Role of Ki-67 in Differentiating Mucinous Cystadenomas, Borderline Mucinous Tumors and Mucinous Cystadenocarcinoma

Authors
Dr U.Bharathi[1]*, Dr Lovely Jose[2], Dr C F Mathew[3]
[1]Assistant Professor, Department of Pathology, Sri Lakshmi Narayana Institute of Medical Sciences and Research, Puducherry, India
[2]Assistant Professor, Department of Pathology, Government Medical College Thrissur, Kerala, India
[3]Rtd.Professor of Pathology, Government Medical College, Thrissur, Kerala, India
*Corresponding Author
Dr U. Bharathi
Assistant Professor, Department of Pathology, Sri Lakshmi Narayana Institute of Medical Sciences and Research, Puducherry, India

Abstract
Introduction: *Borderline ovarian mucinous tumors are the grey zone areas in histopathology. Differentiating borderline mucinous tumors from malignant cystadenocarcinoms is a diagnostic challenge to a histopathologist. Mitotic rate is a traditional and practical method to determine proliferative activity but it's hampered by several factors. This study is designed to evaluate if Ki-67 labelling index is useful in determining the grade of primary mucinous ovarian neoplasms and its superiority over mitosis*
Materials and Methods: *A cross-sectional study was conducted in the department of pathology, in a tertiary health care centre over the period of 5 years. The study includes Cases of mucinous cystadenomas, borderline mucinous tumors and cystadenocarcinomas. Formalin fixed and paraffin embedded sections are reviewed, with representative sections being selected for IHC. Labeling index is determined with a light microscope.The number of mitosis is calculated in 10 high power fields (40X) in the most mitotically active area. Data was collected & entered in Microsoft office excel 2007 sheet. This was then analysed using software SPSS version 19.0.The statistical test used was Fischer's exact test.*
Results: *The mean Ki-67 labeling index in benign, borderline and malignant tumors were 1.74 %, 26.45 %, 62.71 %respectively. A Ki-67 labeling index of 42% may be used to discriminate between borderline and malignant tumors. Mitotic rate between benign, borderline and malignant tumors was statistically significant (p value <0.001). However 2 borderline tumors did not show mitosis.*
Conclusions: *There is a superiority in using ki67 labelling index over mitosis for differentiating benign borderline and malignant tumors. Ki 67 can be used as an additional diagnostic tool to differentiate mucinous tumors*
Keywords: *Ki 67 labelling index, Mitotic rate, Mucinous ovarian tumor.*

Introduction

Mucinous tumors account for 15% of ovarian tumors. Mucinous Cystadenomas account for 10% of benign ovarian tumors and mucinous carcinomas for 2.5-10% of ovarian cancers[1]. Changing biologic potential of tumor cells with different growth patterns produces a number of structural variants that are defined as benign, borderline and

malignant. One of the most controversial topics in gynecologic oncology is borderline tumors and is confusing to both clinicians and patients. These tumors in contrast to typical ovarian carcinomas, do not invade the ovarian stroma and therefore they are considered to be noninvasive. Most importantly, these noninvasive tumors have a markedly superior prognosis when compared with ovarian carcinomas. The category has led to a clinical dilemma because many patients with borderline tumors are young and wish to preserve their fertility. Borderline tumors are regarded as a subset of carcinomas and their treatment has often been more aggressive than is necessary considering their behavior, which is usually benign. A lack of unequivocal prognostic criteria in ovarian neoplasms provokes a continuous search for new criteria to evaluate the advancement of neoplastic process. Recent interest has been focused on IHC determination of cell proliferation associated antigens; one of the most widely used reagents in this field is antibody Ki67. Immunohistochemical detection of proliferating cells is a way to determine the proliferative potential of a tumor, and the expression of Ki-67 antigen has become a widely used marker. This antigen is expressed during all active phases of the cell cycle (G1, S, G2, and mitosis). The monoclonal Ki-67 antibody (MIB-1) reacts with the nuclear Ki-67 antigen expressed in cycling cells. High expression of Ki-67/MIB-1 has been found to indicate a poor prognosis in several cancers, including ovarian cancer. Mitotic rate is a traditional and practical method to determine proliferative activity, but its hampered by several factors. The prognosis is still poor in carcinomas despite improvements in diagnostic methods and chemotherapeutic agents over the past few years Our primary research focus is to develop a high-quality risk prediction model for ovarian cancer to guide personalized therapy[2,3]. Though histological criteria have been suggested for differentiating the various histological types of primary mucinous tumors, the practical application of these is often difficult. It would be useful if markers independent of histology could be developed to categorize these neoplasms.

Application of Ki-67 labelling index could be useful in this regard. Despite the difficulty in histopathological diagnosis of ovarian mucinous neoplasms it still remains the gold standard. This study is designed to evaluate if Ki-67 labelling index is useful in determining the grade of primary mucinous ovarian neoplasms using routine histology as gold standard. If outcome of the study points to labelling index as a useful marker this could be used in addition to histology to determine the grade of primary ovarian mucinous tumors and this could help in prognostication and management of these tumors.

Materials and Methods

Study Design: Cross-sectional study

Study Period: 1.2 2014 – 30.6.2015

Study Setting: Department of Pathology, Government medical college, Thrissur

Study Population: All mucinous carcinomas and borderline mucinous tumors received during the period of study are included. Since about 15 times number of mucinous cystadenomas was expected during the study period a sample of every 10th case of these tumors will be included so that the number will not be disproportionate to the other types of mucinous tumors.

Inclusion Criteria: Primary mucinous tumors received in the Pathology department of Govt Medical College Thrissur are to be included in the study. Mucinous tumors diagnosed as borderline cystadenomas and mucinous cystadenocarcinomas will be studied from material available in the department from the year 2010. Mucinous cystadenomas will be included, sampling every 10th case as they form a large number.

Exclusion Criteria: Ovarian tumors eg. Other tumor considered to be of epithelial origin like serous, endometrioid, clear cell and Brenner tumor will be excluded from the study.

Methodology

The study includes cases of primary mucinous ovarian tumors diagnosed in the Pathology department of Govt. Medical College Thrissur; a

tertiary care institution. Cases of mucinous cystadenomas, borderline mucinous cystadenomas and cystadenocarcinomas are to be included both retrospectively and prospectively. Formalin fixed and paraffin embedded sections are reviewed, with representative sections being selected for IHC. Briefly, 4 microns thick sections are deparaffinized and dehydrated. Antigen retrieval is performed by pressure cooking. The slides are then incubated with the MIB1 antibody dilution. Assessment of stained tumor cell nuclei are performed by the following method. All nuclei with detectable staining above the background level are scored as positive. Labelling index is determined with a light microscope; with high objective, counting a total of 1000 tumor cells within at least 5 different view fields; (having maximum proliferation of cells) and expressing the results as the percentage of positive cells by the formula

Labelling index= [no. of positive tumor cells/total no of cells counted]

The number of mitosis is calculated in 10 high power fields (40X) with the highest mitotic activity.

Data entry and analysis

Data was collected & entered in Microsoft office excel 2007 sheet. This was then analysed using software SPSS version 19.0. The statistical test used was Fischer's exact test. A

P value of <0.005 was considered statistically significant. The findings were presented in appropriate charts & tables

Observations and Results

Table.1 Mitotic rate of mucinous tumors

Mitosis	Frequency	Percent
No mitosis	17	53.1
0-4/10 HPF	8	25
>5/ 10 HPF	7	21.9

Table 2 Mitotic rate in benign, borderline and malignant tumors

Mitosis	Nil	1-4/10 hpf	>5/10hpf
Benign	15	0	0
Borderline	2	8	0
Malignant	0	0	7

Table 3 Ki 67 labelling index in mucinous tumors

Ki 67 labeling index	Frequency	Percent
0-5%	15	46.9
6-40%	10	31.2
>40%	7	21.9

Table 4 Ki- 67 labelling index in benign cystadenomas

Ki-67 Labeling Index	Frequency	Percentage
0%	3	9.4
0.1%	1	3.1
0.3%	1	3.1
0.5%	1	3.1
0.8%	1	3.1
0.9%	1	3.1
1.5%	1	3.1
2.5%	1	3.1
3%	2	6.2
4%	1	3.1
4.5%	1	3.1
5%	1	3.1

The mean Ki-67 expression in benign cystadenomas is 1.74%

Table 5 Ki-67 Labelling index in borderline tumors

Ki-67 Labeling Index	Frequency	Percentage
10%	1	3.1
12.5%	1	3.1
15%	1	3.1
20%	1	3.1
28%	1	3.1
35%	3	9.4
36%	1	3.1
38%	1	3.1

The mean Ki-67 labeling index in borderline tumors is 26.45%

Table 6 Ki-67 Labelling index in malignant tumors

KI-67 Labeling Index	Frequency	Percentage
46%	1	3.1
52%	1	3.1
60%	2	6.2
65%	1	3.1
75%	1	3.1
81%	1	3.1

The mean Ki-67 labeling index in mucinous cystadenocarcinoma is 62.71%

**Table 8** Ki-67 Labelling index in borderline and malignant tumors

Ki-67 labeling index	Sensitivity	Specificity
5.45	1.000	0.909
13.75	1.000	0.273
31.5	1.000	0.818
42.00	1.000	1.000
56.00	0.714	1.000
70.00	0.236	1.000
82.00	0.000	1.000

On analysing the specificity and sensitivity of Ki-67 labeling index of borderline and malignant mucinous tumors it is found that a labeling index of 42% may be used to discriminate the two

Ki-67 labelling index between borderline and malignant tumors

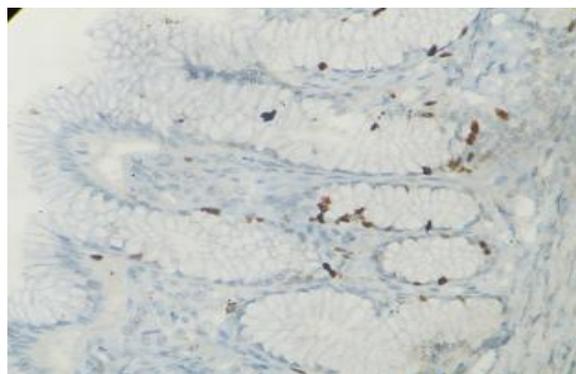Fisher's exact test value - 5.142×10^{-5}

There is a difference in Ki-67 labeling index between borderline and malignant mucinous tumors and this is statistically significant (p value <0.001)
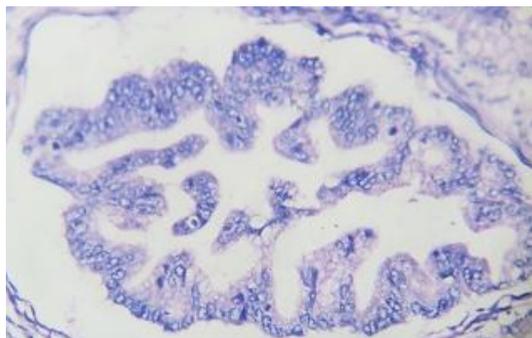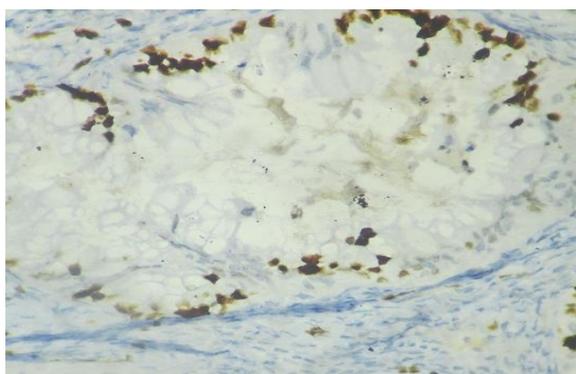

Figure 1: Mitotic activity in malignant cystadenocarcinomas 3 mitosis /HPF (40X)
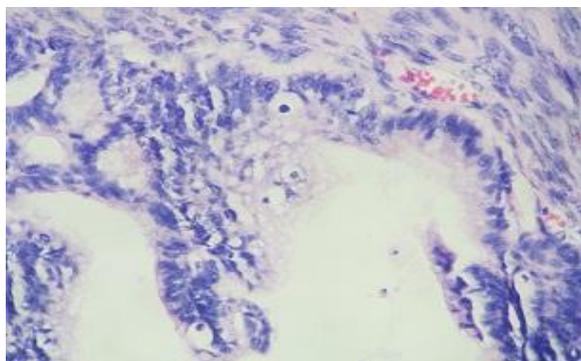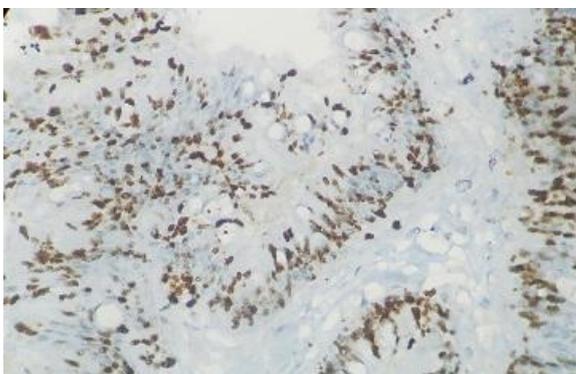

Figure 2: Mitotic activity in borderline cystadenomas 1 mitosis /hpf

Figure 3: Ki - 67 staining in benign mucinous cystadenoma. LI- 3% (40X)

Figure 4: Ki - 67 staining in borderline mucinous tumors LI- 36% (40 X)

Figure 5: Ki - 67 staining in mucinous cystadenocarcinoma. LI-81%(40 X)

Discussion

Morphological changes in the nuclei of neoplastic cells and their proliferative activity are a significant element of histopathological examination. In many cases, traditional techniques cannot define precisely the grading of malignancy, the best evidence of which is the borderline tumors. In a study by Guro Aune et al[4] there was a statistically significant difference in mitosis between benign, borderline and malignant tumors. In the present study, 53.1%

of tumors showed no mitosis. Among these, there were 15 benign and 2 borderline tumors. 8 borderline cases (25%) showed mitosis in the range of 1-4/10 HPF. All the 7 malignant tumors had mitosis of >5 /10 HPF. There is a statistically significant difference in mitosis between benign and borderline tumors (p value< 0.001). There is a difference in mitosis between borderline and malignant tumors also.The division of the various epithelial subtypes into benign, borderline, and malignant forms is based on the premise that tumors with architectural and cytologic features that are intermediate between those of clinically benign and malignant tumors of the same epithelial cell type have a significantly better prognosis[5]. Rice et al studied 80 patients with mucinous borderline ovarian tumors; none of the patients had greater than stage I disease and there was no recurrence in these group. The prognosis for these patients were excellent[6]. The standard therapy for older patients is abdominal hysterectomy, bilateral salpingooophorectomy, omentectomy and appendectomy. Many young patients who have not completed childbearing can be safely treated with unilateral salpingo-oophorectomy thereby preserving fertility potential. The proliferative fraction of ovarian carcinomas have been investigated immunohistochemically by means of antibodies that recognize the nuclear antigen Ki-67 expressed in proliferating cells. Ki-67 is expressed in all the phases of cell cycle. The Ki-67/MIB-1 expression is an established method for evaluation of proliferation in ovarian tumors. The main aim of our study is to evaluate whether the Ki-67 labelling index can be useful in differentiating between benign borderline and malignant tumors with routine histology as gold standard.

In the present study, the mean Ki-67 labelling index in benign tumors was 1.74% (0-5 %). The mean Ki-67 labelling index in borderline tumors was 26.45% (10-38%). The mean Ki-67 labelling index in malignant tumors was 62.71% (46-81%). Compared to benign tumors the mean Ki-67 in borderline tumors was significantly increased. There is a difference in Ki-67 expression between benign and borderline tumors and this is statistically significant (p value <0.001). There is also a difference in Ki-67 labelling index between borderline and malignant tumors and this is statistically significant (p value <0.001). The findings are almost similar to a study by Neserin Gursan et al[7] where mean Ki-67 labelling index in benign, borderline and malignant tumors are 14.9%, 22.8% and 42.8% respectively. In our present study, the Ki-67 labelling index was positively correlated with the mitotic count. This is similar to a study by Guro Aune et al. In his study, there was a statistically significant difference in the expression of Ki-67/MIB-1 between carcinomas, borderline tumors, and benign tumors and the mean labelling index in carcinomas, borderline and benign tumors were 36.7%, 20%, and 1.85% respectively.

In a study by Luminita et al higher Ki-67 labeling index were observed in malignancies (61.53%), most of them in higher stages; borderline cases (13.3%) and benign (9.09%)cases presented with low Ki-67 labeling index[8]. Min KW et al were analyzing Ki-67 expression and they reported that the expression of Ki-67 correlates with the type and grade of the tumor in ovarian cancer. The study concluded Ki-67 labeling index is low in borderline tumors and high in ovarian carcinomas[9]. In the present study, on analysing the sensitivity and specificity of Ki-67 labelling index between borderline and malignant tumors it is found that a value of 42% can be used as a cut off to discriminate the two.

Conclusion

The immunohistochemical study of the mucinous ovarian tumors indicated significant differences of the Ki-67 labelling index in relation to the grade of the tumor and this demonstrates that these markers can be used as an additional indicator in differentiating carcinomas and borderline mucinous ovarian tumors. The present study looked at the utility of mitosis versus Ki-67 labelling index as a possible indicator of grade of mucinous ovarian tumor. It was found from the statistical analysis that Ki- 67 has a possible slight advantage over mitosis.

During this study, it was also found that it is more difficult to observe mitosis than positive Ki-67 labelling index and it was also seen that in 2 of the borderline tumors included no mitosis could be observed. A Ki-67 labelling index of 42% may be used to discriminate between borderline and malignant tumors.

Acknowledgment

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