Proliferative Marker in Distinction between Benign and Malignant Mesothelial Proliferations

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ABSTRACT

Background: *Differentiation of benign from malignant mesothelial proliferations is a major problem in the pathology of the serosal membranes, particularly in small biopsy specimen.*

This study was conducted for the evaluation of proliferative marker for distinction between malignant mesothelioma (MM) and mesothelial hyperplasia (MH).

Materials and Methods: *Thirty six cases of malignant mesothelioma (MM) with the mean age of 62.94 years (range: 36-80 years, M/F: 3.58) and 22 cases of mesothelial hyperplasia (MH) were evaluated for proliferative status by immunohistochemical (IHC) method with monoclonal antibody, Ki-S5 (Ki-67); the labeling indices (LI) were evaluated.*

Results: *Average count revealed a significant increase in MM as compared with reactive MH (p value <0.0001). Considering a threshold of 9% for ki-67, a sensitivity of 88% and specificity of 94% were resulted.*

Conclusion: *Proliferative marker of Ki-67 can be useful in distinction between malignant mesothelioma and mesothelial hyperplasia (p-value <0.0001).* **(Tanaffos 2006 5(2); 9-12)**

Key words: *Mesothelial proliferation, Mesothelioma, Proliferative index, Ki-67, Ki-S5*

INTRODUCTION

During 30 years, many studies have been conducted to demonstrate definite patterns of malignant proliferation of mesothelial cells, and also to establish the criteria to differentiate between malignant mesothelioma (MM) and metastatic tumors of pleural surfaces. At present, it is easy to differentiate between the two processes by IHC (1-4). Differentiating benign mesothelial hyperplasia (MH) from MM has been of little concern with a few articles in the literatures in this regard, while in many instances this differentiation is much more important than finding the source of a malignant tumor involving the pleura. In a study by American-Canadian Panel of Mesothelioma on 217 cases, a consensus could not be reached regarding the benign or malignant course of proliferations in 22% of cases(5). To solve this problem, different methods including flowcytometry, DNA ploidy, telomerase activity, and IHC for P53 were suggested, but none showed reproducible results (6-14). Many proliferation markers have been used to diagnose benign and malignant lesions, as a prognostic marker

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and to find a correlation with survival rate in different tumors, but there are only a few markers used for mesothelial lesions (15-18). Ki-67 antigen is present in all states of cell cycle except for G_0 which can be used to assess the growth fraction (the number of cells in cell cycle) of normal, reactive and neoplastic tissues (19).

In this study, the growth rate of proliferating cells for differentiating MM from MH was evaluated with IHC method for proliferative marker of Ki-67 using Ki-S5 antibody.

MATERIALS AND METHODS

Thirty six malignant mesotheliomas and 22 cases of reactive mesothelial hyperplasia were selected from the files of pathology department of Masih Daneshvari Hospital (a referral respiratory center) between 1999 and 2004. The slides were reviewed by two pathologists and all cases with adequate material for IHC studies were selected. The mesotheliomas were diagnosed based on morphology, clinical, and IHC profiles.

Staining Methods:

From the paraffin blocks, 5 μm sections were prepared, dried overnight at 37˚C and then incubated at 60˚C for one hour. Internal peroxidase was inactivated by methanol-peroxidase before using first antibodies (Ki-S5); epitopes were recovered by boiling the slides in Tris-HCl, pH 9 in autoclave (120 atm pressure for 10 minutes). After cooling, monoclonal antibody Ki-S5 was applied to the slides. Immunoreaction was done using streptavidin complex method with peroxidase and biotinylated rabbit anti mouse antibody according to Dako kit (Table 1). For each step, one section of tonsil was used as positive and negative control. In the final step, all sections were counterstained and dehydrated.

Evaluation Method:

The immunohistochemical stains were evaluated using light microscopy at × 40 and × 400 magnifications. First, areas of most proliferative activity were selected at × 40 magnification, then 300

consecutive cells were counted using × 400 magnification. The positively stained cells, regardless of degrees of staining, were counted for proliferative marker and labeling indices (LIs) in every cases and were determined as percent of immunoreactive cells. The difference between LIs for each case was mostly lower than 5% (28 from 35 cases) between the pathologists. In these cases, the mean of two LIs was chosen for the next analysis. If the differences between the two pathologists were more than 5%, the evaluation would be repeated using two-headed microscope. Immune reaction was limited to mitotic figures and negative staining of cells in intermediate phase and/or when mitotic figures did not stain were all considered false negative.

The results were analyzed by Spearman correlation and Mann-Whitney U test using SPSS version 12.

**Table 1.** Primary antibody dilutions, commercial sources, and positive control

Antibody	Antigen	Clone	Dilution	Source	Positive	Retrieval
mAb Ki-S5	Ki- 67	Monoclonal	1/25	DAKO	Tonsil	Tris HCL pH:9 Autoclave

RESULTS

The study included 36 cases of malignant mesothelioma, including 26 men and 10 women (male/female ratio 3.85) with the mean age of 62.9 yrs. (range 36-80 years) and 22 cases of reactive mesothelial hyperplasia. Mean LIs for Ki-67 in mesothelioma and mesothelial hyperplasia was 24.6% (range 1-66%) and 6.33% (range 0-25%) respectively. (Table 2, Fig 1). There were statistically significant differences for LIs between mesothelioma and MH (p-value < 0.0001).

Table 2. Mean and range of proliferative marker for MH and MM (MH: mesothelial hyperplasia, MM: malignant mesothelioma).

Pathology	ki-67	
	Mean	Range
MH	6.23 (%)	0-25 (%)
MM	24.6 (%)	1-66 (%)
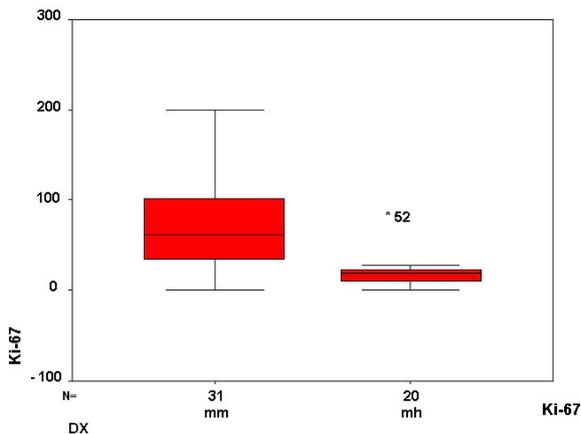
Figure 1. Box plots of ki-67 LI average and LI max in benign and malignant pleural disease.

By use of Roc curve and choosing cut off point of 9%, sensitivity and specificity were 88% and 94% for Ki-67 respectively.

DISCUSSION

The diagnostic distinction between reactive and neoplastic mesothelial proliferations is problematic; particularly in small biopsy specimen. Whilst true stromal invasion of mesothelial cells is recognized to be a useful indicator of malignancy. This can be very difficult to differentiate from reactive mesothelial cells entrapment in fibrous tissue in recurrent organizing effusions. To date, there is no immunohistochemistry marker which can confidently differentiate between reactive and neoplastic mesothelial cells.

Mitotic count, long used by surgical pathologists as a diagnostic and prognostic criterion in malignant tumors, may be subjective and furthermore, the mitotic phase constitutes only a small part of the cell proliferation cycle (20).

The diagnostic and prognostic value of Ki-67 immunostaining of human tumors has been widely documented and accepted (17, 21)

In review of English Medical literature, only two studies have investigated the role of proliferative markers in differentiation between malignant mesothelioma and mesothelial hyperplasia (22, 23). Sington et al. (22) demonstrated that MCM2 and Ki-67 can differentiate malignant from reactive proliferation of mesothelial cells. Schonherr et al. (23) 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 our study in regard to using the proliferative marker of Ki-67 on benign and malignant lesions, we showed that by taking 9% as 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%. A 20% cut off level, could diagnose all hyperplasias except for one case who was a young man with spontaneous pneumothorax; the ILs for Ki-67 was 25%. We believe the increased proliferation index in this case was due to acute injury to the pleura. Further studies with larger sample size are required to evaluate this assumption. The false negative results in our study could be due to the lack of expression of proliferative markers in the cell cycle where they are expected to be present.

CONCLUSION

Proliferative marker of Ki-67 can be useful in distinction between malignant mesothelioma and mesothelial hyperplasia (p value <0.0001).

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