Original Article

Diagnostic value of E-cadherin and fibronectin in differentiation between reactive mesothelial and adenocarcinoma cells in serous effusions

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Abstract

Background: One of the problems in studying serous effusion cytological samples is differentiation of reactive mesothelial cells from metastatic adenocarcinoma cells.

Materials and Methods: In this study, the immunohistochemical diagnostic value of E-cadherin and fibronectin markers for differentiation of these 2 groups of cells was studied. 50 cell block samples prepared from serous effusions were examined. Based on clinical and histological studies, 25 cases had primary carcinoma, and the other 25 were proved to be benign effusion cases. All the cases were studied for E-cadherin and fibronectin immunostaining using an envision technique. Statistical analyzes were performed employing Chi-square and exact Fisher tests, using SPSS software (version 16).

Results: 24 of the 25 benign cases were stained with fibronectin and 2 with E-cadherin, whereas from among the 25 metastatic cases, 2 reacted to fibronectin and 22 to E-cadherin. Considering the staining of the 2 markers under conditions that the cells were stained with fibronectin but not with E-cadherin, positive predictive value (PPV) and negative predictive value (NPV) to identify reactive mesothelial cells were 100% and 92.5% while under conditions that had not been stained with fibronectin but with E-cadherin, PPV and NPV to detect adenocarcinoma cells were 95.2% and 82.1%, respectively.

Conclusion: Employing this short panel can be helpful for better differentiation of adenocarcinoma and reactive mesothelial cells in serous fluids.

Key Words: Adenocarcinoma, E-cadherin, fibronectin, immunohistochemistry, reactive mesothelial cell, serous effusion

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INTRODUCTION

Cytologic examination of the serous fluid is very important because the specimens represent a significant percentage of non-gynecologic samples, and this cytologic examination may be the first, best or only chance for making the diagnosis of an underlying malignancy.[1] The major purpose of cytologic examination of serous effusions is to determine whether malignant cells are present. This is an extremely

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important task since in most cases the presence of malignant cells in effusions indicates an advanced or terminal stage of malignancy, and it is associated with poor survival.[2] Whenever the serous membranes are irritated in a process of inflammation or longstanding effusion, mesothelial cells proliferate, shed in the fluid, and show morphological changes in nucleus and cytoplasm including enlargement of the nucleus binucleation or multinucleation and mitotic figures. In some cases, morphological differentiation of reactive mesothelial cells from adenocarcinoma in serous effusions is extremely difficult.[3] Therefore, adoption of complementary methods will increase diagnostic accuracy.[4] Nowadays, immunocytochemistry (ICC) is one of the suggested methods, which helps distinguishing between reactive mesothelial and adenocarcinoma cells.[5,6]

Employing the immunocytochemical method to help differentiation of the 2 groups of cells has been investigated in many studies, in some of which the markers have been found to be helpful.[6-17] In these studies, the markers have been used separately or in multiple panels. The differentiative significance of some of which are controversial.[8,9,15,18,19]

The aim of this study was to evaluate the effectiveness of 2 combined markers for fibronectin and E-Cadherin for discriminating between reactive mesothelial cells and adenocarcinoma cells obtained from serousal cavity fluids.

MATERIALS AND METHODS

Tissue samples

Paraffin-embedded cell blocks and H&E-stained slides of peritoneal and pleural fluid were retrieved from cytology archive of Alzahra Hospital, Medical University of Isfahan, between 2009 and 2011. From among 1450 slides which were screened to ascertain their appropriate diagnoses. Among of them, 50 paraffin-embedded cell blocks, 25 cases for each reactive, and adenocarcinoma groups were selected. The cases of reactive mesothelial cells were confirmed with review of the previous and/or current medical records without any past history or clinical or imaging documents in favor of malignancy. Adenocarcinomacases had confirmatory biopsy specimens. Only cases with cellular cell blocks were selected for immunocytochemical (ICC) staining.

Immunocytochemistry

For immunocytochemistry (ICC) staining with fibronectin and E-cadherin markers, monoclonal antibody avidin-biotin method was performed. At first step, 3 μm thin sections were obtained from selected blocks, and then the specimens underwent de-paraffinization and hydration. Then, antigen retrieval was done with citrate buffer 1% (PH = 6) in microwave for 20 minutes. Slides were incubated with fibronectin monoclonal antibody, clone 568 with 1:200 dilution (Navacastra Co., U.K.) E-cadherin monoclonal antibody, clone M3612 with 1:400 dilution (Dako Co., Denmark) at room temperature. All cases were blindly examined by 2 pathologists. According to previous studies, membrane staining for E-cadherin marker and membrane and cytoplasm reaction for fibronectin was considered as positive.[8,10,12]

Immunoreactivity determination by pathologist

All cases were blindly examined by 2 pathologists. The immunoreactivity of cells was evaluated with high power field (×400) Zeiss microscope, in 0.46 millimeters dimension.[20] The slides were counterstained with hematoxylin to allow evaluation of cells' morphology and assessment of the localization of staining on

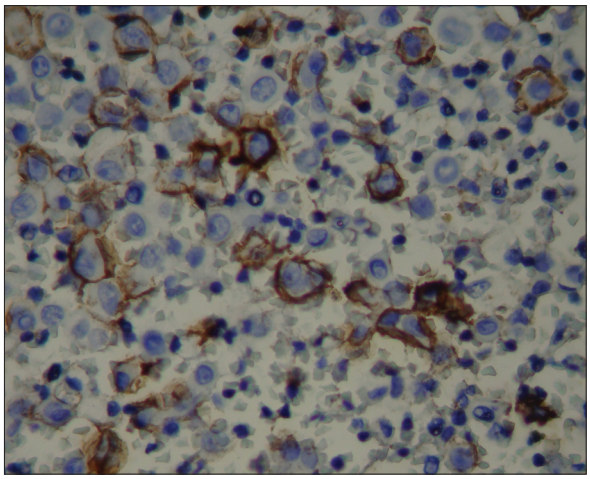

Figure 1: Membranous pattern of staining of adenocarcinoma cells with E-cadherin

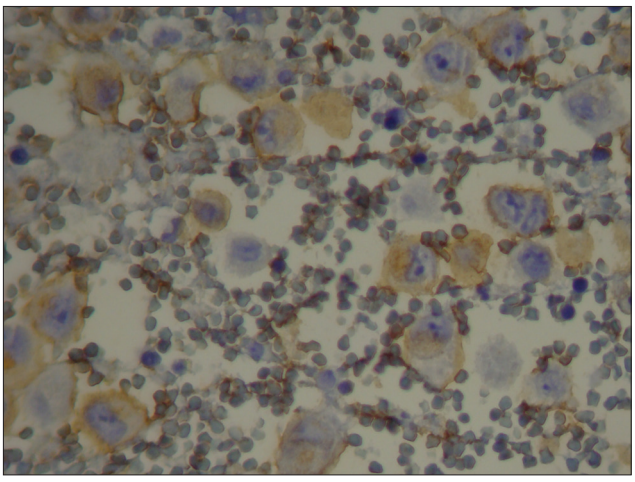

Figure 2: Membranous - cytoplasmic pattern of staining of reactive mesothelial cells with fibronectin

routine light microscopy. On immunohistochemical stains for E-cadherin, the colored (brown) reaction product at the antigen site was in the cell membrane and membrane - cytoplasm for Fibronectin[5,7,9] [Figures 1 and 2]. Immunocytochemical reactivities were evaluated by calculating the proportion of positively-stained cells in at least 10 visual fields, and definite staining of moderate intensity in more than 10% of cells were considered positive.

Data analysis

Statistical analyzes were performed employing Chi-square and exact Fisher tests, using SPSS software (version 16).

RESULTS

The results of the study are summarized and presented in Table 1. In the reactive mesothelial cells group, 24 out of 25 cases reacted positively to fibronectin, whereas only 2 out of 25 adenocarcinoma cases reacted positively to this marker. As for the E-cadherin marker, 2 out of 25 of reactive cases largely reacted focally and the 22 adenocarcinoma cases reacted. The 3 cases, which did not react, were poorly-differentiated according to their later biopsy specimen. The differences between immunostaining results of E-cadherin and fibronectin in malignant and benign cells were statistically significant ($P < 001$ for E-cadherin and $P < 001$ for fibronectin).

Table 1: Results of IHC for E-cadherin and fibronectin antibodies

	Reactive		Malignant	
	Peritoneal (%)	Pleural (%)	Peritoneal (%)	Pleural (%)
E-Cad (-)	13 (52)	10 (40)	2 (8)	1 (4)
E-Cad (+)	2 (8)	0	15 (60)	7 (28)
Fib (-)	1 (4)	0	15 (60)	8 (32)
Fib (+)	14 (56)	10 (40)	2 (8)	0
E-cad (+)/ Fib(-)	1 (4)	0	13 (52)	7 (28)
E-cad (-)/ Fib(+)	13 (52)	10 (40)	0	0
Total	15	10	17	8
	25		25	

(-) = Negative, (+) = positive *P* value = < 0.001

Table 2: Specificity, sensitivity, predictive values, and efficacy of immunostaining in serous fluid

	Specificity (%)	Sensitivity (%)	PPV (%)	NPV (%)	Efficacy (%)
E-Cad (for ACA)	92	88	91.6	88.4	90%
Fib (for RMC)	92	96	92	95	94%
E-Cad(+)/Fib(-) [for ACA]	96	80	95.2	82.7	88%
E-Cad(-)/Fib(+) [for RMC]	100	92	100	92.5	96

PPV = Positive predictive value; NPV = Negative predictive value; (-) = Negative; (+) = Positive; ACA = Adenocarcinoma; RMC = Reactive mesothelial cells

Table 2 illustrates the sensitivity, specificity, efficacy, and predictive values of the 2 markers separately. Fibronectin as a mesothelial marker has shown a sensitivity of 96% and specificity of 92%, and E-cadherin has had a sensitivity of 88% and specificity of 92% for metastatic adenocarcinoma.

In cases where immunoreactivity was positive for E-cadherin and negative for fibronectin, the specificity for adenocarcinoma diagnosis was 96% whose positive and negative predictive values were 95.2% and 82.1%, respectively; whereas, in negative E-cadherin and positive fibronectin cases, we had 100% specificity for adenocarcinoma diagnosis with positive and negative predictive values of 100% and 92.5%.

DISCUSSION

Based on morphologic features alone, the cytologic differentiation of reactive mesothelial cells from adenocarcinoma can be difficult. Because of a number of reasons, both artifactual and attributable to the nature of lesions, there could be a significant overlap between benign and malignant conditions. Various cytologic features are characteristic of, but not specific for, mesothelial cells. For example, intercellular spaces (windows), commonly seen in cellular aggregates of mesothelial cells, also can be identified in 13% of cases of metastatic adenocarcinoma.[13] Therefore, ancillary studies often are performed to assist in the differential diagnosis. Many studies have been conducted for differentiation between the 2 groups, and various markers have been suggested;[6-17] however, cytopathologists still encounter difficulties in effusion cytologic diagnosis.[15,20]

In the present study, we have used E-cadherin and fibronectin as a short panel. In a number of studies, investigators have examined the E-cadherin marker as a cell adhesion molecule, which exists in epithelial and not in mesotelial cells,[9,10,12,16] and also fibronectin, which is mesenchymal cells glycoprotein and exists in cytoplasm and in membrane of mesotelial cells.[7,8]

E-cadherin is a one member of a family of intracellular calcium-dependent adhesion molecules; a transmembrane protein-expressed inepithelial cells. Its extracellular amino terminal binds to the same structure of neighboring homotypic cells when calcium ion exists, mediating the epithelial cell-cell adhesion.[10,16,21] Many studies have shown alterations in E-cadherin expression in many types of cancer, specifically, lobular carcinoma of the breast and poorly-differentiated gastric carcinomas. Theoretically, only the exfoliated cells originating from epithelial tissues can express E-cadherin, therefore, detection

of E-cadherin expression is helpful for determining cells from epithelia. Because no epithelial cells were in benign effusions, the appearance of epithelial cells in effusions means a metastasis of carcinoma developed from epithelia. Our results showed that E-cadherin of the exfoliated cells were valuable for the diagnosis of malignant effusions, with a high sensitivity, specificity, positive predictive value, negative predictive value rate similar to other studies.[22,23]

In some previous studies, 85% of adenocarcinoma cells have reacted to E-cadherin marker, which almost complies with our findings (88%).[23,24] 3 adenocarcinoma samples, which reacted negatively to the E-cadherin, included 2 metastatic poorly-differentiated colonic adenocarcinoma toward peritoneum and 1 metastatic poorly-differentiated gastric adenocarcinoma to pleura.

Fibronectin was another marker that has been evaluated in conjunction with E-cadherin in our study. It is a recently-studied marker for mesothelial cells. It is a multifunctional adhesive protein whose primary role is to attach cells to variety matrices. It is a 450 KDa glycoprotein composed of 2 chains linked by a disulfide bond whose primary function is adhering cells to a matrix.[7,8] Several investigators have studied fibronectin expression in mesothelial cells. Earlier studies concerned the measurement of fibronectin in body fluids. Fibronectin is produced by fibroblasts, monocytes, and endothelial cells. It is thought to be directly involved in attachment, spreading, and migration of cells. It serves to enhance the sensitivity of certain cells to the proliferative effects of growth factors.[25]

Athanassiadou *et al.* were the first to show monoclonal fibronectin positivity in reactive mesothelial cells of serous effusions.[26]

Lee *et al.* used a panel consisting of cytokeratin, carcinoembryonic antigen, epithelial membrane antigen, and fibronectin to distinguish between carcinoma and reactive mesothelial cells in serous effusions.[7] In their study, they found fibronectin to be a highly specific marker for mesothelial cells. In the present study, fibronectin emerged as a 92% specific and 96% sensitive marker for mesothelial cells. This finding suggests that fibronectin positivity in a cell excludes the possibility of it being a carcinoma cell.

In a previous study, 100% of reactive mesothelial cells had reacted to fibronectin marker, in our study, however, one of the peritoneal samples had no reaction, which had simultaneously been negative for E-cadherin marker too.[8] The probable cause of this discrepancy might be due to technical error including prolonged fixation, antigen loss during antigen retrieval, and antibody demaskation.[15]

CONCLUSION

Regarding the results of the present study, using E-cadherin/ fibronectin short panel can be helpful for better differentiation of adenocarcinoma and reactive mesothelial cells in serous fluids, specifically when adenocarcinoms have insufficient differentiation.

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