMMUNOSTAIN IN DIFFERENTIATING REACTIVE MESOTHELIAL CELLS LESIONS FROM MALIGNANT EPITHELIAL CELLS LESIONS IN SEROUS EFFUSIONS

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ABSTRACT

Objective: Parietal and the visceral layers of the mesothelium are separated by a thin layer of lubricating fluid and are made up of a single layer of flat cells. The three body cavities pleura, peritoneum, and pericardium are lined by mesothelium. The collection of fluid inside these cavities is called an effusion and may be due to a variety of inflammatory, infective, and neoplastic causes and often causes reactive mesothelial cells hyperplasia that sometimes is very hard to differentiate from malignant epithelial cells in cytopathological examinations of these fluids.

Methods: We studied the utility of the Human Battifora Mesothelial Epitope-1 (HBME1) immunostain to differentiate these conditions. All the fluids from various effusions collected at the department of pathology, at a tertiary care institute in Lucknow were included in the study. Detailed history, examination findings, blood investigations, imaging findings, and histopathology reports were also noted. Fluids that showed reactive mesothelial hyperplasia and/or malignant epithelial cells on microscopic examination were further analyzed by the application of HBME1 on the cell block.

Results: A total of 50 fluids were studied finally including 30 cases from the positive malignant cells group and 20 cases from the reactive mesothelial cells group. Out of 30 cases included in the malignant cells group, 16 cases (53.33%) were immunoreactive for HBME1, and out of the 20 cases included in the reactive mesothelial cell group, 18 cases (90%) showed immunoreactivity for HBME1.

Conclusion: Observing this we can conclude that HBME1 immunoreactivity was significantly associated with the presence of reactive mesothelial cells compared to the malignant cells group.

Keywords: Human Battifora Mesothelial Epitope- 1, Cellblock, Effusion, Reactive mesothelial cells, Malignant epithelial cells.

INTRODUCTION

The mesothelium is made up of simple squamous epithelium present on the surface of all coelomic organs such as the heart, lungs, and peritoneum [1]. In the absence of any disease, the parietal and the visceral layers of the mesothelium are separated by a thin layer of lubricating fluid. Under pathologic conditions mostly infective, inflammatory, and neoplastic, a large amount of fluid gets accumulated. This accumulated fluid in the serous cavities is defined as effusion. Depending on the serous cavity involved, they are named pericardial, pleural, and peritoneal effusions. Malignant pleural effusions are far more common and they are caused by a variety of cancers; primary or metastatic. On the other hand, benign pleural effusions are caused primarily by infections [2,3]. Sometimes, it is very difficult to differentiate benign reactive mesothelial cells from malignant epithelial cells in effusion fluid, especially in specimens containing abundant reactive mesothelial cells on light microscopic examination. The use of immunocytochemistry in such cases provides significant help to reach the final diagnosis. Immunocytochemistry involves the use of a panel of antibodies that are specific and sensitive for the mesothelial cells and which differentiates between reactive mesothelial cells and cells of adenocarcinoma in serous fluids [4]. One such antibody is human Battifora Mesothelial Epitope-1 (HBME-1) which is a novel monoclonal anti-human antibody developed using human malignant mesothelioma cells as an immunogen; it recognizes an unknown antigen shared by the normal mesothelium, bronchial and endometrial epithelia, and cartilage as well as endocervix [5-7]. It is also expressed in many instances by their malignant counterparts [8].

METHODS

It was a prospective observational study of a 1-year duration carried out in a department of pathology, at a tertiary care institute, Lucknow after taking ethical clearance from the institutional ethical committee and proper informed consent from the patients. Effusion fluids that showed reactive mesothelial hyperplasia and/or malignant epithelial cells on microscopic examination were included in the study and their demographic and detailed clinical data were also obtained. Hematoxylin and eosin (H and E) stain, Giemsa stain, and Papanicolaou stains were used in the staining of smears prepared after centrifugation of effusion fluids. Fluids that were predominantly hemorrhagic, inflammatory, and inadequate for comments were excluded from the study. Fluids that were included in the study were further processed. Cellblock was made, and the following procedure was followed step by step in the preparation of cell block-

1. Around 20 to 30 ml of fluid was collected in a sterile syringe
2. Collected fluid was transferred to a sterile test tube and centrifuged at 4000 rpm for 6 min
3. Supernatant was discarded
4. Pellet was transferred in 9:1 alcohol: formalin solution (9 parts of absolute alcohol + 1 part 40% formalin)
5. Kept for 45 min and then centrifuged at 4000 rpm for 6 min
6. Finally, the pellet was transferred in filter paper and processed in histokinette overnight
7. Paraffin blocks were made, and sections were taken over slides to perform H&E staining and immunocytochemistry with HBME-1.

Immunocytochemistry - Standard protocols were used. For immunocytochemical evaluation, the Streptavidin Biotin immunoperoxidase method was used. The following reagents were used.

Table 1: Distribution of cases as per age

Age group (years)	Total (n=50), n (%)	Malignant epithelial cells group (n=30), n (%)	Reactive mesothelial cells group (n=20), n (%)
<20	2 (4)	0	2 (10)
21–30	4 (8)	1 (3.33)	3 (15)
31–40	7 (14)	1 (3.33)	6 (30)
41–50	15 (30)	12 (40)	3 (15)
51–60	11 (22)	7 (23.33)	4 (20)
>60	11 (22)	9 (30)	2 (10)
Mean±SD (range) in years	49.64±14.35 (12–76)	54.63±10.63 (30–76)	42.15±16.13 (12–65)

t: −3.304, p: 0.002. SD: Standard deviation

Primary antibody - Mouse Mono Anti-mesothelioma (HBME-1), source; mouse, quantity 0.5ml. This antibody reacts with an unknown antigen on the microvilli of mesothelioma cells. It stains normal mesothelial cells as well as epithelial mesotheliomas in a thick membrane pattern due to abundant lung microvilli on the surface of these cells.

Positive control: mesothelioma, Cellular localization: cell membrane.

Secondary antibody: It is used from the Dako EnVision tm FLEX detection system. The staining patterns will be classified as cytoplasmic or membranous (thin and thick) or combined.

Results- The presence of brown-colored end product at the site of the target antigen was indicative of positive reactivity.

Staining characteristics of HBME-1.

Intensity – Positive cases were scored based on the percentage of stained cells and categorized into 3 categories.

Staining Intensity	No of stained cells
1+	<10%
2+	10–50%
3+	>50%.

Staining pattern – Following patterns were identified based on the part of cells stained with HBME-1.

Staining pattern
Membranous (Thick and thin)
Cytoplasmic
Combined (Membranous+Cytoplasmic)

Statistical analysis

The statistical analysis was performed using the Statistical Package for the Social Sciences, version 16 for windows (SPSS, Chicago IL, USA) and Microsoft Excel. The values were represented in number (%) and mean±standard deviation. Various characteristics of the two groups: malignant cells and reactive mesothelial cells were compared to study the association of a variable with a given group. Association was studied through various statistical tests of significance. Univariate analysis was performed by the Chi-square test for non-parametric data and the Student's t-test for independent variables for parametric data. For comparing multiple groups with parametric data, analysis of variance was used. Statistical significance was defined at p-value of <0.05. Statistical analysis was two-tailed.

RESULTS

After the omitting, the cases under exclusion criteria, a total of 50 cases of effusion fluids were enrolled in the study. Of these, 30 were positive for malignant cells and 20 were for benign reactive mesothelial cells group. The mean age of the study population was 49.64±14.35 years with an age range of 12–76 years. The mean age of the population in the malignant epithelial cells group was 54.63±10.63 years with an age range from 30

Table 2: Distribution of subjects according to gender

Gender	Total (n=50), n (%)	Malignant epithelial cells group (n=30), n (%)	Reactive mesothelial cells group (n=20), n (%)
Male	25 (50)	9 (30)	16 (80)
Female	25 (50)	21 (70)	4 (20)

χ^2: 12.00, p: 0.001

Table 3: Human Battifora Mesothelial Epitope 1 immunoreactivity in malignant epithelial cells group and reactive mesothelial cells group

Group	Total (n)	Number of cases HBME1 immunoreactive (n)	Positive percentage (%)
Malignant epithelial cells group	30	16	53.33
Reactive mesothelial cells group	20	18	90

HBME: Human Battifora Mesothelial Epitope

to 76, and in the reactive mesothelial cell group, it was 42.15±16.13 years with an age range of 12–65 years. Statistically, there was a significant difference in the mean age of both groups (Table 1). Overall, there were 25 males and 25 females in the present study. However, the majority of the cases (70%) were female in the malignant epithelial cells group, whereas the majority of cases (80%) in the reactive mesothelial cell group were male. This difference was statistically significant (p=0.001) (Table 2). Cellblock findings were similar to their respective cytomorphological findings. Out of 30 cases included in the malignant epithelial cells group, 16 cases (53.33%) were immunoreactive for HBME1. Out of the 20 cases included in the reactive mesothelial cell group, 18 cases (90%) showed immunoreactivity for HBME1. HBME1 immunoreactivity was significantly associated with the presence of the reactive mesothelial cells group compared to the malignant epithelial cells group (χ^2=5.825, p=0.016) (Table 3). The sensitivity and specificity for HBME1 in distinguishing between reactive mesothelial cell and malignant epithelial cells group was 90% and 46.67%, respectively. For any particular positive test result, the probability that it is true positive predictive value is 52.94%. For any particular negative test result, the probability that it is true negative predictive value is 87.50%. Comparing the staining intensity in both the groups, out of 18 cases testing positive for HBME1 in the reactive mesothelial cell group, 16 cases (80%) had intense staining intensity (3+) and 2 cases (10%) had moderate staining intensity (2+) and only 2 cases in malignant epithelial cells group had intense staining intensity (3+), and other remaining had either (1+) or (2+) intensity. On statistical analysis, a strong grade of staining intensity (3+) was significantly observed in the reactive mesothelial cell group (p=0.000) (Table 4). All HBME1 positive cases in the reactive mesothelial cell group had a membranous pattern, whereas 62.5% of cases in the malignant epithelial cells group had combined, 25% had membranous, and 12.5% had a cytoplasmic pattern. A membranous pattern was significantly noted in the reactive mesothelial cell group (p=0.000) (Table 5).

Table 4: Comparison of staining intensity in malignant epithelial cells group and reactive mesothelial cells group

Staining intensity	Total (n=50), n (%)	Number of cases (%)		χ^2	p-value
		Malignant epithelial cells group (n=30)	Reactive mesothelial cells group (n=20)		
Negative	16 (32)	14 (46.67)	2 (10)	20.174	0.000
1+	4 (8)	4 (13.33)	0		
2+	12 (24)	10 (33.33)	2 (10)		
3+	18 (36)	2 (6.67)	16 (80)		

Table 5: Comparison of staining pattern in malignant epithelial cells group and reactive mesothelial cells group

Staining pattern	Total (n=34), n (%)	Malignant epithelial cells group (n=16), n (%)	Reactive mesothelial cells group (n=18), n (%)
Membranous	22 (64.71)	4 (25)	18 (100)
Cytoplasmic	2 (5.89)	2 (12.50)	0
Combined	10 (29.40)	10 (62.50)	0

χ^2: 20.864, p: 0.000

DISCUSSION

In this study, a total of fifty cases of serous fluids with the demographic characteristics, clinical data, and HBME1 immunoreactivity intensity and pattern were studied. The mean age group in the malignant epithelial cells group was significantly higher in the present study; however, in other studies, it was either equal to or lower than the reactive cells group [9,10]. The explanation for an older population in the malignant epithelial cells group could be the increasing prevalence of various malignancies in the aging population. Female preponderance was seen in the malignant epithelial cells group while male preponderance was seen in the reactive mesothelial cell group in the present study. Similar findings were observed by Afshar *et al.* [10] 90% of cases in the reactive cells group while only 53.33 % of cases in the malignant epithelial cells group showed immunoreactivity with HBME1. Most of the studies done before had similar results [11,12]. Strong intensity (3+) was predominantly associated with the reactive cells group, while in the malignant epithelial cells group, (1+) to (2+) grade of intensity was present in the majority of immunoreactive cases [12]. In our study, all the positive cases with HBME1 in the reactive mesothelial cells group showed a thick membranous pattern and no cytoplasmic pattern was observed in any case. The malignant epithelial cells group exhibited membranous, cytoplasmic but the majority showed a combined pattern. Similar results were seen in the study of Rahmani *et al* and Mocanu *et al.* [9,13]. The sensitivity and specificity for HBME1 in distinguishing between reactive mesothelial cell and malignant epithelial cells group was 90% and 46.67%, respectively, in the present study; similarly, Politi *et al.* observed HBME-1 sensitivity and specificity in the differentiation of reactive mesothelial cells from malignant epithelial cells group was 98% and 71%, respectively [11].

CONCLUSION

HBME-1 can be used to differentiate reactive mesothelial cells from malignant epithelial cells group, but its use is debatable because few cases show overlapping features, as well as few cases, show negative HBME-1 immunoreactivity. However, because of its high sensitivity, it can be used in the panel along with other antibodies to differentiate these two conditions in serous fluids.

AUTHORS CONTRIBUTION

Each author has contributed individually in either research work or manuscript preparation.

CONFLICTS OF INTERESTS

There are no conflicts of interests.

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