Med. J. Cairo Univ., Vol. 88, No. 2, March: 733-738, 2020
www.medicaljournalofcairouniversity.net

Histopathological and Immunohistochemical Study of D2-40 Expression in Malignant Pleural Mesothelioma and Metastatic Adenocarcinoma

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Abstract

Background: Malignant pleural mesothelioma (MPM) is known to be an aggressive malignant tumor due to the difficulty of making early diagnosis and its rapid progression. Its incidence is increasing worldwide but no treatment plans are accepted. D2-40 is an immunohistochemical marker (monoclonal antibody) that has been used as a lymphatic endothelial marker and used in the differential diagnosis of MPM (epithelioid type) versus metastatic adenocarcinoma. Fifty specimens of the pleural biopsy were viewed. They were diagnosed as 40 cases of MPM (epithelioid type) and 10 cases of metastatic adenocarcinoma (unknown primary origin). These diagnoses based on Hematoxylin and Eosin (Hx & E) stained sections and other immunohistochemical markers such as calretinin, carcinoembryonic antigen (CEA), thyroid transcription factor-1 (TTF-1).

Aim of Study: To compare D2-40 immunostaining in MPM (epithelioid type) and metastatic adenocarcinoma with determining its sensitivity and specificity in both types.

Results: All cases of MPM epithelioid type were positive for D2-40 while all cases of metastatic adenocarcinoma were negative (both sensitivity and specificity= 100%). D2-40 staining result was considered positive or negative according to the presence or absence of membranous staining. Statistical analysis was done for assessment of D2-40 expression in both types of tumors by using SPSS version 21.

Conclusion: Routine immunohistochemical work using D2-40 with calretinin is recommended. D2-40 would be superior to calretinin because of its membranous pattern of staining which does not obscure the cytological features of the tumor cells as compared to calretinin.

Key Words: *D2-40 – Mesothelioma – Metastatic adenocarcinoma.*

Introduction

MPM is a highly malignant tumor arising from mesothelium lining the pleura. It usually arises in the parietal surface and then affects the visceral one. Visceral pleural surface involvement means a more advanced stage. This is an important factor in the prognosis of the disease [1].

The global MPM burden is unclear. It was estimated that about 43 thousand people die from the tumor each year worldwide. MPM incidence has been increasing in industrialized countries. The main cause is occupational exposure to asbestos. The latent period between asbestos exposure and the development of MPM is 30-40 years. The incidence peak is expected to be around 2020 [2].

In Egypt, there are many fabricated products reinforced with asbestos. A steady increase in the number of cases in Egypt was detected. The male to female ratio was 1.6:1. Environmental exposure plays a major role in two regions: Helwan and Shubra while in Upper and Lower Egypt the exposure was lower. MPM in Egypt present in high incidence in areas of high pollution, so the environmental control program would benefit it [3].

Diagnosing MPM is difficult because the symptoms are similar to those of other chest diseases. A history of exposure to asbestos may increase suspicion for this tumor. Physical examinations, chest X-rays, C.T and/or MRI with lung function tests were performed [4].

WHO classification of pleural mesothelial tumors, 2015 was: (1) Diffuse MPM, (2) Localized MPM, (3) Well-differentiated papillary mesothelioma, and (4) Adenomatoid tumor. Type (1) and (2) further subdivided into epithelioid, sarcomatoid and biphasic [5].

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The histological variants of epithelioid MPM were: Tubulopapillary, micropapillary, trabecular, acinar, solid, clear cell, deciduoid, adenoid cystic, signet ring cell, small cell, rhabdoid and pleomorphic. Sarcomatoid mesothelioma subclassified into the conventional spindle, desmoplastic and heterologous (e.g. osteosarcomatous) differentiation [6].

The important pathological factors associated with a poor prognosis are:

1- Histological type, especially the desmoplastic variant of sarcomatoid type and the pleomorphic variant of the epithelioid type.
2- Nuclear grading (degree of nuclear atypia, mitosis and/or MIB-1 labeling index) which is a strong predictor of survival in mesothelioma. Other factors of adverse prognosis include: low chronic inflammatory stromal tumor response, high CD 10 expression, and loss of p16 expression but these factors are not the standards of practice [7].

The most important differential diagnosis of MPM is a metastatic tumor that covers the pleural surface. However, many localized tumors that exist in the pleural surface may mimic mesothelioma by microscopic examination. For this reason, the gross picture of the tumor and the findings at thoracotomy are important to make a definite diagnosis [8].

The immunohistochemical panel has been used to differentiate between MPM and other neoplasms. It usually includes two or more mesothelial markers and two or more epithelial/carcinoma markers used to exclude metastatic carcinoma [9].

There are no absolute antibodies that can be used for the diagnosis of MPM. An initial step could include 2 mesothelial markers with 2 markers for the suspected differential diagnosis (e.g. metastatic lung adenocarcinoma). If the results of staining are concordant, the diagnosis of MPM or metastatic adenocarcinoma may be considered established. If they are not, a second step, expanding the panel of immunohistochemical antibodies, may be needed [6].

D2-40 is a monoclonal antibody that has been recently recommended as a new immunohistochemical marker for the diagnosis of MPM [10].

It is a useful positive mesothelial marker in differentiating between MPM epithelioid type and metastatic adenocarcinoma [11].

This marker has been known to stain lymphatic endothelium and mesothelial cells with high sensitivity and specificity [12].

D2-40 stains tumor cells in a membranous pattern of immunostaining that does not obscure the cytological features of these cells, so it is easier to determine the degree of cytological atypia, which may be difficult in cells that are stained by calretinin [13].

Calretinin is a well-established marker for the diagnosis of MPM and the differentiation from metastatic adenocarcinoma. It is a calcium-binding protein of the EF-hand family. It is expressed in both the nucleus and cytoplasm [14].

Material and Methods

All 50 specimens were already fixed in 10% formalin, routinely processed and embedded in paraffin. Specimens were obtained from the Department of Pathology, Abbassia Hospital of Chest diseases from January 2011 to January 2016. They were selected according to the diagnosis based on Hx & E stained slides and previous immunohistochemical studies. They were classified according to WHO classification, 2015 as 40 cases of MPM epithelioid type and 10 cases of metastatic adenocarcinoma (unknown primary origin). All selected cases were previously stained with calretinin (all selected cases of MPM were positive for calretinin while all cases of metastatic adenocarcinoma were negative).

The D2-40 antibody used is a mouse polyclonal IgG antibody; the recommended immunohistochemical-paraffin dilution is 1:200 up to 1:500 (unit size 0.1ml). The staining procedure was conducted using an automated immunostainer. The lymphatic vessels in these sections served as an internal control. D2-40 staining results were considered positive or negative according to the presence or absence of membranous staining. Positive results were evaluated according to intensity as strong, moderate, or weak (compared to the intensity of staining of the internal control).

The percentage of immunoreactive tumor cells was scored as follows: 1+ (5% to 25% of cells), 2+ (26% to 50%), and 3+ (>50%). If a staining result was positive <5% of cells, it was considered negative (score=0) [12].

Statistical analysis was done for assessment of D2-40 expression in both types of tumors and to compare the results with calretinin by using SPSS version 21 [p-value <0.05 was significant].

Results

All cases of MPM epithelioid type were positive for D2-40 while all cases of metastatic adenocarcinoma were negative (both sensitivity and specificity = 100%).

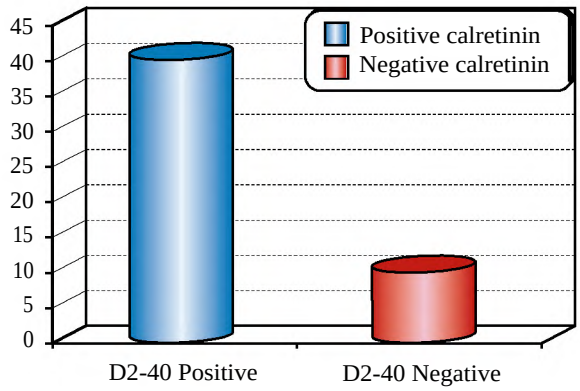
Table (1): Results of D2-40 immunohistochemical staining in each group of tumors.

Histopathological type	D2-40 results	Frequency
MPM epithelioid type	Positive	40
	Negative	0
	Total	40
Metastatic adenocarcinoma	Positive	0
	Negative	10
	Total	10

Table (2): Relation between D2-40 and calretinin results in each group.

D2-40 results	Calretinin results	Percent
Positive (MPM)	Positive	100%
	Negative	0%
	Total	100%
Negative (metastatic adenocarcinoma)	Positive	0%
	Negative	100%
	Total	100%

[With high significant p-value (0.000)].



Graph (1): D2-40 results with calretinin expression in both types of tumors.

Score 3 is the most common score among positive D2-40 cases (28/40) and the strong intensity of the positive staining is the most prevalent (24/40).

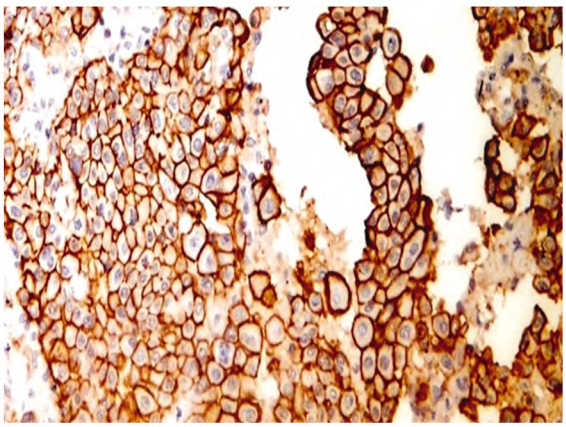


Fig. (1): MPM epithelioid type showing positive D2-40 staining, (original magnification x200), with strong membranous staining and score 3.

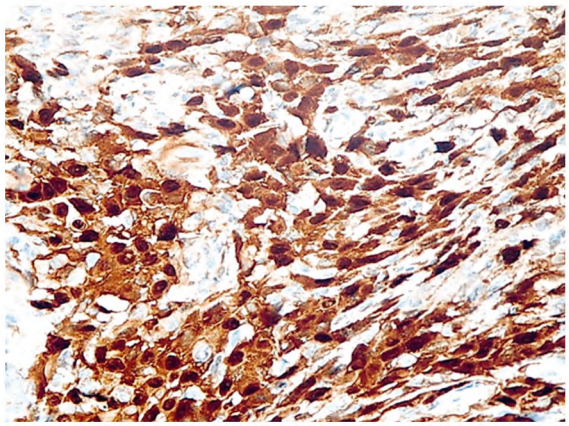


Fig. (2): A case of MPM epithelial type showing positive calretinin staining, by high power (original magnification x200), note the pattern of staining which masking nuclear details.

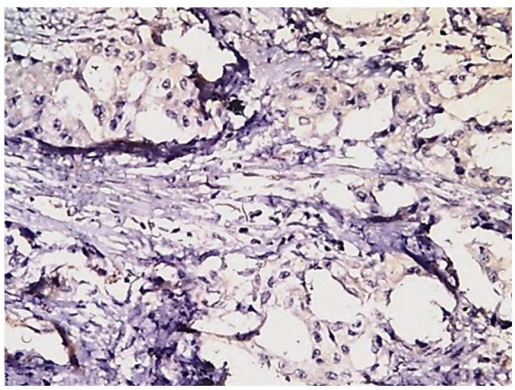


Fig. (3): A case of metastatic adenocarcinoma showing negative D2-40 staining, score 0, at high power (original magnification x200).

Discussion

The International Mesothelioma Panel recommended that 2 positive mesothelioma markers and 2 positive carcinoma markers together with a pan-cytokeratin are sufficient in the majority of cases [6]. The extended panel should include 4 first-line positive mesothelial markers: Calretinin, D2-40, WT1, and cytokeratin 5/6 together with 6 positive carcinoma markers: CEA, TTF-1, CD15, MOC-31, BG8, and Ber EP4 [12].

In the current study, D2-40 was found to be expressed in all cases of malignant mesothelioma epithelioid type (40/40) with 100% sensitivity and specificity.

Albert et al., [15] study also showed that D2-40 positive results were present in 51 of 53 MPM cases, 33 cases were epithelioid type and all were positive (100%). These results were also similar to results obtained by Muller et al., [16] who reported that all the 18 cases of MPM epithelioid type in their study had a strong membranous pattern of D2-40 staining (100%), and results of Bhalla et al., [17] study which showed that D2-40 was positive in all cases of MPM epithelioid type.

On the other hand, the results were little different from Ordonez [18] study who found that D2-40 was positive in 25 out of 29 cases of MPM (86%). In the study of Sienko et al., [19] 32/45 cases of MPM epithelioid type were positive for D2-40 (71.1%). Hinterberger et al., [14] found that 84 out of 112 cases of MPM epithelioid type were positive to D2-40 (75%). Esheba [20] study found that the cases of MPM examined showed positive D2-40 staining in 87% of cases. This is maybe explained by the larger number of cases examined in these studies.

The expression of D2-40 in metastatic adenocarcinoma cases in this work was negative for all selected cases (0/10). This was similar to results of Ordonez [18] study which included 34 cases of lung carcinomas and 70 cases of other adenocarcinomas (17 ovarian, 10 mammary, 10 colonic, 10 renal, 5 endometrial, 5 gastric, 5 pancreatic, 5 prostatic, 3 thyroid) and none of these carcinomas were positive to D2-40.

Bhalla et al., [17] found that the positivity of D2-40 in metastatic adenocarcinoma cases was 0%. Deniz et al., [21] study was done only on lung adenocarcinoma cases and all the 36 cases were negative to D2-40.

Teresa et al., [22] study on formalin-fixed, paraffin-embedded cell blocks of pleural effusions of metastatic adenocarcinoma (21 cases) showed that none of the tested cases stained with D2-40. Albert et al., [15] studied 30 cases of lung adenocarcinoma, 35 cases of renal carcinoma, 26 cases of ovarian carcinoma, 16 cases of breast carcinoma, 11 cases of prostatic carcinoma, and 7 cases of bladder carcinoma. Their results were different from the current study as they illustrated that 17 of 26 (65%) ovarian carcinomas were positive to D2-40 but it was negative in other tumors examined.

Travis et al., [5] illustrated that according to WHO 2015 the sensitivity of D2-40 as the mesothelial marker is 90-100% while specificity versus lung adenocarcinoma is 85%.

In the current study, calretinin was found to be expressed in 40/40 of these cases with 100% sensitivity and specificity.

This is similar to Comin et al., [23] study, in which all the 42 cases of MPM epithelioid type were stained positive for calretinin. In Ordonez [24] study which included 60 cases of MPM epithelioid type, all of them reacted for calretinin. Albert et al., [15] study showed that 33/33 cases of MPM epithelioid type were positive for calretinin.

But in Yaziji et al., [25] study, 65 MPM epithelioid type cases stained with calretinin showed that the sensitivity for mesothelioma was 95% while the specificity was 87% and this could be due to a larger number of cases. While Esheba [20] found that among 15 MPM epithelioid type cases, calretinin immune-expression was positive in 87% of cases.

The expression of calretinin in metastatic adenocarcinoma cases in this work was negative for all selected cases (0/10). This is similar to what was obtained by Seda et al., [26] who found that no positive results were observed in other malignant tumors used in the study rather than MPM. David et al., [27] found the same result, as all adenocarcinoma specimens were classified as negative for calretinin staining.

But this is different from what was obtained by Comin et al., [23] as 23 cases of lung adenocarcinoma were stained by calretinin, 2 cases showed positive reactivity for calretinin (8.7%). In Ordonez [24] study, among 50 cases of pulmonary adenocarcinoma, 8% were positive for calretinin. This is also could be explained by a large number of cases. Esheba [20] study illustrated calretinin was positive in 2 out of ten cases of lung adenocarcinoma.

Travis et al., [5] illustrated that according to WHO 2015 the sensitivity of calretinin as a mesothelial marker is more than 90% while specificity versus lung adenocarcinoma 90-95%.

These differences may be due to the different numbers of cases, different staining techniques (as using autostainer or not) or due to the difference in the antibodies used (poly or monoclonal).

In this work, cases of MPM which stained positive for D2-40 showed that the strong intensity of the membranous pattern of staining is the most prevalent (24/40) and score 3 is the most common. This is similar to what was reported by Hanna et al., [12] study at which the strong intensity of staining was the most common among MPM cases (44%) and more than 50% of malignant cells expressing positive staining in 94% of cases (score 3).

Conclusions: D2-40 and calretinin immunostaining are recommended as routine positive markers for MPM. Both have the same high specificity and sensitivity but D2-40 would be superior to calretinin because of its membranous pattern of staining which does not obscure the cytological features of the tumor cells.

Conflict of interest:

None declared

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دراسة باثولوجية وهستوكميائية مناعية لاستخدام (دى ٢-٤٠) فى فحص ورم الظهارة المتوسطة الخبيث وثانويات الاورام الغدية الخبيثة

تشمل هذه الدراسة ٥٠ حالة من أورام الغشاء البللورى تم تجميعها من قسم الباثولوجى بمستشفى الأمراض الصدرية بالعباسية وقد شخصت هذه الحالات بناء على الصبغة المعتادة (هيموتكسيلين وايوسين) وكذلك صبغات مناعية أخرى سابقة كالتالى:٤٠ حالة ورم ظهارة متوسطة خبيث و ١٠حالات أورام غدية ثانوية خبيثة.

وتم تنفيذ صبغة دى ٢-٤٠ المناعية على جميع الحالات وقد قيمت النتائج حسب وجود (موجبة) أو عدم وجود (سالبة) الصبغ الغشائى للخلايا السرطانية وقد قسمت درجة وجود الصبغ الغشائى إلى قوية، متوسطة وضعيفة بالمقارنة بالنسيج الضابط الداخلى. ويتم تقييم نسبة الخلايا التى تظهر دى٢-٤٠فى كل حالة على حدا بناء على التقييم التالى:

- ١+ (٥-٢٥٪ من الخلايا يظهر الصبغة)
- ٢+ (٢٦-٥٠٪ من الخلايا يظهر الصبغة)
- ٣+ (أكثر من٥٠٪ من الخلايا يظهر الصبغة)

وفى حالة أقل من ٥٪ من الخلايا يظهر الصبغة تعتبر النتيجة سالبة (تقييم صفر)

وقد تم التحليل الإحصائى المطلوب للنتائج ووجد أنه فى جميع حالات أورام الظهارة المتوسطة الخبيثة كانت النتائج موجبة لصبغة دى ٢-٤٠أما فى حالات الأورام الغدية الثانوية الخبيثة كانت جميعها سالبة (خصوصية وحساسية ١٠٠٪).

وكذلك فى جميع حالات أورام الظهارة المتوسطة الخبيثة كانت النتائج موجبة لصبغة (كالريتينين) أما فى حالات الأورام الغدية الثانوية الخبيثة كانت جميعها سالبة (قيمة ص =٠،٠٠٠وخصوصية وحساسية ١٠٠٪ أيضا).

معظم حالات أورام الظهار المتوسطة الخبيثة قد أظهرت صبغة غشائية قوية الدرجة وكذلك التقييم الثالث (+٣) فى نسية الخلايا المصبوغة فى كل حالة.

وقد وجد أن صبغة دى ٢-٤٠ قد تكون أكثر فائدة فى التشخيص من صبغة (كالريتينين) وذلك لطبيعة صبغتها الغشاء الخلوى فقط بدون التأثير على شكل انوية الخلايا السرطانية والخصائص الستيولوجية لهذه الخلايا.