Original Article

Proliferative index using Ki-67 index in reactive mesothelial versus metastatic adenocarcinoma cells in serous fluid

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Abstract

Background: The cytological diagnoses of serous effusions are usually made by routine cytomorphology with certainty. However, overlapping cases sometimes exist between reactive mesothelial and adenocarcinoma cells. We tried to evaluate the diagnostic utility of proliferative index using a Ki-67 monoclonal antibody in distinguishing between reactive mesothelial cells and adenocarcinoma in serous effusions.

Materials and Methods: Paraffin blocks and H and E stained slides of peritoneal and pleural fluid cell blocks were retrieved from cytology archive of Alzahra Hospital, Medical University of Isfahan, between 2006 and 2010, from among 1025 slides which were screened to ascertain their appropriate diagnoses. Among of these 80 paraffin-embedded cell blocks, 40 cases for each reactive and adenocarcinoma groups were selected. The proliferative index was calculated by using the Ki 67 monoclonal antibody against nuclear proteins.

Results: The mean ages of the patients in the reactive mesothelial and adenocarcinoma groups were 60.58 and 58.45 years, respectively. The gender distribution for the malignant group included 23 cases (%57.5) of females and 17 cases (42.5%) of males. This ratio for reactive group included 14 cases (35%) and 26 cases (65%). The mean of Ki-67 index in adenocarcinomatous cells was 17.15 (SD=15.11) and in reactive mesothelial cells was 3.58 (SD= 3.59) (P=0.001). We consider to using the proliferative marker of Ki-67 on benign and malignant lesions revealed 12% as cut off level. The means of Ki-67 index according to serousal spaces were included: Pleura: 10.56 (SD= 13.06) and peritoneum: 10.03 (SD= 12.78), (P=0.9).

Conclusion: Ki-67 index is useful immunostaining panel for differentiation of mesothelial and adenocarcinoma cells in malignancy like ovarian carcinoma that sometimes mimics mesothelial morphology.

Key words: Adenocarcinoma cell, Ki-67 antibody, mitosis, 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 nongynecologic 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 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]

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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. Occasional mitotic figures are often present and do not indicate malignancy, but the presence of numerous mitoses is suspicious, particularly in a highly cellular effusion. In some cases, morphological differentiation of reactive mesothelial cells from adenocarcinoma in serous effusions is extremely difficult.[3] So adoption of complementary methods will increase diagnostic accuracy.[4] Nowadays immunocytochemistry (ICC) is one of the suggested methods which helps distinguishing between mesothelial and adenocarcinoma.[5,6] It seems counting the cellular proliferation by monoclonal antibody against Ki-67 is a reliable method to evaluate fractions of rapidly growing cells in both reactive mesothelial and carcinoma cells.[7]

The expression of the human protein Ki-67 is associated with cell proliferation. The fact that Ki-67 protein is present during all the active phases of the cell cycle (G1, S, G2, and mitosis), and absent from the G0 phase, has made it an excellent marker for determining the growth fraction of a determined cell population (normal or tumoral).[8-11]

The aim of this study was to evaluate whether immunocytochemical expressions of proliferation markers for Ki-67 in reactive mesothelial cells and adenocarcinoma cells obtained from serousal fluids could be useful for their differential diagnosis.

MATERIAL AND METHODS

Tissue samples

Paraffin-embedded cell blocks and HandE-stained slides of peritoneal and pleural fluid were retrieved from cytology archive of Alzahra Hospital, Medical University of Isfahan, between 2006 and 2010, from among 1025 slides which were screened to ascertain their appropriate diagnoses. Among these 80 paraffin-embedded cell blocks, 40 cases for each reactive and adenocarcinoma groups were selected. The cases of reactive mesothelial 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 a Ki-67 monoclonal antibody, ht avidin-biotin method was performed. In the first step, 3 μm thin sections were obtained from selected blocks, and then the specimens underwent deparaffinization and hydration. Exposure of the antigen to citrate buffer 1% (PH=6) was done in microwave for 20 minu. Slides were incubated with the Ki-67 anti-human monoclonal antibody, clone MIB-1, code no: M7249 with 1/50 dilution (DAKO Co., Denmark) at room temperature. The staining intensity of cells was evaluated with a high power field (×400) Zeiss microscope, in 0.46 mm dimension.[12]

Ki-67 determination by pathologist

Immunoreactivity was evaluated as the percentage in all adequate specimens by counting all positive epithelial cells' nuclei over the total number of epithelial cells. Sections stained for Ki-67 were initially screened microscopically. The areas with the highest numbers of Ki-67-labeled nuclei were encircled, and the Ki-67 index was calculated by the percentage of positive cells. On immunohistochemical stains for Ki-67, the colored (brown) reaction product at the antigen site was in the cell nucleus, and the slide was counterstained with hematoxylin to allow evaluation of cells' morphology and assessment of the localization of staining on routine light microscopy. At least 8 fields were chosen within previously encircled "hotspots" to be evaluated at 20× to obtain the percentage of cells positive for Ki-67 (the Ki-67 index).[13]

Data analysis

For data analysis, the Mann-Whitney test was used. This test is an alternative for t-test that used to compare two independent groups of sampled data.

RESULTS

The mean ages of the patients in the reactive mesothelial and adenocarcinoma groups were 60.58 and 58.45 years, respectively. The gender distribution for malignant group included: 23 cases (%57.5) female and 17 cases (42.5%) male. This ratio for reactive group included: 14 cases (35%) and 26 cases (65%).

The mean of Ki-67 index in adenocarcinomatous cells was: 17.15 (SD=15.11) and in reactive mesothelial cells was: 3.58 (SD= 3.59) (P=0.001). The mean of Ki-67 index according to serousal spaces were included: Pleura: 10.56 (SD= 13.06) and peritoneum: 10.03 (SD= 12.78), (P=0.9).

The descriptive statistics of Ki-67 index according to origin of metastatic adenocarcinomatous are shown in Table 1. We considered to use the proliferative marker of Ki-67 on benign and malignant lesions, revealed 12% as cut off level.

Table 1: Comparison of mean and SD of Ki-67 index in metastatic adenocarcinomas by specific origin (*P* value=0.5)

Origin	Case number	Mean of Ki-67 index	SD
Unknown	14	9.86	7.94
Ovary	10	21.77	18.85
Lung	9	24.67	20.01
Breast	3	14.67	9.07
Urinary bladder	1	-	-
Colon	1	-	-
Pancreas	1	-	-
Cholangiocarcinoma	1	-	-

DISCUSSION

Although native serous cavity cells "mesothelial cells" do not proliferate in effusions after exfoliation, they may complete an already started mitotic division. The presence of mitotic figures in effusion cytology suggests a process that is capable of causing significant proliferative activity in response to whatever in causing the effusion. As with cytopathological evaluation in general, the presence of mitotic figures should not lead to a false interpretation of malignancy.[14,15] Recently, benign mesothelial proliferative conditions are increasingly reported, because benign mesothelial conditions are occasionally difficult to distinguish from malignant cells[16] due to their severe nuclear changes, including enlargement and irregularity of nuclei with coarse chromatin and conspicuous nucleoli and the presence of mitotic figures. Clinical data with respect to such diseases as anemia, cirrhosis, systemic lupus erythematosus, pulmonary infarction, renal failure, and AIDS can help interpretation of these conditions; however, in many cases, especially outpatients, clinical data is not easily available.[3]

Several methods are available for the evaluation of the degree of cellular proliferation. The older and still widely used method is mitotic count in a certain number (usually 10 to 50) of consecutive "high-power" fields (40× objective).[17] The mitotic phase constitutes only a small part of the cell proliferation cycle. Several factors may have contributed to the superiority of the Ki-67 index in predicting cellular proliferation in our study. Mitotic count has been shown to be influenced by multiple factors, including tissue fixation and thickness of tissue sections. It can be difficult to differentiate mitotic figures from apoptosis. Furthermore, immunohistochemical staining for Ki-67 identifies proliferating cells in the G1, S, G2, and mitosis phases of the cell cycle, whereas mitotic count identifies only a small proportion of the proliferating cells in the mitosis phase.[17,18] So in this study, we evaluated cellular proliferation using immunocytochemistry with the monoclonal antibody Ki-67 that reacted with a nuclear antigen present in all proliferating cells.[19-21]

The diagnostic and prognostic value of Ki-67 immunostaining of human tumors has been widely documented and accepted.[22,23] In review of English Medical literature investigated the role of proliferative markers in differentiation between malignant mesothelioma and mesothelial hyperplasia,[24,25] Sington *et al.*[24] demonstrated that Ki-67 can differentiate malignant from reactive proliferation of mesothelial cells; Schonherr *et al.*[25] in their study on pleural cytology specimens showed 100% specificity for Ki-67 to differentiate MM from MH taking a cut off level of 20% for proliferating cells. In a study in regard to using the proliferative marker of Ki-67 on benign and malignant lesions, Taheri *et al.* showed that by taking 9% as a cut off level for proliferating cells in regions with most proliferative activity, differentiation between MM and MH is possible with sensitivity and specificity of 88% and 94%, respectively. A 20% cut off level could diagnose all hyperplasias except for one case who was a young man with spontaneous pneumothorax; the Ki-67 was 25%. They believed that the increased proliferation index in this case was due to acute injury to the pleura,[26] but in most studies material were histologic sections instead of cytologic preparation and a few studies have investigated the role of proliferative markers in differentiation between reactive mesothelial cells and metastatic adenocarcinoma.

Immunocytochemical profiles for Ki-67 of normal and reactive mesothelial cells are unknown. In Terada's study, the proliferative index for normal mesothelium and reactive one were respectively: 1% and 20%.[27] Schönherr *et al.* demonstrated highly significant difference (P=0.001) between Ki-67 proliferation rates of mesothelial cells from patients with malignant tumors other than mesothelial origin (7.0% to 25.5%) and mesothelial cells of patients without any malignant disease (1.8% to 16.3%). Setting a threshold at 10% for identification of a malignant disease,[28] Kimura *et al.* showed significantly higher Ki-67 index in malignant cells as compared with reactive mesothelial cells (cut-off value = 30%).[29] In Hasteh *et al.* study for reactive cases, the proliferative index was high in 6 of 64 (9%) cases, moderate in 22 of 64 (34%) cases, and negative to low in 36 of 64 (56%) cases.[30]

It is possible that reactive mesothelial cells like any other reactive cells show higher proliferation index. The other possibility is contamination with many lymphocytes in the effusion specimens, which can cause difficulty in estimating the proliferative index by immunostain.

CONCLUSION

Ki-67 index is a useful immunostaining panel for

differentiation of mesothelial and adenocarcinoma cells in malignancy like ovarian carcinoma that sometimes mimics mesothelial morphology, but the proliferative index alone, however, cannot be used to discriminate benign lesions from malignant ones.[31] We recommend panel of immunocytochemical markers and study the larger sample for determining a Ki-67 index in gastrointestinal malignancy.

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