Ki-67 and p53 - Novel Prognostic Biomarkers in Surface Epithelial Ovarian Tumors

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ABSTRACT

Background: 70% of the women diagnosed with ovarian carcinoma have advanced disease because of ineffective screening tools. The proliferative activity of ovarian carcinoma is a valuable indicator of tumor aggressiveness. Ki-67 is an excellent marker to determine the growth fraction. p53 suppressor gene has an essential role in controlling cell cycle and initiating carcinogenesis.

Aim: To evaluate the proliferative activity using Ki-67 index and to correlate with histological subtype, grade, Federation of Gynecology and Obstetrics (FIGO) stage, and p53 overexpression by immunostaining in surface epithelial ovarian tumors.

Materials and Methods: This 3 year prospective study was undertaken in the Department of Pathology, Vinayaka Missions, Salem and approved by ethical committee. 47 cases of surface epithelial ovarian tumors were evaluated in this study. The proliferation expression related to Ki-67 antigen was evaluated by immunohistochemical monoclonal MIB-1 antibody. In each case, p53 immunostaining was done along with Ki-67 labeling index (Ki-67 LI) which was calculated as percentage of positively stained cells using high power objective of the microscope (x400).

Results: Among the 47 cases, the final histopathological diagnosis showed that 16 were benign, 10 were borderline, 16 were malignant and 5 were undifferentiated tumors. Immunostaining for Ki 67 and p53 was done for all selected 47 Surface Epithelial Ovarian Tumors (SEOT) cases. The difference in the mean Ki-67 labelling index between benign (2.9%), borderline (7.2%), and malignant epithelial tumors (37.5%) were statistically significant (p =<0.001). High labeling index of Ki-67 was noted with advanced FIGO stage. p53 was positive in 25 cases which was more frequent in serous adenocarcinomas than mucinous adenocarcinomas. p53 positivity was statistically significant between benign, borderline, and malignant epithelial tumors (p=<0.001).

Conclusion: In surface epithelial tumors of ovary histological grade and FIGO stage when combined with Ki-67 LI and p53 in histopathology report would help in diagnostic differentiation of subtypes, prognosis, deciding the need for adjuvant chemotherapy and in predicting the survival analysis. Both these biomarkers are very much useful to identify borderline tumors which are likely to behave in a malignant fashion.

Keywords: Surface epithelial ovarian tumors, Ki-67, p53.

INTRODUCTION:

Ovarian tumors accounts for 6% of all cancer in women. Ovarian tumors are the 5th leading cause of cancer death in women in India. Ovarian surface epithelial tumors represent the most common lethal gynecologic neoplasms for women of reproductive age and older and continue to present a challenge despite advances in our knowledge of the disease over the...
These tumors display biological behaviors that follow their histopathological grading of malignant, borderline or low malignant potential (LMP), or benign. Ovarian borderline (low malignant potential) tumors are a puzzling group of neoplasms that do not fall neatly into benign or malignant categories. Their behaviour is enigmatic, their pathogenesis unclear, and their clinical management controversial, especially for serous borderline tumors the most common type of ovarian borderline tumor. Multiple factors such as age, race, histologic type, grade, FIGO stage, residual disease, CA125 levels and performance status at the time of diagnosis influence survival of SEOC. These factors failed to explain the biological behaviour of ovarian cancer and hence, more objective ways to establish the prognosis are needed.

Recent years have witnessed significant development in the use of immunohistochemistry (IHC) in diagnostic ovarian pathology. Immunostaining is now employed not only for diagnosis but also for other parameters including prognosis, microscopic tumor staging, prediction of response to therapy, and for the selection of therapeutic agents. The proliferative activity of ovarian carcinoma is considered to be a valuable indicator of tumor aggressiveness. Determination of proliferative activity has been reported to be of a diagnostic with prognostic value and many methods are used to estimate the number of proliferating cells.

Ki-67 is an excellent marker to determine the growth fraction of a given cell population. The fraction of Ki-67 positive tumor cells is often correlated with the clinical course of cancers. Immunostaining with monoclonal antibody Ki-67, detecting all phases of the cell cycle except G0, allows assessment of the growth fraction of both normal and neoplastic cells.

p53 suppressor gene has an essential role in controlling cell cycle and initiating carcinogenesis. The loss of p53 function with resultant overexpression of p53 protein detected by immunohistochemical staining was described in approximately 50% of ovarian cancers. Unlike normal p53 protein, rapidly removed from the nucleus, mutant forms have a prolonged half-life, which favors intranuclear accumulation, becoming detectable immunohistochemically. To evaluate the proliferative activity using Ki-67 index and to correlate with histological subtype, grade, Federation of Gynecology and Obstetrics (FIGO) stage, and p53 overexpression by immunostaining in surface epithelial ovarian tumors.

**MATERIALS AND METHODS:**

This 3 year prospective study was undertaken in the Department of Pathology and approved by ethical committee. We received 239 ovarian lesions during this study period out of which 200 were ovarian neoplasms after exclusion of non-neoplastic lesions. Oopherectomy and panhysterectomy specimens with primary neoplastic lesions were included in this study. Among 200 ovarian tumors studied, 155 cases were surface epithelial ovarian tumors [SEOT] (77.5%), 15 cases were sex cord stromal tumors (7.5%), 22 cases were germ cell tumors (11%), 3 cases were metastatic tumors and 5 cases come under unclassified category.

After adequate fixation, representative bits were taken & processed. Paraffin sections & slide were then stained with hematoxylin and eosin. Out of 155 SEOT, Ki-67 and p53 immunohistochemical
marker study was performed on selected (47 cases) formalin-fixed, paraffin embedded tissue material.

Ki-67 immunohistochemical proliferative marker study using peroxidase-antiperoxidase technique was done. Positive Ki-67 staining was observed as brown granular nuclear staining. For Ki-67 scoring the most positive area of the tumor was selected avoiding foci of inflammation. The distribution of Ki-67 immunoreactivity in surface epithelial tumors of ovary were quantitatively assessed as -ve (less than 1% are negative cells) and +ve (equal or more than 1% are positive cells) and the positive cases were graded as + (1-30%), ++ (30-50 %) and +++ ( more than 50%) are positive cells. The number of positive nuclei is counted in 500 tumor cells in a high power field(x 400 magnification). The average of 3 counts over the same slide was taken and expressed as the percentage of Ki-67 positive cells in the tumor.

The distribution of p53 immunoreactivity in surface epithelial tumors of ovary were quantitatively assessed as –ve (less than 10% are negative cells) and +ve (equal or more than 10% are positive cells) and the positive cases were graded as + (10-30%); ++ ( 30-50%); and +++ ( more than 50%) are positive cells.

STATISTICAL ANALYSIS

The Chi-square test for contingency tables were performed to determine the significance of results. p value < 0.05 was considered significant.

RESULTS:

Surface Epithelial tumors was the commonest group encountered in the present study, 155 cases out of 200 were SEOT (77.5%), 31 cases showed papillary pattern. The distribution of SEOT tumors encountered in this study has been illustrated in the table 1. Benign serous cystadenoma was the most common SEOT (50/155).

Table 1. Distribution of Surface Epithelial tumors

<table>
<thead>
<tr>
<th>S.No</th>
<th>Types of cases</th>
<th>No. of cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Benign cyst (n=9)</td>
<td>Serous</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mucinous</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Benign cystadenoma (n=88)</td>
<td>Serous</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mucinous</td>
<td>38</td>
</tr>
<tr>
<td>3</td>
<td>Borderline cystadenoma (n=10)</td>
<td>Serous</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mucinous</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>Malignant cystadenocarcinoma (n=43)</td>
<td>Serous</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mucinous</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>Undifferentiated tumors (n=5)</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td>155</td>
<td>77.5</td>
</tr>
</tbody>
</table>

The present study consisted of 47 selected SEOT cases. Among the 47 cases, the final histopathological diagnosis showed that 16 were benign lesions, 10 were borderline epithelial neoplasms, 16 were malignant tumours and 5 were undifferentiated tumors. [Table 2 & Figure 1]

Table 2. Distribution of Histological subtypes of Surface epithelial ovarian tumors

<table>
<thead>
<tr>
<th></th>
<th>Serous</th>
<th>Mucinous</th>
<th>Total (n=47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>8</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Borderline</td>
<td>1</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Malignant</td>
<td>8</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Undifferentiated tumors</td>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Immunostaining for Ki-67 was done for all selected 47 SEOT cases. The difference in the mean Ki-67 labelling index between benign, borderline, and malignant epithelial tumors were statistically
significant (p =<0.001). [Table 3 & Figure 2] High labeling index of Ki-67 was noted with advanced FIGO stage. [Table 4]

**Table 3. Distribution of Ki-67 immunoreactivity in various histopathological subtypes of surface epithelial tumors of ovary**

<table>
<thead>
<tr>
<th>Histological types of ovarian tumors</th>
<th>Ki-67 index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Benign</td>
</tr>
<tr>
<td>Serous</td>
<td>2.8%</td>
</tr>
<tr>
<td>Mucinous</td>
<td>3%</td>
</tr>
<tr>
<td>Undifferentiated tumors</td>
<td>-</td>
</tr>
</tbody>
</table>

**DISCUSSION:**

Ovarian tumors pose a biggest diagnostic challenge in the field of gynaecological oncology and also major problem to the gynaecologist due to their higher complication rate. The 10 years survival rate is estimated to be 15%–30% at later stages of ovarian cancer compared to 90% survival rate for early stage. Over 70% of the women diagnosed with ovarian carcinoma have advanced disease at the time of diagnosis because of asymptomatic nature and ineffective screening tools. Inspite of the current considerable progress in the management of ovaian tumors, early diagnosis is unsuccessful; moreover the recurrence rate is increased due to resistant to chemotherapy and residual tumour. Important prognostic factors include stage of disease, age at diagnosis, histological type and grade, ploidy, and the amount of residual disease after primary surgery. In recent times, various molecular and proliferative markers have been reported as an important prognostic factor for women with the disease along with response to various therapeutic modalities. The growth fraction estimated by the proliferative markers reflects more closely the clinical stage of tumor than the degree of differentiation of tumors and useful to predict the clinical behavior and in identifying ovarian tumors especially borderline tumors which are likely to behave in a malignant fashion.

Similarly immunostaining for p53 was done for all selected 47 SEOT cases and showed positivity in 25 cases and the distribution is shown in Table 5 & Figure 2. p53 positivity was statistically significant between benign, borderline, and malignant epithelial tumors (p =<0.001).

**Table 5. Distribution of p53 immunoreactivity in various histopathological subtypes of surface epithelial tumors of ovary**

<table>
<thead>
<tr>
<th>Histological types of ovarian tumors</th>
<th>p53 positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Benign</td>
</tr>
<tr>
<td>Serous</td>
<td>0</td>
</tr>
<tr>
<td>Mucinous</td>
<td>0</td>
</tr>
<tr>
<td>Undifferentiated tumors</td>
<td>-</td>
</tr>
</tbody>
</table>

Determination of proliferative activity of the tumour has been reported to be of a diagnostic and prognostic value with other known clinicopathologic features in several types of cancers including those of the lymphatic system, lung, brain, breast, cervix, uterus, ovary, prostate and in soft tissue sarcoma. Ki-67 is the most commonly
Ki-67 index provides insight into nuclear proliferation and predict the clinical behaviour of ovarian tumors.\textsuperscript{18,19} We evaluated Ki 67 expression in 47 selected cases which included 16 benign cystadenoma (8 serous, 8 mucinous), 10 borderline cystadenomas (1 serous, 7 mucinous), 16 carcinoma (8 serous, 8 mucinous adenocarcinoma). A statistically significant difference (p =<0.001) was obtained between the mean Ki 67 indices of benign, borderline & malignant tumors. These findings are in close agreement with Monisha chowdhury et al and with Garzetti et al.\textsuperscript{17,20} Ki 67 index is especially useful in borderline epithelial tumors.

\begin{figure}
\centering
\begin{subfigure}{0.49\textwidth}
\centering
\includegraphics[width=\textwidth]{A.png}
\caption{A Mucinous borderline tumor –intestinal type.high power view showing goblet cells (H&E-45X)}
\end{subfigure}
\begin{subfigure}{0.49\textwidth}
\centering
\includegraphics[width=\textwidth]{B.png}
\caption{B: Mucinous cystadenocarcinoma showing confluent pattern of growth with back-to-back glands with mucinlakes (H&E 100X)}
\end{subfigure}
\begin{subfigure}{0.49\textwidth}
\centering
\includegraphics[width=\textwidth]{C.png}
\caption{C: Serous borderline tumor with papillary fronds lined by cells with mild to moderate atypia without stromal invasion. (H&E-10X).}
\end{subfigure}
\begin{subfigure}{0.49\textwidth}
\centering
\includegraphics[width=\textwidth]{D.png}
\caption{D: Papillary serous carcinoma showing tumor cells arranged in papillary pattern with fibrovascular core (H&E x 100x)}
\end{subfigure}
\caption{Ki-67 and p53- prognostic biomarkers in surface epithelial ovarian tumours}
\end{figure}
Figure 2. A Benign serous cystadenoma showing p53 negative (40x) B: Borderline mucinous showing p53 positivity (40x) C: Serous cystadenocarcinoma showing strong p53 positivity (10x) D: Benign serous cystadenoma, ki-67 index 2.8% (10x) E: Borderline serous tumor ki-67 index 8.3% (40x) F: Mucinous cystadenocarcinoma ki-67 index 8.3% (10x)

Lavanya Rajagopal, et.al: Ki-67 and p53- prognostic biomarkers in surface epithelial ovarian tumours

Ovarian tumors of low malignant potential. The malignant tumors expresses high Ki-67 LI than benign and borderline tumors. Sylvia et al., studied 60 consecutive cases of epithelial ovarian tumours and found that Ki-67 LI was highest in malignant tumours (Mean PI – 48.6±26.76) followed by borderline and lowest in benign. 22
In our study, we checked if there is a correspondence between p53 and Ki-67 positivity and the pathogenic model described before. We observed in our research none p53 positive reaction in benign, low immunoreaction in borderline tumors, and high positive reactions in malignancies. Our study results show that p53 overexpression is more frequent in serous adenocarcinomas than mucinous adenocarcinomas which coincides with the study results of Morita K et al who observed p53 overexpression more frequently in serous adenocarcinomas (5/8, 63%) than in mucinous adenocarcinomas (2/9, 22%) and was correlated with the malignant potential of serous tumors. Sylvia et al also showed a higher p53 expression in malignant tumors. Kmet et al found that the estimated prevalence of p53 mutation as 45%, 5%, and 1%, respectively, for invasive, borderline and benign tumors. Marks et al analyzed p53 staining in ovarian tissue and found positive staining in 50% of malignant tumors but no staining in benign tissue. The presence of mutant p53 protein has been significantly associated with high histological grade.

Our observations indicate that an increased proliferative activity seems usually to involve immunohistochemically detectable alterations in the p53 gene, contributing to the progression of ovarian carcinoma. These findings also suggest that the most critical factor for cell proliferation could be the loss of p53 function. Recent studies suggest that Ki-67 is potentially an attractive therapeutic target in cancer due to the ubiquitous expression in all proliferating cells. Inactivation of the proliferation marker Ki-67 will lead to cell death specifically in proliferating cells and thus could be a potential strategy for the treatment not only of ovarian cancer but also of numerous other malignancies.

### Table 6. Comparative analysis of the results of p53 immunostaining with other studies

<table>
<thead>
<tr>
<th>Author</th>
<th>No. of cases</th>
<th>Tumor type with p53 positive status</th>
<th>Percentage of p53 positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyperi et al.</td>
<td>140</td>
<td>poorly differentiated (52/82 p53 +) Moderately differentiated (36/46 p53 +) Well differentiated (9/12 p53 +)</td>
<td>69.28%</td>
</tr>
<tr>
<td>Handl et al.</td>
<td>60</td>
<td>malignant (49/53+) borderline (5/53-) benign (6/53-)</td>
<td>81.60%</td>
</tr>
<tr>
<td>Ayad et al.</td>
<td>57</td>
<td>42/57 malignant p53+ borderline and benign tumors were not included</td>
<td>73.68%</td>
</tr>
<tr>
<td>Kaprinaczky et al.</td>
<td>38</td>
<td>(26/38) tumors p53+ (9/38) p53+ (3/38) p53- undetermine.</td>
<td>68.42%</td>
</tr>
<tr>
<td>Present study</td>
<td>47</td>
<td>Benign (6/47) p53+ borderline (5/47) p53+ malignant (20/47) p53+</td>
<td>53.2%</td>
</tr>
</tbody>
</table>
CONCLUSION:

In surface epithelial tumors of ovary histological grade and FIGO stage when combined with Ki-67 LI and p53 in histopathology report would help in diagnostic differentiation of subtypes, prognosis, deciding the need for adjuvant chemotherapy and in predicting the survival analysis. Both these biomarkers are very much useful to identify borderline tumors which are likely to behave in a malignant fashion.

REFERENCES:


