Immunohistochemical Differentiation between Reactive and Malignant Mesothelial Proliferations in Pleural Effusion

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Abstract

Background: The differentiation between benign and malignant mesothelial cells in pleural effusion in some cases can be a challenge. In the current study, we investigated the value of immunohistochemistry (IHC) in making that differentiation.

Aim of Study: This study aims to examine the value of IHC expression of desmin, EMA, GLUT-1, p53, Ki67, and BAP1 in discrimination between benign and malignant mesothelial proliferation in pleural effusions and compare their results regarding sensitivity and specificity.

Material and Methods: Pleural fluids from 30 cases diagnosed as malignant meothelioma (MM) epitheloid type and 20 cases diagnosed as reactive mesothelial hyperplasia (RMH) were selected, and stained with immunohistochemical stains included BAP-1, desmin, epithelial membrane antigen (EMA), glucose-transport protein 1 (GLUTÅ]1), Ki67, and p53.

Results: BAP-1 was negative in 5% (1 of 20) cases of reactive MH and in 63.3% (19 of 30) of MM cases ($p<.005$). GLUTÅ]1 was positive in 15% (3 of 20) of benign and 70% (21 of 30) of malignant cases ($p<.005$). Desmin was positive in 80% (16 of 20) cases of reactive MH and in 10% (3 of 30) of MM cases ($p<.001$). EMA was positive in 10% (2 of 20) of benign and 96.7% (29 of 30) of malignant cases ($p<.001$). P53 showed strong nuclear positivity in 5% (1 of 20) of benign and 53.3% (16 of 30) of malignant cases ($p<.001$). Ki67 showed strong nuclear positivity in >40% of mesothelial cells in 10% (2 of 20) of benign and 16.7% (5 of 30) of malignant cases ($p=0.40$). EMA negativity and desmin positivity were found in 75% (15 of 20) of reactive MH cases and 3.3% (one of 30) of MM cases. EMA positivity and desmin negativity were found in 5% (1 of 20) of reactive MH cases and 90% (27 of 30) of MM cases ($p<.001$). Positivity for both of Desmin and BAP-1 was detected in 80% (16 of 20) of RMH cases. The combination of Desmin and BAP-1 negativity was detected in 60% (16 of 30) of MM cases (p-value $p<.001$).

Conclusions: Cases showed positivity for EMA and negativity for desmin strongly favors malignant mesothelioma. On the contrary, cases showed negative EMA and positive desmin strongly favors Reactive mesothelial hyperplasia. also the combination of Desmin and Bap-1 postivity favors diagnosis of RMH, and the combination of Desmin and BAP-1 negativity favors the diagnosis of MM. Similarly, cases showed strong membranous expression of GLUT-1 and/or strong nuclear expression of p53 strongly favors diagnosis of malignant mesothelioma. Proliferative index showed detected by Ki67 showed no significant difference between reactive and malignant cases.

Key Words: BAP-1 – P53 – Ki67 – Desmin – EMA – GLUT-1 – Reactive mesothelial hyperplasia (RMH) – Malignant mesothelioma (MM) – Immunohistochemistry (IHC).

Introduction

MALIGNANT mesothelioma is a highly aggressive neoplasm with a worse outcome, usually linked to asbestos. The neoplasm arises from the mesothelial cells that line the serous cavities, especially the pleura. Mesothelioma has a poor prognosis [1,2]. Survival rates are low with a median survival of 4 to 14 months. The cases are usually diagnosed late, and the available therapeutic regimens are limited. The incidence of malignant mesothelioma has been increasing all over the world since the mid 20th century [3].

Malignant mesothelioma (MM) has many different morphologic types which commonly difficult to be differentiated from reactive proliferation of mesothelial cells or tumors of nonmesothelial origin arising form serous membranes [4].So it is difficult to diagnose a case as malignant mesotheliom with great confidence, especially if the biopsy is small on using routine light microscopy alone [5]. For that cause, additional techniques for diagnosis as histochemistry, EM, and IHC, usually needed help the pathologistto have a more confident diagnosis and support him to identify the (1) Mesothelial origin and (2) Malignant nature of the lesion [6].

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The identification of the nature of proliferated mesothelial cells benign or malignant is essential for the patient treatment, but the pathologist even experts find such differentiation, in some cases, so difficult [7,8]. Stromal invasion is considered the clue for diagnosing proilfertaed mesothelial cells as malignant in tissue biopsy, but in pleural effusion it is not applicable [9,10]. The cellular morphological criteria that used to define cells as malignant are pleomorphism, macronucleoli, large cellular aggregates, papillary-like tissue fragments, and cell-in-cell engulfment. These are important criteria, but of limited value in cytologic effusion as they may be present in cases with RMH. RMH may be associated with variable degrees of cyto-logical atypi, conversely some cases of MM may be associated with bland cytological features [11,12].

BAP1 gene encodes BRCA1-associated protein 1 (BAP1) which acts as nuclear hydrolase included in many cellular processes, as chromatin remode-ling. BAP1 acts as a true tumor suppressor gene [13,14]. Families with increased incidence of MM, have shown the presence of germline BAP1 mu-tations. Recently, this led to the identification of the BAP1 tumor predisposition syndrome that is inherited in an AD manner and is characterised by uveal melanoma, mesothelioma, cutaneous melano-cytic lesions, renal cell carcinoma, basal cell car-cinoma, and may be associated with intrahepatic cholangiocarcinoma [15,16]. Similarly, Sporadic BAP-1 was also detected in some neoplasms as uveal melanoma, MM, and cutaneous melanocytic tumors [17,18].

Malignant cells supply their energy needs through increased glucose consumption, producing large quantities of lactic acid via glycolysis. Glu-cose transporters (GLUTs) and monocarboxylate transporters (MCTs) [19]. It can usually be detected in erythrocytes, the blood-brain barrier, and the placenta but rarely in other organs. It is commonly up-regulated in human malignancies to mediate glucose influx and lactic acid efflux, respectively [20].

P53 is a 53-kDa nuclear protein. It is encoded by tumor suppressor gene p53 which lies on short arm of chromosome 17. P53 has many roles in the cell as transcription, cell cycle regulation, repair of DNA, and induce apoptosis of cells with dam-aged DNA to keep the genetic stability [21]. P53 gene deletions or mutations are common event in many human neoplasms (60%) that causing tumor growth. The product of mutated p53 gene has a longer half life than the normal protein and more stable, so this mutated protein is easily identified by IHC. Thus cells of malignant tumors not benign tumors have high levels of such mutated proteine [22].

Ki67 is a nuclear protein that regulate cell proliferation, can be identified by monoclonal antibody MIB-1. its expression is nuclear in the active cell phase (G1, S, G2, M) not in the resting phase (G0) [23]. Detection of Ki67 is used to detect the growth fraction (the number of cells in cell cycle) of normal, reactive, and neoplastic tissue. The labeling index (The percentage of ki67 positive cells) is of low values in benign lesions, but of high values in malignant neoplasms. High labeling index is a very good indicator for high proliferation of cells that indicates malignant nature of such proliferation, and affects the rate of recurrence and outcome [24].

Epithelial membrane antigen (EMA) is one of several glycoproteins found in human milk fat globule membranes which are packaged in the Golgi apparatus, so globular reactivity of the Golgi apparatus may be seen. The glycoprotein identified with EMA is now known to be one of a series of glycoproteins or mucins and is designated MUC1 [25].

Desmin is a protein that is encoded in humans by the DES gene. Desmin is a type III intermediate filament found near the Z line in sarcomeres. Desmin is only expressed in vertebrates, however homologous proteins are found in many organisms. It is a 52kD protein that is a subunit of intermediate filaments in skeletal muscle tissue, smooth muscle tissue, and cardiac muscle tissue [5].

Material and Methods

This prospective study included 20 cases of non-neoplastic reactive mesothelial proliferations, selected 30 cases of malignant mesothelioma epi-thelioid type. Pleural effusions were collected from the Chest Department, Benha Faculty of Medicine -Benha University and the International Medical Center (IMC) in the period (June 2015–June 2017). Paraffin-embedded cell blocks of pleural effusion were prepared. The cases of reactive MH were confirmed with review of the previous and/or current medical records.

The confirmation of MM diagnosis was through IHC stains as caleritin, WT-1, CK5/6, TTF-1 and Leu M1. All the patients were associated with clinical and readiological features refaring to MM.

Immunohistochemical staining:

The cell blocks of the cases were cut 4μm thick, then mounted on positively-charged slides, we

follow slandered ABC staining protocol (avidin-biotin complex) that uses the Ultra Vision Detection System (Anti-polyvalent, HRP/DAB, ready-to-use, Lab Vision corporation). For antigen retrieval, microwave treatment in 10mM citrate buffer (Neo-Markers, Cat. # AP-9003), pH 6.0 was done and stained with desmin (Dako, Carpentaria, Calif; 1:100 dilution), EMA (Dako; 1:2 dilution), GLUT-1 (rabbit polyclonal antibody, Thermo Scientific, Waltham, Mass; 1:200 dilution), p53 (Vector Laboratories, Burlingame, Calif; 1:30 dilution), Ki67 proliferation index (Dako, 1:100 dilution), and BAP-1 (clone C-4, cat no sc-28383, Santa Cruz Biotechnology, USA; 1:100 dilution) (Table 1). The incubation period was thirty for all IHC stains. The suitable positive and negative controls were used. We apply the freshly prepared DAB-substrate-chromogen solution as a counterstain.

A single pathologist interpreted both the IHC stains in association with the H&E stained slides. The pathologist did not know the diagnosis of cases at time of interpretation the IHC stained slides. The atypical mesothelial cells were the target if they can be recognized from the other reactive mesothelial cell, but if such differentiation was difficult; all mesothelial cells were the target.

Table (1): Antibody sources, dilutions, and fixation conditions.

Antibody	Source	Catalogue No.	Dilution	Fixation
Desmin	Dako	M0760	1:100	10% formalin
EMA	Dako	N1504	1:2	10% formalin
GLUT-1	Thermo Scientific	RB9052	1:200	10% formalin
p53	Vector Laboratories	VPp958	1:30	10% formalin
Ki67	Dako	M7240	1:100	10% formalin
BAP-1	Santa Cruz Biotechnology	SC-28383	1:100	10% formalin

EMA   : Indicates epithelial membrane antigen.
GLUT-1 : Glucose-transport protein 1.
BAP1   : BRCA-1 associated protein.

Interpretation of IHC staining:

Desmin immunoreactivity: Recorded as negative when no immunoreactivity was seen, considered as positive if showed strong membranous positivity and cytoplasmic staining. Focal/weak if <20% of cells were positive or showed only blush positivity, and positive if strong positivity was seen in ≥20% of cells [26].

EMA and GLUT-1 immunoreactivity: Were recorded as negative (no staining), focal/weak positive if there were a few (<20%) scattered cells that showed a membranous staining pattern or if

there was only blush cytoplasmic staining but no membranous staining, and positive if there were ≥20% of mesothelial cells showed strong membranous accentuation with or without cytoplasmic staining [26].

Ki67: The mesothelial cells that showed positivity were calculated in RMH cases and MM cases. The cases were considered negative if (0% of nuclei staining), low (<10%), moderate (10%-40%), and high (>40%) [22].

P53: Cases were considered as negative if there was no staining or only faint nuclear staining, focal if there was strong nuclear staining in <10% of cells, and positive if the strong nuclear staining was detected in ≥10% of cells [22].

BAP1: The cases were recorded as negative if the nuclear staining was totally absent in all the target cells, associated with positive internal control as lymphocytes or stromal cells in the background. The cases were recorded as positive if at least 5% of the target cells showed strong nuclear staining. Negative cases in absence of internal positive control were not considered [27].

Data analysis was performed with the statistical package for social sciences (version 16.0.1; SPSS Inc., Chicago, Illinois, USA). Descriptive analysis of the variables and statistical significance of the tests were expressed in *p*-value. *p*-value less than 0.05 (<0.05) was considered significant and <0.01 was highly significant.

Results

Among the patients with MM, the mean age was 65 years (range, 37-75 years); 20 were men, and 10 were women. Regarding the patients with RMCs, the mean age was 38 years (range, 25-73 years); 16 were men, and 4 were women. The cases were examined histologically and immunohisto-chemically for EMA, Desmin, P53, Ki67, GLUT-1, and BAP-1. The results are shown in Table (2).

To distinguish MM from RMCs, immunohisto-chemical positivity for P53, GLUT1, EMA, and ki67 had sensitivities of 53%, 70%, 97%, and 63% and specificities of 100%, 85%, 90%, and 70%, respectively, and negativity for BAP-1 and desmin with 63%, 80% sensitivity and 95%, 90% specificity respectively. Immunostaining for detection of GLUT1, EMA, and was generally concentrated around the cell membrane, with only occasional cytoplasmic staining. Desmin showed cytoplasmic staining in both MM and RMC cases.
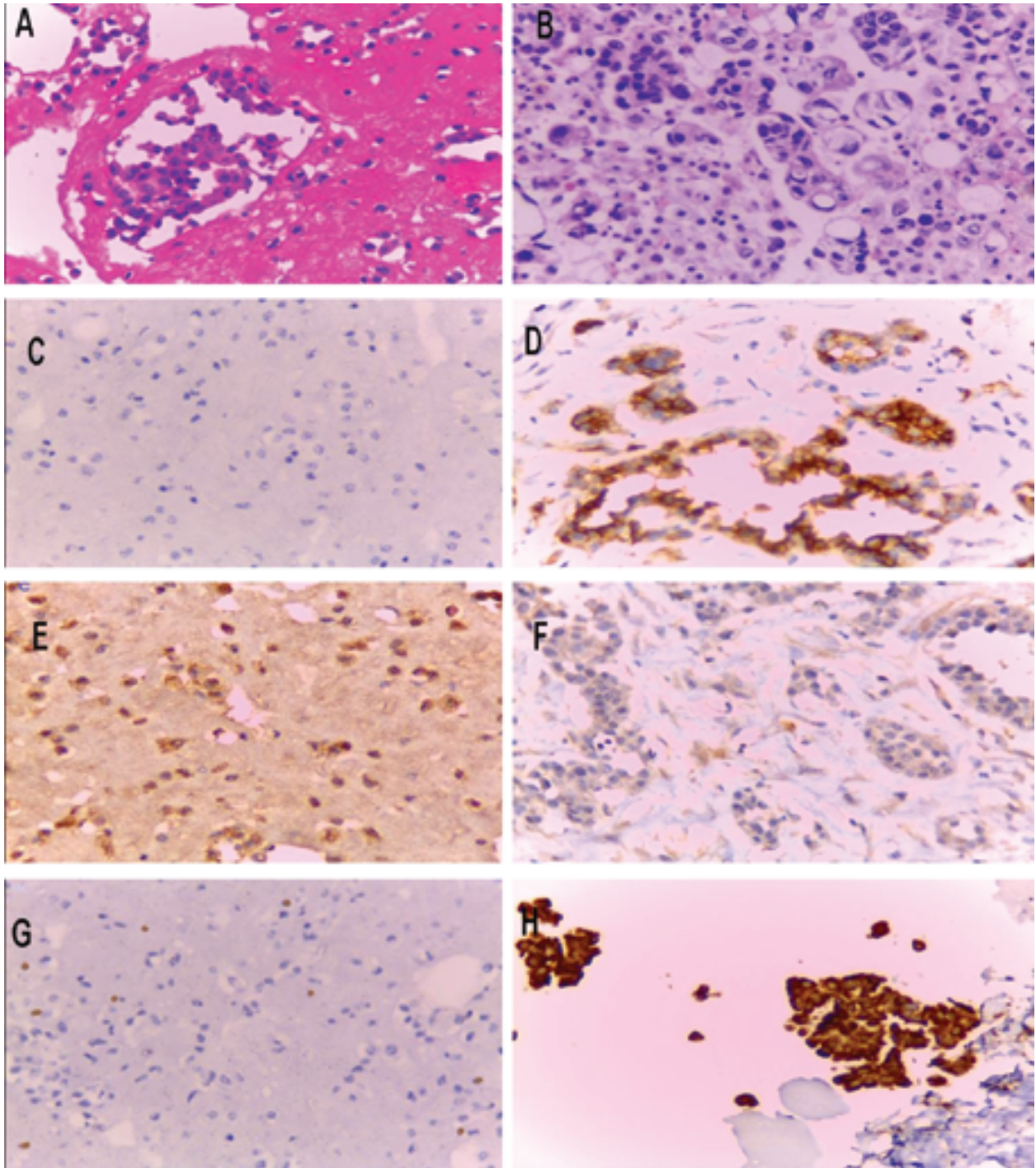
Fig. (1): (A) A case of reactive mesothelial hyperplasia is shown (H & Ex400). (B) A case of malignant mesothelioma is shown (H & Ex400). (C) Epithelial membrane antigen (EMA): Reactive mesothelial cells show no immunoreactivity. (D) EMA: Malignant mesothelial cells show positive membranous and cytoplasmic staining. (E) Desmin: Reactive mesothelial cells show strong positive membranous and cytoplasmic staining. (F) Desmin: Malignant mesothelial cells show no to weak and focal staining. (G) GLUT-1: Reactive mesothelial cells show no immunoreactivity, with positive lymphocytes in the background . (H) GLUT-1: Malignant mesothelial cells show strong membranous positivity with some cytoplasmic staining (IHC, DAB x 400).
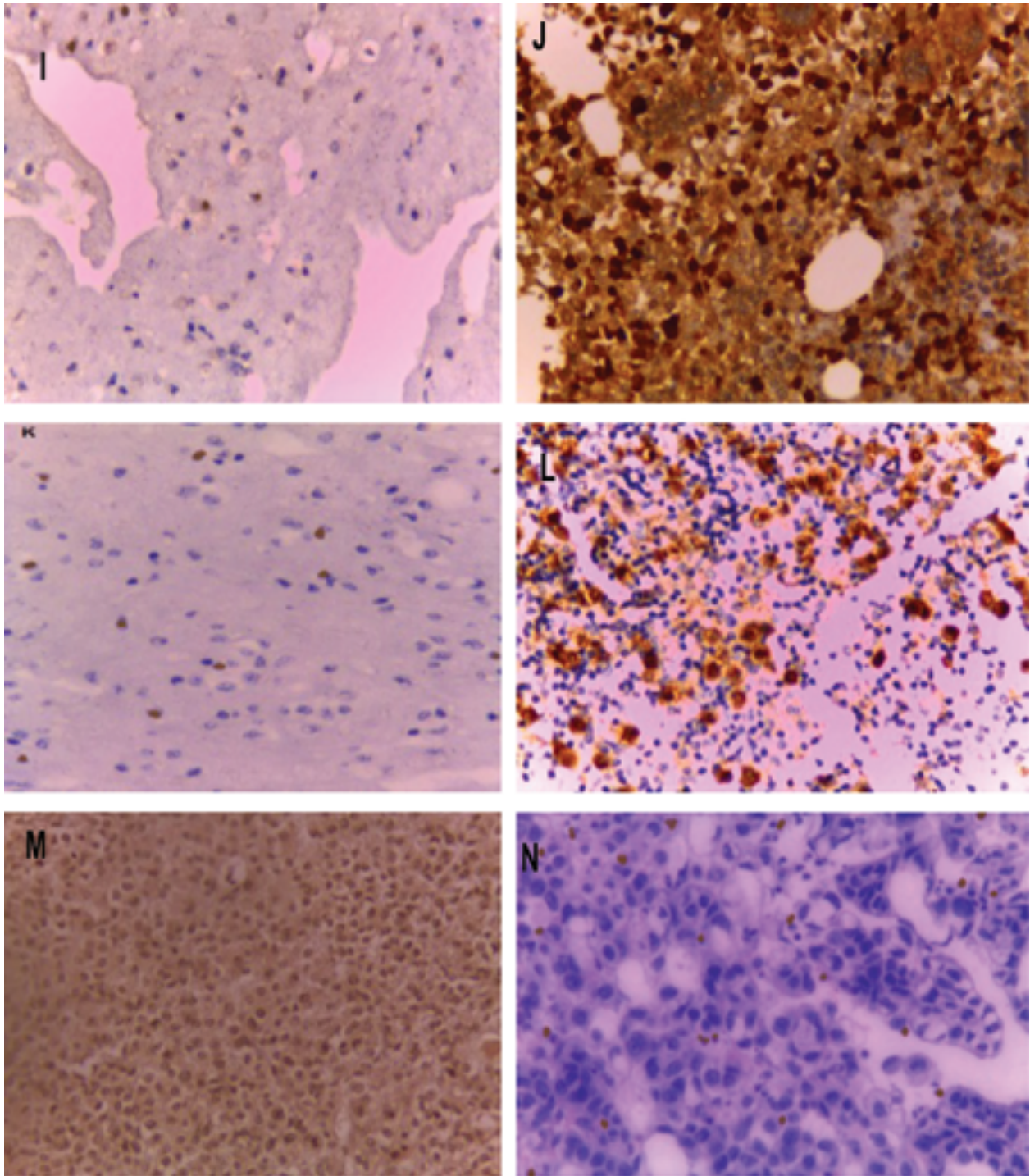
Fig. (2): (I) P53: Reactive mesothelial cells show no immunoreactivity. (J) P53: Malignant mesothelial cells show strong nuclear staining in ≥10% of cells. (K) Ki67: Reactive mesothelial cells showed low nucler staining (<10%) (L) Ki67: Malignant mesothelial cells showed high nucler staining (>40%). (M) BRCA-1 associated protein-1 (BAP-1): Reactive mesothelial cells showed positive nuclear staining in target cells. (N) BAP-1: Malignant mesothelial cells showed absent nuclear staining in the target cells in the presence of a positive internal control, provided by lymphocytes (IHC, DAB x400).

Table (2): Summary of results of immunohistochemical stains.

Stain	Mesotheliom		Reactive		Sensitivity	Specificity	*p*-value
	No.	%	No.	%			
BAP-1:							
Negative	19	63.3	1	5	63%	95%	<.005
Positive	11	36.7	19	95			
Total	30		20				
GLUT-1:							
Negative to focal<20%	9	30	17	85	70%	85%	<.005
Positive (≥20%)	21	70	3	15			
Total	30		20				
Desmin:							
Negative	22	73.3	3	15	80%	90%	<.001
Focal (<20%)	5	16.7	1	5			
Positive (≥20%)	3	10	16	80			
Total	30		20				
EMA:							
Negative	0	0	14	70	97%	90%	<.001
Focal (<20%) or weak	1	3.3	4	20			
Positive(≥20%)	29	96.7	2	10			
Total	30		20				
P53:							
Negative to focal (<10%)	14	46.7	19	95	53%	100%	<.001
Positive	16	53.3	1	5			
Total	30		20				
Ki67 proliferative:							0.040
Negative to low (<10%)	11	36.6	14	70	63%	70%	
Moderate (10%-39%)	14	46.7	4	20	17%	90%	
High (>40%)	5	16.7	2	10			
Total	30		20				

Discussion

Discrimination between malignant mesothelioma of the pleura from reactive proliferation of mesothelial cells is still a big challenge. Most cases of MM present with pleural effusion, and the diagnosis of mesothelioma in such effusion is not an easy task for the pathologist, even the experts. It is essential to differentiate MM from reactive proliferation of mesothelial cells, as the later is non-neoplastic lesion that may associate many other conditions as pleuritis, peritonitis, serosal invasion of other cancers. There is a great resemblance of MM to RMH, so it is difficult to differentiate between them by routine histological examination alone. Many novel IHC markers have been used to differentiate between MM from other non-serosal malignancies and RMH.

BRCA1-associated protein 1 (BAP1) is a tumour suppressor gene frequently inactivated in mesothelioma. In the present study, out of 30 cases of MM, 19 cases (63.3%) showed loss of expression of BAP-1, however only one case (5%) of RMH showed loss of expression of BAP-1. These results were in agreement to Andrici et al., [27]. That revealed BAP-1 loss in 57% of cases of MM, also Righi et al., [28] found negative nuclear staining for BAP1 occurred in 62% of MPMs (including

27% with a cytoplasmic pattern). Hida et al., [29] reported that immunohistochemical detection of loss of BRCA1 associated protein 1 (BAP1) is a reliable markers for MPM diagnosis. Kushitanii et al., [24] reported that BAP1 had 66.2% sensitivity and 100% specificity for the differentiation of MM from RMH. Cozzi et al, [30] found that in malignant mesothelioma cases, there was absent nuclear staining of BAP1 76.5% of the cytologic effusion and 47.5% of the tissue biopsies but all cases of reactive mesothelial hyperplasia showed nuclear expression for BAP1. Also he concluded that cases with absent expression of BAP1 IHC is strongly supported to be malignant mesothelioma. BAP1 may be used as one of the IHC panels for diagnosis of MM in cytologic effusion with high specificity and sensitivity.

In the present research, we found that 70% of MM cases showed positive expression of GLUT-1, however among the RMH cases, 15% of cases showed positive expression of GLUT-1. These results were supported by Minato et al., [20] who found that the sensitivity of GLUT-1 was 85%. Also, Kato et al., [31] found that Immunohistochemical GLUT-1 expression was seen in (100%) of MMs cases and in 100% of cases showed linear membranous staining +/- cytoplasmic staining.

Conversely, all cases of reactive mesothelial proliferation showed negative expression for GLUT-1, concluding that GLUT-1 may be used as an IHC marker, with high sensitivity and specificity for differentiation between reactive mesothelilal hyperplasia and malignant mesothelioma cases. Similarly, Mog et al., [19] concluded that mestoelioma cases showed more levels of IHC expression of GLUT-1 than RMH. Also, found that higher levels ofGLUT-1, mRNA in mesothelioma cases than in non-neoplastic mesothelial cell lines. Conversely, Chang et al., [32] reported that the sensitivity for GLUT-1 was 29%. Hasteh et al., [26] documented 47% sensitivity of GLUT-1 in his study, and that 12% of benign cases showed positive staining for GLUT-1. Also, Stephen et al., [33] reported that The sensitivity of GLUT-1 in epithelioid malignant mesothelioma was 49%.

As regard Desmin staining, 80% of RMH showed positive staining, however only 10% of MM cases showed positive staining, and this difference was statistically significant (p-value <.001). Concerning EMA staining, 10% of RMH cases showed positive staining, however nearly all cases (97%) showed positive staining of it. These results were in agreement to our results in a previous study done on tissue biopsy for differentiation between reactive and malignant mesothelial proliferation [25], as 88.2% of RMH cases (15 of 17), and 7.7% of MM cases (1case of 13 cases) showed positive expression of Desmin. However as regard the EMA staining results, 5.9% of RMH (1 case of 13 case), and 92.3% of MM cases (12 of 13) showed positive expression. Also Minato et al., [20] reported EMA as a positive marker for MM cases. Chang et al., [32]. Found that the sensitivity of EMA was 46% and 100% specificity in differentiation between RMH and MM cases. In a study of Hasteh et al., [26] it was found that 9% (6 of 64) of benign cases showed positivity for EMA, but all the malignant cases 100% (52 of 52) showed such EMA positivity (p<.001). Arslan et al., [34] found in his study on tissue biopsy that staining with EMA was observed in 68.7% of cases (45 of 67), whereas weak positivity was detected in only one case with RMH, and this study concluded that EMA has an important role in differentiation between reactive and malignant mesothelila proliferation. Conversely, Salman et al., [35] reported in his study a case of primary MM of the peritoneum that showed positivity for desmin and negative expression of EMA.

The great conflict in the results may be explained by many causes, as specimen of different types, different patient population or scoring systems. Also, antibodies used mono or polyclonal, the antigen retrieval methods, the histologic type of MM used may also affect the results.

As regard p53 immunostaining, it was found that strong nuclear positivity was detected in 5% of RMH cases (1 of 20), however in MM cases, 53% (16 of 30) of cases showed strong positive nuclear staining, and that difference was statistically significant (p-value <0.05). Our results matched to results reported by Hasteh et al., [26] who found strong nuclear expression of P53 was found in 2% (1 of 46) of RMH and 47% (7 of 15) of MM cases (p<.001). Also, Hafez and Tahoun, [22] reported that positive nuclear expression for p53 was found in 31 out of 41 MM cases (75.6%) and in 3 out of 50 RMH cases (6%), p<0.005. p53 had 75.6% sensitivity, 94% specificity. Conversely, Koo et al., [21] concluded that there was no great difference in the extent of nuclear expression of P53 between RMH and MM cases.

Ki67 IHC staining in this study revealed that Ki67 showed strong nuclear expression in >40% of mesothelial cells in 10% (2 of 20) of RMH and 16.6% (5 of 30) of MM cases (p=.38). Similarly, Hasteh et al., [26] who found that Ki67 showed strong nuclear positivity in >40% of mesothelial cells in 9% (6 of 64) of benign and 16% (8 of 49) of malignant cases (p=.38). These results were conflicting to results reported by Hafez and Tahoun, [22] who found that ki67 immunostaining was positive in 30 out of 41 malignant effusions (73.2%) and in 17 out of 50 benign effusions (34%), p<0.05. ki67 had 73.2% sensitivity, 66% specificity. Kushitani et al., [24] concluded that Ki-67 (cut-off value: 10%) had 85.1% sensitivity and 87.5% specificity in differentiation between reactive and malignant mesothelial proliferation.

Disclosures: No relevant conflicts of interest to declare.

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التمايز المناعى الكيميائى بين التكاثرات الظهارية المتوسطة التفاعلية والخبيثة فى الانصباب الجنبى

المقدمة: يمكن للتمييز بين الخلايا الظهارية المتوسطة والخبيثة فى الانصباب الجنبى فى بعض الحالات أن يكون تحديا. فى الدراسة الحالية، قمنا بدراسة قيمة الكيمياء المناعية (IHC) فى عمل هذا التمايز.

طرق البحث: تم اختيار السوائل الجنبية من ٣٠ حالة تم تشخيصها على أنها ورم الظهارة المتوسطة الخبيثة (MM) ونوع الظهارة الخبيثة و٢٠ حالة تم تشخيصها على أنها تضخم روماتيزمى تفاعلى (RMH)، وتم تلوينها بقع مناعية كيميائية مناعية تشمل BAP-1، desmin، غشاء ظهارى مستضد (EMA) البروتين ١ (GLUT-1) وKi67 وp53.

النتائج: كان BAP-1 سالباً فى ٥٪ (١ من ٢٠) من حالات MH التفاعلية وفى ٦٣.٣٪ (١٩ من ٣٠) من حالات MM (p<.005). كان GLUT-1 موجباً فى ١٥٪ (٣ من ٢٠) من الحميدة و ٧٠٪ (٢١ من ٣٠) من الحالات الخبيثة (p<.005). كان ديسين موجباً فى ٨٠٪ (١٦ من ٢٠) من حالات MH الفاعلية وفى ١٠٪ (٣ من ٣٠) من حالات (p<.001). كان EMA إيجابياً فى ١٠٪ (٢ من ٢٠) من حميدة و ٩٦.٧٪ (٢٩ من ٣٠) من الحالات الخبيثة (p<.001). أظهرت P53 إيجابية نووية قوية فى ٥٪ (١من ٢٠) من حميدة و ٥٣.٣٪ (١٦ من ٣٠) من الحالات الخبيثة (p<.001). أظهر Ki76 أيجابية نووية قوية فى < ٤٠٪ من الخلايا الظهارية فى ١٠٪ (٢ من ٢٠) من الحميدة و ١٦.٧٪ (٥ من ٣٠) من الحالات الخبيثة (p<0.40). تم العثور على سلبية EMA وأيجابية desmin فى ٧٥٪ (١٥ من ٢٠) من MH الحالات التفاعلية و ٣.٣٪ (واحدة من ٣٠) من حالات MM. تم العثور على الإيجابية والسلبية desmin فى ٥٪ (١ من ٢٠) من حالات MH التفاعلية و ٩٠٪ (٢٧ من ٣٠) من الحالات (p<.001). تم الكشف عن إيجابية كل من Desmin وBAP-1 فى ٨٠٪ (١٦ من ٢٠) من الحالات RMH. تم أكتشاف مزيج من Desmin وBAP-1 سلبية فى ٦٠٪ (١٦ من ٣٠) من الحالات MM (قيمة p<.001).

الخلاصة: أظهرت الحالات الإيجابية لـ EMA والسلبية لـ desmin تؤيد بشدة ورم الظهارة المتوسطة الخبيثة. على العكس من ذلك، أظهرت الحالات سلبية EMA وإيجابية desmin تؤيد بقوة تضخم ورم الظهارة المتوسطة رد الفعل. أيضاً مزيج من إيجابية ديسمين و Bap-1 تفضل تشخيص RMH، ومزيج من سلبية Desmin وBAP-1 تفضل تشخيص ورم الظهارة المتوسطة الخبيثة. وبالمثل، أظهرت الحالات تعبيراً غشائياً قوياً عن GLUT-1 و / أو تعبيراً نووياً قوياً عن p53، ويفضل بشدة تشخيص ورم الظهارة المتوسطة الخبيث. أظهر مؤشر التكاثرى الذى أكتشفه Ki76 عدم وجود فرق كبير بين الحالات التفاعلية والخبيثة.