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ORIGINAL ARTICLE

Diagnostic Utility of BRCA1-Associated Protein 1 (BAP1), Programmed Cell Death 4 (PCD4) And Epithelial Membrane Antigen (EMA) Expression In Differentiation Of Malignant Mesothelioma From Reactive Mesothelial Cell Hyperplasia In Both Cytology And Cell Block

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ABSTRACT

Background: Malignant pleural mesothelioma (MPM) is a fatal tumor that originate from the mesothelial cells. Sometimes, it is difficult to be diagnosed based on morphology alone as reactive mesothelial hyperplasia (RMH) and some metastatic carcinomas may be confused with mesothelioma . Our study aims to adjust diagnostic value of BRCA1 "Breast Cancer gene" associated protein-1 (BAP1), Programmed Cell Death4 (PCD4), and Epithelial membrane antigen (EMA) in differentiation of malignant mesothelioma (MM) from reactive mesothelial hyperplasia (RMH) by immunohistochemistry (IHC).

Methods: This retrospective study, include 60 patients, was carried out in Chest and Pathology Departments of Zagazig University from October 2016 till August 2020. The expression levels of BAP1, PCD4 and EMA were investigated using cytological analysis compared with cell block method in all cases of MPMs and RMH.

Results: BAP1loss was detected in cases of malignant mesothelioma confirmed by cytology in 19 out of 20 patients with sensitivity of 95%, specificity 92.5%. PCD4 was positive in 39 out of 40 patients of RMH and can diagnose reactive mesothelioma with sensitivity of 85.7% and specificity 100%. EMA was positive 95% in MM confirmed by cytology and can diagnose malignant mesothelioma with a sensitivity of 95% and specificity 97.5%.

Conclusions: Cell block method increases the sensitivity of diagnosis in cases that were recorded as reactive mesothelial hyperplasia by conventional cytological smears. BAP1 loss, negative PCD4 and positive EMA immunostaining can differentiate and diagnose MM from RMH with improved diagnostic accuracy.

Keywords: BAP1; PCD4; EMA; Malignant mesothelioma; Diagnosis

INTRODUCTION

Malignant pleural mesothelioma (MPM) is a fatal tumor that originate from the mesothelial cells; the incidence of disease increased in last years. According to registry of the Egyptian National Cancer Institute (NCI) 2020, MPM constitute 0.1% and 0.17% of cancers among male and females respectively **[1].**

The diagnosis of MPM is vague on morphology alone, atypical reactive mesothelial hyperplasia and some metastatic carcinomas may be confused with mesothelioma **[2].** Most patients about 54–89% presented with pleural effusion**.** Identification of benign or malignant mesothelial cells in pleural fluid smear cytology is essential for the treatment [3].

Use of IHC markers with cytological smear contributed to increase of diagnostic accuracy. Fluid cytology and immunocytology on cell block is being essential for detection of MPM in problematic cases [4]. The molecular pathways involved in MPM are still unidentified regarding

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gene alteration that triggers tumor genesis and progression **[5].**

BAP1 [BRCA1-associated protein 1] is a tumor suppressor gene involved in gene expression, transcription and DNA repair. BAP1 mutations are initial step for the development of MM. BAP1 mutations have been recorded in 23 - 81% MM and it increased especially in cases of epithelioid MM **[6]**. Germ line BAP1 mutations occur in 2% of MM patients [7].

Programmed Cell Death 4 (PDCD4) is an onco-suppressor gene whose expression is frequently altered in cancer. PDCD4 plays its role by affecting both mRNA transcription and translation [8].

PDCD4 suppress many oncoproteins by interfering the activity of eukaryotic initiation factors 4A and 4G (eIF4A, eIF4G) and interact with the JNK/c-Jun/AP-1 pathway implicated in gene transcription. Decreased nuclear PDCD4 expression is a marker for malignant transformation [9]. PCD4 had been used to distinguish MPM from benign mesothelial conditions [10]

Epithelial membrane antigen (EMA) is a glycoprotein found in the Golgi apparatus of human milk fat membrane. It can promote invasion of extracellular matrix by malignant cells [11].

Our study aims to adjust diagnostic value of BRCA1 associated protein-1 (BAP1), Programmed Cell Death (PCD4) and Epithelial membrane antigen (EMA) in differentiating malignant mesothelioma (MM) from reactive mesothelial hyperplasia (RMH) by immunohistochemistry (IHC).

METHODS

Study design

Retrospective cohort study was run over a period of 34 months, from October 2016 till August 2020.It was carried out in Chest and Pathology Departments of Zagazig University, Zagazig, Egypt. Cases were collected from archive of Pathology. Written informed consent was obtained from all participants, the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Methods

This study included 60 accessible patients of exudative pleural effusion, from those admitted to chest and oncology departments. The Full clinical medical history and a detailed clinical examination was done for all patients, routine hematological investigations: complete blood picture, liver, kidney function tests, erythrocyte sedimentation rate (ESR), prothrombin time concentration, partial thromboplastin time, fasting and 2 h postprandial blood glucose. Radiology was done using plain chest radiography: posteroanterior and lateral views in addition to chest ultrasonography. Conventional contrast enhanced computed tomography.

Pleural fluid aspiration (pleural fluid was aspirated from the patients and sent for full pathological, chemical, bacteriological, adenosine deaminase (ADA) according to Weinberger et al [12].

Cytological examination of pleural fluid specimen from every patient with an exudative pleural effusion, which is suspected to be mesothelioma should be sent for cell block [13]. Inclusion criteria include cellular smears with suspected mesothelioma.

Exclusion criteria include, other variants of mesothelioma, metastatic lung adenocarcinoma and cytology specimens were excluded if cell count was not satisfactory

The confirmation of MM diagnosis was through IHC stains as calretinin, WT-1, CK5/6, CK7 and 20, TTF and desmin. All the patients were associated with clinical and radiological features diagnosed as MM.

Preparation for cell block

Fluid intended for CB was subjected to fixation for 1 h by adding 5ml of 10% alcohol–formalin for one hour then fluid was centrifuged 15 min, the sediment was put on a filter paper and processed as routine histopathological specimen

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in which 4-µm section from each submitted paraffin block specimens stained by H&E [18].

Immunohistochemical smears preparation

Stained smear preparations incubated with antihuman BAP1(rabbit polyclonal, ab245391, diluted 1/100, Abcam), Anti-PCD4(mouse monoclonal ab9G6, diluted 1/100, Abcam) and antiEMA (mouse monoclonal ab546-2, diluted 1:200, Abcam). Antibody binding was detected by Dako's HRP Envision Kit (Dako Cytomation, Denmark) and then visualized with 3,3'diaminobenzidine and counterstained with Mayer's hematoxylin.

Inflammatory cells acted as positive control for BAP1.Normal human tonsil tissue was used as positive control for PCD4 and positive control for EMA was breast carcinomas.

Interpretation of immunohistochemical staining

BAP1 was recorded as negative if the nuclear staining was totally absent in all the cells, and positive if at least 50% of the atypical mesothelial cells showed nuclear immune staining [15]

For PCD4 evaluation: Negative if cells show no staining and weak positive if <30 of cells shows immunostaining [16].

As regards EMA evaluation; it considered negative if cells show no staining, focal/weak positive if there was (<20%) scattered cells that showed membranous staining pattern [17].

Statistical analysis

Data analysis was performed using the software SPSS (Statistical Package for the Social Sciences) version 20. using their means and standard deviations were described for quantitative variables. Absolute frequencies of categorical variables were described. Kappa Cohen coefficient was used to measure interrater reliability between biopsy and cytology. Medcalc software was used to calculate performance of each marker in identifying nature of mass. Statistical significance was set at 5% ($P<0.05$).

RESULTS

Clinicopathological characteristics

The study was conducted on 60 patients among them 35 were males and 25 were females, with a mean age of 54.2 ± 10.91 years. Malignant mesothelioma as evident in 33.3% of patients by cytology, which increased to 68.3% on doing cell block. BAP1 loss, EMA and PCD4 were evident in 36.7%, 33.3% and 30% of patients respectively **(Table1.Fig 1,2).**

Immunohistochemical results

BAP1 loss was detected in malignant mesothelioma in 19 out of 20 patients confirmed by cytology and 20 out of 21 confirmed by cell block. Absence of BAP1 loss excludes reactive mesothelial cells in 37 out of 40 patients confirmed by cytology. However, it was absent in 37 out of 39 patients with reactive mesothelioma by cell block. BAP1 loss can diagnose malignant mesothelioma (compared to cytology) with a sensitivity of 95%, specificity 92.5%, PPV 86.4%, NPV 97.4% and accuracy 93.3%. BAP1 loss can diagnose malignant mesothelioma (compared to cell block) with a sensitivity of 95.2%, specificity 94.9%, PPV 90.9%, NPV 97.4% and accuracy 95%. There is almost perfect agreement between BAP1 loss and detection of mesothelioma by each of cytology and cell block **(Table 2. Fig 3,4).**

Negative PCD4 present in malignant mesothelioma in 17 out of 20 patients confirmed by cytology and 18 out of 21 confirmed by cell block. Presence of PCD4 rule out reactive mesothelial cells in 39 out of 40 patients confirmed by cytology and 39 out of 39 confirmed by cell block. Negative PCD4 can diagnose malignant mesothelioma (compared to cytology) with a sensitivity of 85%, specificity 97.5%, PPV 92.9%, NPV 94.4% and accuracy 93.3%. Positive PCD4 can diagnose reactive mesothelial cells (compared to cell block) with 85.7% sensitivity, 100% specificity, 100%PPV, 92.9% NPV and 95%accuracy. There is substantial agreement between PCD4 (positive) and detection of reactive mesothelioma by each of cytology and cell block **(Table 3. Fig 5,6)**.

EMA detects malignant mesothelioma in 19 out of 20 patients confirmed by cytology and 20 out

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of 21 confirmed by cell block. Absence of EMA excludes reactive mesothelial cells in 39 out of 40 patients confirmed by cytology and all those confirmed by cell block. EMA can diagnose malignant mesothelioma (compared to cytology) with a sensitivity, specificity, PPV, NPV and accuracy 95%, 97.5%, 95%, 97.5% 96.7% respectively. Absent EMA can diagnose reactive mesothelial cells (compared to cell block) with a 95.2% sensitivity 100%specificity, 100% PPV, 97.5% NPV and 98.3% accuracy. There is almost perfect agreement between EMA (positive) and detection of mesothelioma by each of cytology and cell block **(Table 4. Fig 7).** There is statistically significant perfect agreement between cytology and cell block in malignant mesothelioma diagnosis **(Table 5)**.

Table 1: Clinicopathological data of the studied patients

Data	N=60	%
Age (year):		
Mean ± SD	54.2 ± 10.91	
Range	38 – 70	
Gender:		
Male	35	58.3
Female	25	41.7
Result of cell block:		
Reactive	40	66.7
Malignant	20	33.3
Result of cytology:		
Reactive	41	68.3
Malignant	19	31.7
BAP1 loss:		
Positive	22	36.7
Negative	38	63.3
EMA		
Positive	20	33.3
Negative	40	66.7
PCD4:		
Positive	18	30
Negative	42	70

Table 2: Performance of BAP1 loss in diagnosis of malignant mesothelioma in comparison to result of cytology and biopsy.

BAP1 loss	Mesothelioma by cytology		Mesothelioma by cell block		Total
	Malignant	**Reactive**	**Malignant**	**Reactive**	
Positive	19	3	20	2	22
Negative	1	37	1	37	38
Total	20	40	21	39	60
	Cytology		**Cell block**		
Sensitivity	95%		95.2%		
Specificity	92.5%		94.9%		
PPV	86.4%		90.9%		

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NPV	97.4%	97.4%
Accuracy	93.3%	95%
Kappa	0.854 (almost perfect agreement)	0.889 (almost perfect agreement)

Table 3: Performance of PCD4 in diagnosis of reactive mesothelioma in comparison to result of cytology and biopsy

PCD4	Mesothelioma by cytology		Mesothelioma by cell block		Total
	Malignant	**Reactive**	**Malignant**	**Reactive**	
Positive	3	39	3	39	42
Negative	17	1	18	0	18
Total	20	40	21	39	60
	Cytology		**Cell block**		
Sensitivity	85%		85.7%		
Specificity	97.5%		100%		
PPV	92.9%		100%		
NPV	94.4%		92.9%		
Accuracy	93.3%		95%		
Kappa	0.69 (substantial agreement)		0.723 (substantial agreement)		

Table 4: Performance of EMA in diagnosis of malignant mesothelioma in comparison to result of cytology and biopsy.

EMA	Mesothelioma by cytology		Mesothelioma by cell block		Total
	Malignant	**Reactive**	**Malignant**	**Reactive**	
Positive	19	1	20	0	20
Negative	1	39	1	39	40
Total	20	40	21	39	60
	Cytology		Cell block		
Sensitivity	95%		95.2%		
Specificity	97.5%		100%		
PPV	95%		100%		
NPV	97.5%		97.5%		
Accuracy	96.7%		98.3%		
Kappa	0.886 (Almost perfect agreement)		0.923 (Almost perfect agreement)		

Table 5: Agreement between cytology and biopsy in diagnosis of malignant mesothelioma

Cytology	Cell block		Test	
	Reactive N= (%)	**Malignant N= (%)**	**Kappa**	**p**
Reactive	**40 (97.6)**	**0 (0)**	**0.962**	**0.038***
Malignant	**1 (2.4)**	**19 (100)**		

*p<0.05 is statistically significant

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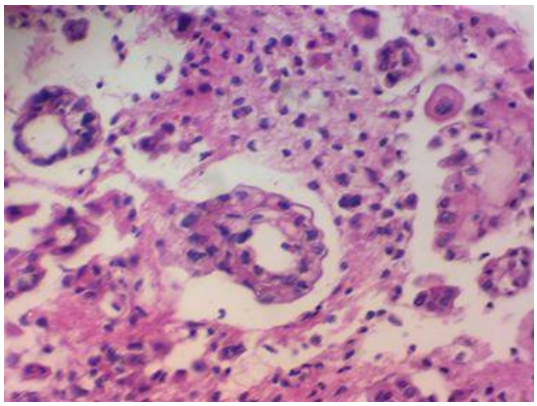
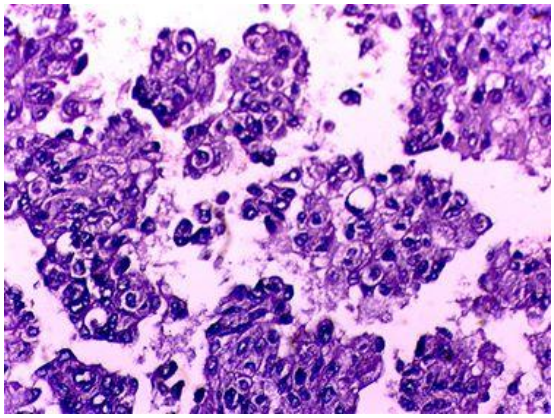


Fig 1



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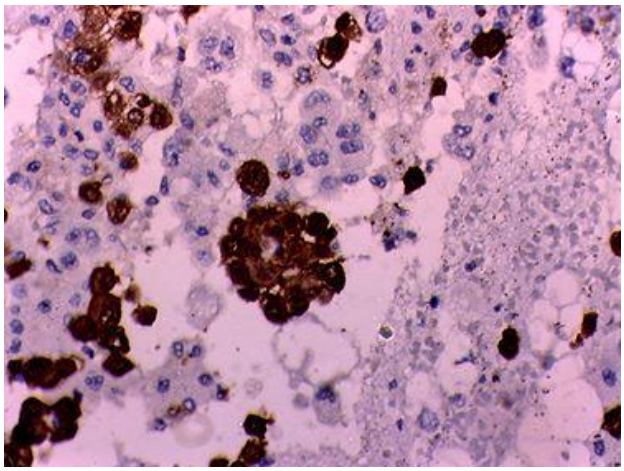

Fig 3

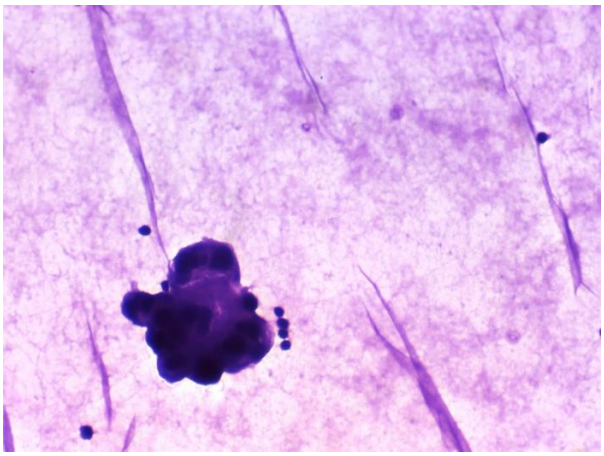

Fig 4

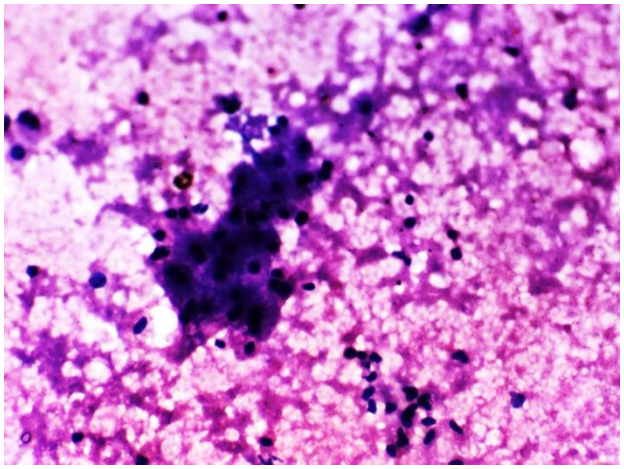

Fig 5

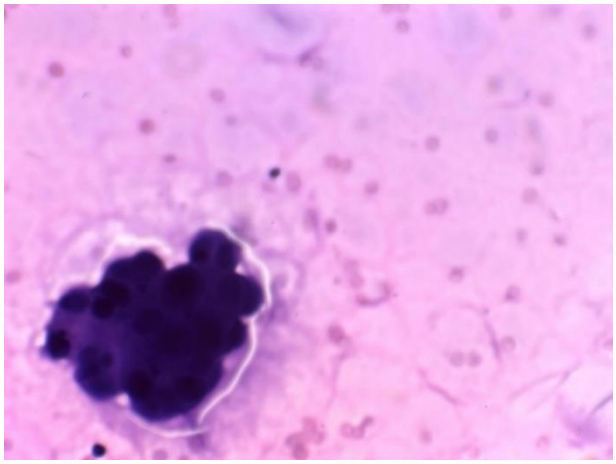

Fig 6

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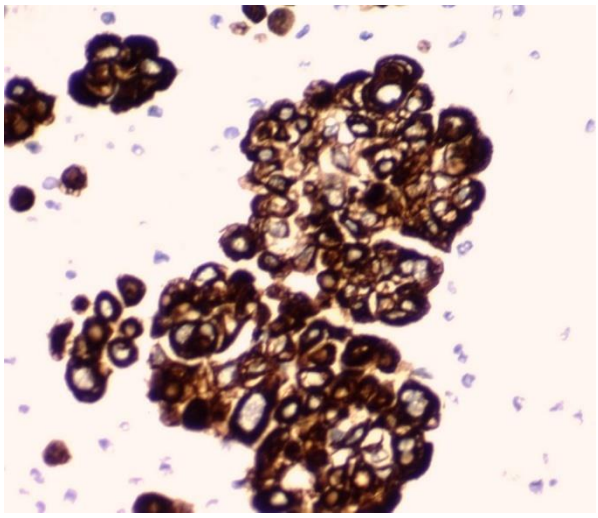

Fig 7

Fig 1: Cell block from reactive mesothelial cell hyperplasia (H&Ex100).
Fig 2: Cell block from malignant mesothelioma epithelioid type (H&Ex400).
Fig 3: Cell block showing nuclear staining of BAP1 in reactive mesothelial cells (immuneperoxidase x400).
Fig 4: Cytological smear of MM with loss of BAP1 nuclear staining (immuneperoxidase x400).
Fig 5: Cell block of reactive mesothelial hyperplasia showing positive PCD4 immunestaining (immune peroxidase x100).
Fig 6: Cytological smear Low expression of PCD4 immunostaining in malignant mesothelioma (immune peroxidase x400).
Fig 7: Cytological smear showing strong membranous EMA staining in malignant mesothelioma (immune peroxidase x400).

DISCUSSION

We found that BAP1 loss was detected in malignant mesothelioma confirmed by cytology in 19 out of 20 patients with sensitivity of 95%, specificity 92.5%. PCD4 was positive in 39 out of 40 patients of RMH confirmed by cytology and can diagnose reactive mesothelioma with a sensitivity of 85 % and specificity 97.5%. EMA was positive 95% in MM confirmed by cytology and can diagnose malignant mesothelioma with a sensitivity of 95%, specificity 97.5%. There is almost perfect agreement between both BAP1 loss and positive EMA in detection of mesothelioma by each of cytology and cell block.

The cytomorphological features found in MPM , reactive mesothelial cells hyperplasia and metastatic carcinoma usually overlap, so guidelines for malignant mesothelioma International by Academy of Cytology and the Papanicolaou Society of Cytopathology [19] including cytomorphological criteria as: (1) highly cellular sample; (2) Larger mesothelial cells (3) molules with a scalloped surface; (4) acidophilic extracellular matrix cores; (5) large nucleoli; (6) cell membrane protrusion ; (7) multinucleated giant cells; and (8) vacuoles overlapping nuclei .

BAP1 mutations were detected in some melanocytic tumors, breast, lung, and renal cell carcinomas. [20]. Reported cases of mesothelioma (48.8%) showed a loss of nuclear BAP1 expression. In contrast, all RMC showed nuclear BAP1 expression. [21].

In our current study, BAP1 loss was positive in 19 out of 20 patients confirmed by cytology in cases of MM. Near results to our results was given by studies by Cigognetti et al. [20]. Hwang et al. [22] and Önder et al. [24] also reported similar findings.

Results were consistent with studies by Hwang et al. [22] and Sheffield et al. [23] who reported that BAP1 loss was more in epithelioid variant of mesothelioma.

This study showed BAP1 IHC in cytological samples had a sensitivity of 95 %, specificity 92.5 %, PPV 86.4%, NPV 97.4% and accuracy 93.3%. With cell block had a sensitivity of 95%, specificity 94.9%, PPV 90.9 %, NPV 97.4% and accuracy 95 % respectively. These results were close to that obtained in a study by Önder et al. [24].

PDCD4 can inhibit the translation of several oncoproteins by suppression eIF4A and eIF4G factors. In addition, it can affect gene transcription by interacting with the JNK/c-Jun/AP-1 pathway down-regulation and this is correlated with tumor progression in different tumors of thyroid, colon, esophagus and ovary [25].

Our study showed PCD4 expression of 20 % of cases of mesothelioma and in 19 out of 20 reactive mesothelial cells confirmed by cytology and 19 out of 19 confirmed by cell block.

This study showed that PCD4 IHC results in cytological samples with sensitivity, specificity, PPV NPV and accuracy tests were 85 %, 97.5 %, 92.9%, 94.4% and 93.3% respectively. Meanwhile, with cell block the sensitivity was 85.7%, specificity 100 %, PPV 100 %, NPV 92.9 % and accuracy 95 %.

The current study showed PCD4 is higher in reactive than mesothelioma, this is consistent with another similar study by Nicolè et al. [16] who reported decreased PDCD4 immuno-staining in MPM compared to non-neoplastic samples.

PDCD4 has the ability of malignant behavior inhibition by the enhancement of both apoptosis and chemo-sensitivity, so can be used as a potential regulatory marker for novel therapeutic strategies [26].

Epithelial membrane antigen (EMA) immunostaining was negative in reactive mesothelial cells and positive in 92.3% malignant cells. The current study showed EMA expression in cytological samples had a sensitivity of 95 %, specificity 97.5 %, PPV 95%, NPV 97.5% and accuracy 96.7%. With cell block had a sensitivity of 95.2%, specificity 100 %, PPV 100 %, NPV 97.5 % and accuracy 96.3 %.

On routine cytological examination 11 cases (46%) of pleural fluid effusion were reported positive for malignancy while with cell block increased to 13 cases (54.1%). An additional increase of two cases of malignancy (8.3%). Near similar findings of Bhanvadia et al. [27] who observed an additional increase of 14% by cell block over routine cytological examination. Similarly, Udasimath et al.[28] who studied cell block sections of pleural fluid and were able to diagnose six additional cases thus increasing diagnostic yield for malignancy by 14%.Similar findings by Arslan et al. [29] who found that staining with EMA was observed in 45 of 67 (68.7%) of malignant mesotheliomas.

The results were consistent with Al Mehy et al. [11] who reported that EMA staining results was 5.9% of RMH and 92.3% of MM cases. In a study of Hasteh et al. [30] and Minato et al. [31] it was found that 9% (6 of 64) of benign cases showed positivity for EMA. All MM showed EMA positivity.

Reported EMA as a positive marker for MM cases in another study by Chang et al. [32].

The results were similar to another study by Gouda et al. [33] who reported that EMA sensitivity was 97% and specificity was 90%. 10% of RMH cases showed positive staining, however, nearly all cases showed positive staining of it.

Conversely, Salman et al., [34] reported in their study a case of primary MM of the peritoneum that showed positivity for desmin and negative expression of EMA.

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Mesothelioma incidence was increased in Egypt last years, there is an urgent need for early diagnosis of malignant mesothelioma which mimics metastatic carcinoma or reactive mesothelial cells hyperplasia in many cases. Immunocytology of pleural cytology or cell block, which is simple and cheap method in our developing countries, can help in such cases and this is the main reason for conducting this study.

Limitations of the study

First, the small number of cases. Second, we didn't fulfill the cytomorphological criteria for diagnosis for every case. Third we did not study the correlation between the expression of the markers and the prognosis of patients with MM.

Conclusions

Cell block method increases the sensitivity of diagnosis in cases that were recorded as reactive mesothelial hyperplasia by conventional cytological smears. BAP1 loss, negative PCD4 and positive EMA immunostaining can differentiate and diagnose MM from RMH with improved diagnostic accuracy.

Conflict of interest: None

Financial disclosure: None

REFERENCES

1- Ibrahim A., Khalid H., Michail N., Baraha H., Kamel H.: Cancer incidence in Egypt: Results of the National Population- Based Cancer Registry Program. J Can Epidem 2020; 34:205–16.

2-Hwang H, Tse C., Rodriguez S., Gown A., Churg A.: p16 FISH deletion in surface epithelial mesothelial proliferations is predictive of underlying invasive mesothelioma. Am J Surg Pathol 2014; 38:681–688.

3-Savic S., Franco N., Grilli B.. Fluorescence in situ hybridization in the definitive diagnosis of malignant mesothelioma in effusion cytology. Chest 2010; 138: 137–44.

4-Bruno R., Alì G, Fontanini G: Molecular markers and new diagnostic methods to differentiate malignant from benign mesothelial pleural proliferations: A literature review. J Thorac Dis. Jan. 2018, 10 (Suppl 2): S342-S352.

5-Sekido Y.: Molecular pathogenesis of malignant mesothelioma. Carcinogenesis. 2013; 34:1413–19.

6-Kindler HL. Peritoneal mesothelioma: The site of origin matters. Am Soc Clin Oncol Educ Book 2013;182–188.

7-Dey A., Seshasayee D., Noubade R.. Loss of the tumor suppressor BAP1 causes myeloid transformation. Science 2012; 337:1541–1546.

8-Asangani IA., Rasheed SA., Nikolova DA., Leupold JH., Colburn NH., Post S., Allgayer H. MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. Oncogene. 2008; 27:2128–36.

9-Cappellesso R., Tinazzi A., Giurici T., Simonato F., Guzzardo V., Ventura L. Programmed cell death 4 and microRNA 21 inverse expression is maintained in cells and exosomes from ovarian serous carcinoma effusions. Cancer Cytopathol. 2014; 122:685–93.

10-Cappellesso R., Nicolè L., Caroccia B., Guzzardo V., Ventura L., Fassan M., Fassina A. Young investigator challenge: MicroRNA-21/MicroRNA-126 profiling as a novel tool for the diagnosis of malignant mesothelioma in pleural effusion cytology. Cancer Cytopathol. 2016; 124:28–37.

11-Al Mehy GF., Abd EL Fattah GA., Gouda MH., El Sawy R.M. and Amer M.M.: Combined serum and immunohistochemical differentiation between reactive , and malignant mesothelial proliferations, 2015. Egypt J. Chest Dis. Tuberc.

12-Weinberger SE, Cockrill BA and Jess Mandel: pleural diseases in principles of pulmonary medicine, 6th edition, 2014 chapter 15.p.202-209.

13-Sahn SA. and Heffner JE: Pleural fluid analysis in Textbook of pleural diseases 2nd Edition, Light RW, Lee YGC (Eds), Hodder, Arnold, London, part two, 2008, chapter 17, p.220-222.

14-Mootha VK., Agarwal R., Singh N. Medical thoracoscopy for undiagnosed Pleural Effusions: experience from a tertiary care hospital in NorthIndia, Indian J. Chest Dis. Allied ,2011 Sci.;53:21-24.

15-Andrici J., Sheen A., Sioson I., Wardell K2, Clarkson C., Watson N. Loss of expression of BAP 1 is a useful adjunct, which strongly supports the diagnosis of mesothelioma in effusion cytology. Modern Pathology, 28: 1360-1368, 2015.

16-Nicolè L., Cappellesso R., Sanavia T., Guzzardo V. and Fassina A.: MiR-21 over-expression and Programmed Cell Death 4 down-regulation features malignant pleural mesothelioma. Oncotarget, 2018, Vol. 9, (No. 25), pp: 17300-17308.

17-Hasteh FL., Lin GY., Weidner N. and Michael CW: The use of immunohistochemistry to distinguish reactive mesothelial cells from malignant mesothelioma in cytologic effusions. Cancer Cytopathol., 25; 118 (2): 90-6, 2010

18-Shivakumarswamy U., Arakeri SU., Karigowdar MH., Yelikar BR.: Diagnostic utility of the cell block method versus the conventional smear study in pleural fluid cytology.2012, J Cytol 29:11–15.

19-Hjerpe A., Ascoli V., Bedrossian C. Guidelines for the cytopathologic diagnosis of epithelioid and mixed‐type malignant mesothelioma. Complementary statement from

the International Mesothelioma Interest Group, also endorsed by the International Academy of Cytology and the Papanicolaou Society of Cytopathology. Acta Cytol 2015; 59: 2–16.

20. Cigognetti M., Lonardi S., Fisogni S. BAP1(BRCA1-associated protein 1) is a highly specific marker for differentiating mesothelioma from reactive mesothelial proliferations. Mod Pathol 2015; 28:1043–1057.

21-Matsumoto S., Hamasaki M., Kinoshita Y. Morphological difference between pleural mesothelioma cells in effusion smears with either BAP1 loss or 9p21 homozygous deletion and reactive mesothelial cells without the gene alterations 2019. Pathology International. 2019; 69:637–645.

22-Hwang HC., Sheffield BS., Rodriguez S. Utility of BAP1 Immunohistochemistry and p16 (CDKN2A) FISH in the diagnosis of malignant mesothelioma in effusion cytology specimens. Am J Surg Pathol. 2016;40(1):120‐126.

23-Sheffield BS., Hwang HC., Lee AF. BAP1immunohistochemistry and p16 FISH to separate benign from malignant mesothelial proliferations. Am J Surg Pathol. 2015;39(7):977‐982.

24-Önder S., Özogul E., Koksal D., Ulasli S., Sevinc and Firat P.: Diagnostic value of BRCA1associated protein1, glucose transporter1 and desmin expression in the discrimination between reactive mesothelial proliferation and malignant mesothelioma in tissues and effusions. Cytopathology. 2019;3 0:592‐600.

25-Li JZ., Gao W., Ho WK., Lei WB., Wei WI., Chan JY., Wong TS. The clinical association of programmed cell death protein 4 (PDCD4) with solid tumors and its prognostic significance: a meta-analysis. Chin J Cancer. 2016; 35:95.

26-Pratheeshkumar P., Son YO., Divya SP., Wang L., Zhang Z., Shi X: ncogenic transformation of human lung bronchial epithelial cells induced by arsenic involves ROS-dependent activation of STAT3-miR-21-PDCD4 mechanism. Sci Rep. 2016; 6:37227.

27-Bhanvadia VM., Santwani PM., Vachhani JH. Analysis of Diagnostic value of cytological smear method versus cell block method in body fluid cytology: study of 150 cases. Ethiop J Health Sci. 2014;24(2):125-39.

28-Udasimath S., Arakeri SU., Karigowdar MH.: Diagnostic utility of the cell block method versus the conventional smear study in pleural fluid. J Cytol. 2012; 29:1115.

29-Arslan S., Bakır K., Elbeyli L.: Epithelial membrane antigen in differential diagnosis of malignant mesothelioma, metastatic adenocarcinoma, and reactive mesothelial hyperplasia Turk Gogus Kalp Dama 2016;24(1):108-112.

30-Hasteh F1., Lin G.Y., Weidner N. And Michaelc.W.: The use of immunohistochemistry to distinguish reactive mesothelial cells from malignant mesothelioma in cytologic effusions. Cancer Cytopathol. 2010; 25; 118(2): 90-6.

31-Minato H1., Kurose N., Fukushima M., Nojimat., Usuda K., Sagawa M. Comparative immunohistochemical analysis of IMP3, GLUT1, EMA ,CD146, and desmin for distinguishing malignant mesothelioma from reactive mesothelial cells. Am. J. Clin .Patho, 2014Jan., 141 (1): 85-93.

32-Chang S1., Oh M.H., Ji S.Y., Han J., Kim T.J., Eom M. Practical utility of insulin-like growth factor II mRNA-binding protein 3, glucose transporter 1, and epithelial membrane antigen for distinguishing malignant mesotheliomas from benign mesothelial proliferations . Pathol. Int., 2014; 64 (12): 607-12.

33-Gouda M, Elmahdy M, and Eloseily G Immunohistochemical Differentiation between Reactive and Malignant Mesothelial Proliferations in Pleural Effusion. Med. J. Cairo Univ., 2019;87, 7: 4345-4353.

34-Salman W.D., Eyden B., Shelton D., Howata., Al-Dawoud A. and Twaij Z.: An EMA negative, desmin positive malignant mesothelioma: limitations of immunohistochemistry? J. Clin. Pathol. 2009;62 (7): 651-2.

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