

## Review Article:

# D2-40 Expression in Malignant Pleural Mesothelioma (Epithelioid Type)

KARIMAN H. ABDELWAHAB, M.Sc.\*; BADAWIA B. IBRAHIM, Ph.D.\*\*;  
SAMAR A. EISHEIKH, M.D.\*\* and AMAL A. HAREEDY, M.D.\*\*

*The Department of Pathology, Faculty of Medicine, Helwan\* and Cairo\*\* Universities*

### Abstract

Malignant pleural mesothelioma (MPM) is known to be a clinical and pathological challenge. Its incidence is continued to increase worldwide including Egypt unfortunately. There is a positive link between the residential location and the incidence of mesothelioma. It is commonly occurring in areas of heavy pollution and environmental exposure to asbestos. Most of MPM cases are strongly suspected on routine hematoxylin & eosin staining. They exhibit a variety of histologic subtypes: Epithelioid, sarcomatoid or biphasic type. Epithelioid type of MPM can be easily confused with metastatic adenocarcinoma. Although immunohistochemistry has proven to be valuable in the differentiation of epithelioid mesothelioma from metastatic adenocarcinoma, no single antibody has demonstrated absolute sensitivity or specificity in making this distinction. Using immunohistochemical analysis with D2-40, a monoclonal antibody that has been used as a lymphatic endothelial marker may be useful in the differential diagnosis of epithelioid mesothelioma versus metastatic adenocarcinoma because of its staining of mesothelial cells. D2-40 had been reported to have strong membranous immunostaining in up to 96% of MPM and reactive mesothelial processes but negative staining in adenocarcinoma.

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

### Review Article

Malignant pleural mesothelioma is an aggressive malignancy arising from mesothelial cells lining the pleura. It is usually developed on the parietal pleural surface and later spreads to visceral pleura. Visceral pleura involvement entails a more advanced disease stage and is, therefore, an important prognostic factor [1].

---

**Correspondence to:** Dr., Kariman H. Abdelwahab,  
The Department of Pathology, Faculty of Medicine,  
Helwan University

The global mesothelioma burden is unclear. It was estimated that as many as 43,000 people worldwide die from the disease each year. However, there is no established global baseline that can be used to evaluate trends in disease occurrence. MPM incidence rates have been increasing throughout the industrialized world. This reflects the industrial exposure to asbestos, combined with a latent period between exposure and the development of mesothelioma averaging 30-40 years. The incidence is predicted to peak around 2020 [2].

In Egypt, the most famous factories are in Shubra El-Kheima district, the 10<sup>th</sup> of Ramadan city and Helwan. These factories produce both cement pipes and corrugated roofing panels reinforced with asbestos. A steady increase in the number of cases in Egypt was detected.

The male/female ratio was 1.6/1. As mesothelioma in Egypt is mainly concentrated in areas of high environmental pollution, a better environmental control program would be of great value [3].

In pathology-based cancer registry done in Ain Shams Faculty of Medicine from 2001 to 2010, there were 81 cases of pleural malignant tumors. MPM was diagnosed in 63 cases and constituted 6.8% of malignant respiratory system tumors and 0.5% of the total malignancies [4].

While in the cancer pathology registry done in the department of pathology, National cancer institute from 2000 to 2011, pleural malignant tumors formed 1.28% of total malignancies. MPM was the most common primary malignant pleural tumor, forming more than half the cases, followed by metastatic carcinoma (13.14%). Epithelioid type

mesothelioma was the most frequent histologic subtype forming 76.2% of all mesotheliomas [5].

Mesotheliomas often present with recurrent pleural effusion, which is submitted for cytologic evaluation. The sensitivity of cytologic diagnosis of MPM ranges between 32% and 76%. This broad range of sensitivity (high false-negative rate) is probably related to sampling rather than interpretation [6].

The most useful cytologic features of epithelioid MPM are the presence of numerous relatively large (>50 cells) balls of cells with berry-like external contours and enlargement of cytoplasm, nucleus, nucleolus (macro-nucleoli) and nuclear atypia if present. However, prominent nucleoli can be present in reactive mesothelial cells [7].

Using molecular techniques [such as fluorescence in situ hybridization (FISH)] in demonstrating homozygous deletion of the p 16 gene which was detected in about 70% of mesothelial proliferations are particularly promising (as reported specificity is 100%) [8].

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 [9].

Epithelioid mesotheliomas with marked nuclear pleomorphism greater than 10% of the tumor were recognized as a "pleomorphic" variant and had been shown to behave similarly to sarcomatoid and biphasic variants (an adversely prognostic pattern) [10].

The most important differential diagnosis of MPM is metastatic or locally invasive (from lung or chest wall) tumor that covers the pleural surface. However, various localized tumors also exist in the pleura and some mimic mesothelioma microscopically. For this reason, knowledge of the gross distribution of tumor, the operator's description of the findings at thoracotomy or thoracoscopy is crucial to making a proper diagnosis [11].

The diagnosis of malignant mesothelioma is rendered with the aid of immunohistochemistry to demonstrate the presence of "mesothelial" or "epithelial" differentiation. Antibody panels that have been proposed for the distinction between MPM and other neoplasms usually include 2 or more epithelial markers used to exclude the diagnosis

of carcinoma, and 2 or more mesothelial markers used to confirm the diagnosis of MPM [12].

There is no single antibody, which can diagnose mesothelioma; therefore, the comprehensive judgment by the results using these combinations of antibodies is required [13].

There is no absolute number of antibodies that can be recommended for the diagnosis of malignant mesothelioma. Workup can be done in stages. An initial workup could use 2 mesothelial markers and 2 markers for the other suspected tumor (i.e. metastatic lung adenocarcinoma). If results are discordant, a second stage, expanding the panel of antibodies, may be needed [7].

D2-40 is a monoclonal antibody that has been recommended as a marker for the diagnosis of malignant mesothelioma [14]. It has been recognized to stain mesothelial cells and lymphatic endothelium with high sensitivity and specificity [15].

It had been reported to have strong membranous immunostaining in up to 96% of mesothelioma and reactive mesothelial processes but negative staining in adenocarcinoma [16].

It is a commercially available monoclonal antibody directed against M2A antigen [17]. The M2A antigen is a 40 kDa O-linked sialoglycoprotein found on the cell surface of testicular gonocytes, germ cell tumors, lymphatic endothelium and mesothelial cells [18].

Since D2-40 has demonstrated selective immunoreactivity for lymphatic endothelium, its clinical uses also include the demonstration of lymphatic invasion by primary tumors and it is used as a marker of certain vascular lesions [17].

Potential interpretive pitfalls exist as it is constantly expressed in lymphatic endothelium and serves as an internal positive control in tissues. So the pathologist mustn't over-interpret focal linear membrane expression within lymphatics [19].

The existence of mesothelin antibodies as D2-40 in mesothelioma patients have raised significant interest in the potential of immunotherapy in MPM. Clinical studies including trials with anti-mesothelin monoclonal antibodies and gene delivery strategies are ongoing [20].

## References

- 1- PINELLI V., LAROUMAGNE S., SAKR L., MARCHETTI P., TASSI F. and ASTOUL P.: Pleural fluid cytological yield and visceral pleural invasion in patients with epi-

- thelioid malignant pleural mesothelioma. *J. of Thorac. Oncol.*, Vol. (7), Issue: (3): 595-598, 2012.
- 2- MAHMOUD H.: Early detection of malignant pleural mesothelioma, Abbassia Chest Hospital, Cairo, Egypt. *Egypt J. of Bronchol.*, Vol. (4), Issue: (1): 1-9, 2014.
  - 3- YOSRI A., SAFY K. and AHMED A.: Epidemiology of mesothelioma in Egypt. A ten-year (1998-2007) multicentre study. *Arch. Med. Sci.*, Vol. (6), Issue: (6): 926-931, 2010.
  - 4- HELAL T., EZZ EL ARAB S. and SALMAN M.: Pathology based cancer registry 2001-2010 Ain Shams Faculty of Medicine. *Cancer Register*, 87: 88, 2015.
  - 5- MOKHTAR N., SALAMMA A., BADAWY O., et al.: Cancer pathology registry 2000-2011, Cairo University, National cancer institute, pathology department, *Canc. Register*, 135, 2016.
  - 6- SHEAFF M.: Should cytology be an acceptable means of diagnosing malignant mesothelioma? *Cytopathol.*, Vol. (22), Issue: (1): 3-4, 2011.
  - 7- HUSAIN A., COLBY T., ORDONEZ N., KRAUSZ T., ATTANOOS R. and BEASLEY B.: Guidelines for pathologic diagnosis of malignant mesothelioma. 2012 update of 2009 consensus statement from the International Mesothelioma Interest Group. Published by College Amr Pathol, online 2013 at [www.thefreelibrary.com](http://www.thefreelibrary.com), Vol. (133), Issue: (8): 1317-1331, 2013.
  - 8- MONACO E., SHUAI Y., BANSAL M., KRASINSKAS M. and DACIC S.: The diagnostic utility of p16 FISH and GLUT-1 immunohistochemical analysis in mesothelial proliferations. *Am. J. Clin. Pathol.*, Vol. (135), Issue: (4): 619-627, 2011.
  - 9- TRAVIS W., BRAMBILLA E., BURKE P., MARX A. and NICHOLSON G.: WHO Classification of Tumors of the Lung, Pleura, Thymus and Heart. IARC, Lyon 4<sup>th</sup> Ed., Vol. (7): 153-71, 2015.
  - 10- ORDONEZ N.: Pleomorphic mesothelioma: Report of 10 cases. *Mod. Pathol.*, Vol. (25), Issue: (7): 1011-1022, 2012.
  - 11- CHURG A., COLBY V., CAGLE P., et al.: The separation of benign and malignant mesothelial proliferations. *Am. J. Surg. Pathol.*, Vol. (24): 1183-1200, 2004.
  - 12- ALBERTO M.: Application of Immunohistochemistry to the Diagnosis of Malignant Mesothelioma. *Arch. Pathol. Lab. Med.*, Vol. (132): 397-401, 2008.
  - 13- TAKESHIMA Y., AMATYA J., KUSHITANI K., et al.: Value of immunohistochemistry in the differential diagnosis of pleural sarcomatoid mesothelioma from lung sarcomatoid carcinoma. *Histopathol.*, Vol. (54): 667-676, 2009.
  - 14- BETTA P., MAGNANI C., BENSI T., TRINCHERI N. and ORECCHIA S.: Immunohistochemistry and molecular diagnostics of pleural malignant mesothelioma. *Arch. Pathol. Lab. Med.*, Vol. (136): 253-261, 2012.
  - 15- HANNA A., YIJUN P., CARLOS B., et al.: Podoplanin is a useful marker for identifying mesothelioma in malignant effusions. *Diagn. cytopathol.*, Vol. (38), Issue: (4): 264-269, 2010.
  - 16- ORDONEZ N.: D2-40 and podoplanin are highly specific and sensitive immunohistochemical markers of epithelioid malignant mesothelioma. *Hum. Pathol.*, 36: 372-380, 2005.
  - 17- CHU Y., LESLIE L., THERESA P., GEZA A. and PAUL Z.: Utility of D2-40, a novel mesothelial marker, in the diagnosis of malignant mesothelioma. *Mod. Pathol.*, Vol. (18): 105-110, 2005.
  - 18- SCHACHT V., DADRAS S., JOHNSON A., et al.: Up-regulation of the lymphatic marker podoplanin, a mucin-type transmembrane glycoprotein, in human squamous cell carcinomas and germ cell tumors. *Am. J. Pathol.*, Vol. (166): 913-21, 2005.
  - 19- ATTANOOS R. and ALLEN T.: Advances in Surgical Pathology: Mesothelioma, 1<sup>st</sup> Ed., by Lippincott Williams & Wilkins. Ch., (12): p: 97-126, 2014.
  - 20- ZANDWIJK N., CLARKE C., HENDERSON D., MUSK A., et al.: Guidelines for the diagnosis and treatment of malignant pleural mesothelioma. *J. Thorac. Dis.*, Vol. (5): Issue (6): 254-307, 2013.

## مظهر الصبغة المناعية (دي ٢-٤٠) في ورم الظهارة المتوسطة الخبيث

ان ورم الظهارة المتوسطة الخبيث يعتبر الأورام الخبيثة الشرسة التي تتكون في الخلايا المبطنة للغشاء البلوري.

تتزايد الإصابة بهذا النوع من الأورام الخبيثة في مصر بشكل مستمر ويرجع ذلك إلى العلاقة بين الإصابة بالورم والتواجد في أماكن عالية التلوث والتعرض لمادة الاسبستوس.

بالرغم من أن الصبغات المناعية قد أثبتت فاعليتها في التفرقة بين أورام الظهارة الخبيثة وثانويات الأورام الغدية الخبيثة ولكن لا توجد صبغة مناعية بعينها لها مطلق الخصوصية والحساسية لهذا الأورام.

استخدام الصبغة المناعية دي ٢-٤٠ (وهي صبغة تستخدم في إظهار الخلايا المبطنة للأوعية الليمفاوية وكذلك الخلايا المبطنة للغشاء البلوري) قد يكون مفيد في التفرقة بين ورم الظهارة المتوسطة الخبيث وبعض أنواع الأورام الغدية الخبيثة التي تنتشر في الغشاء البلوري.