Immunohistochemical Expression of Ki-67 and p53 in Surface Epithelial Ovarian Tumour

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ABSTRACT

BACKGROUND

Surface epithelial ovarian tumour (SEOT) develops from the outer surface of ovary. It accounts for more than 90 % of all the ovarian tumours. Most of the SEOT are benign. Few SEOT are of low malignant potential or high malignant potential. The purpose of this study was to assess the rate of expression of proliferative marker Ki-67 and staining pattern of p53 in various histological types of SEOT.

METHODS

It was a randomised type of study carried out in the Department of Pathology, GSVM Medical College, Kanpur, for 2 years. It included 100 random patients with surgically resected specimens of SEOT. Ki-67 immunohistochemistry (IHC) was performed on all malignant cases and few random benign cases; and the percentage of immunopositive cells in each case was expressed as Ki-67 labelling index (Ki-67 LI). p53 expression was interpreted as positive when cells showed diffuse and intense nuclear staining in malignant cases.

RESULTS

Out of 100 cases, 70 were benign and 30 were malignant. Cases comprised of serous and mucinous histological subtypes. In all malignant cases, Ki-67 expression was found to be positive (Ki-67 LI > 1 %). Highest Ki-67 LI of 52 % was associated with high grade serous cystadenocarcinoma. High grade serous carcinoma (HGSC) had higher p53 positivity. 88.8 % of HGSC were p53 positive.

CONCLUSIONS

Ki-67 is a cost-effective proliferative marker; therefore, its assessment can be included in routine histopathological report of SEOT for better understanding of the biologic behaviour and aggressiveness of the tumour. p53 expression was more common in HGSC and can help in discriminating between HGSC and low-grade serous carcinoma (LGSC).

KEYWORDS

p53, Ki-67, Ovarian Tumour, Serous, Mucinous, SEOT

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BACKGROUND

Ovarian cancer is the 6th most common tumour among all the tumours in women. It accounts for a total of 30 % of all cancers of the female genital tract and constitutes for total 3 % of all the cancers in women. The incidence of ovarian cancer ranks just after the incidence of carcinoma of the cervix and the endometrium.[1]

Most of the ovarian cancers are diagnosed only in the advanced stages, so it still remains the deadliest where the 5 yr survival rate falls lesser than 20 % despite the fact that new diagnostic and therapeutic strategies are being used to improve the 5 year survival rate of the patient.[2] Ki- 67 is an excellent immunohistochemical marker to determine proliferating cells of the tumour. It is expressed in all active phases of the cell cycle (G1, S, G2 and M phase) except in resting cells (quiescent cells – G_o phase). The monoclonal Ki–67 / MIB-1 antibody reacts with the nuclear Ki-67 antigen expressed in proliferating cells.[3]

The expression reflects tumour proliferation and has been found to indicate tumour aggression, tumour metastasis and known to predict disease outcome in many human malignancies such as central nervous system tumours (meningioma), lymphoproliferative diseases, connective tissue tumours and breast tumours.[4] p53 is a tumour suppressor gene situated on chromosome 17. p53 gene mutation results in uncontrolled cell proliferation. Approximately 50 % of malignant tumours in human have mutations in p53 gene and it is the most common tumour suppressor gene involved with human malignancies.[4]

The purpose of this study was to evaluate the biological significance of reactivity of p53 and also Ki-67 antigen expression in benign cystadenomas, borderline tumours and invasive cystadenocarcinomas for better understanding of the aggressiveness of the tumour and also helps in correct classification of epithelial ovarian tumour which are confusing in routine H & E staining.

METHODS

It was a randomised type of study because 100 random samples were taken into account. Out of 70 benign samples, 10 samples taken for Ki-67 immunoexpression were also random. Estimated target population was 20,00,000.

The study was carried out in the Department of Pathology, GSVM Medical College, Kanpur for over a period of 2 years from July 2018 to October 2020 comprising of 100 random patients with surgically resected specimen of surface epithelial ovarian tumours (SEOT) after taking proper informed consent.

We used extensive samples of tumour with Haematoxylin – Eosin (H & E) classical technique to make diagnosis of neoplasm. For p53 immunoexpression, we used the DO7 monoclonal antibody (Novocastra, Leica Biosystems, New Castle, United Kingdom) with pre-treating in citrate solution (PH6) for 20 minutes and incubation with the primary pre-diluted antibody for 30 minutes, visualization with diaminobezidine (DAB) and counterstained with haematoxylin.

Intensity for staining was reported as negative, weak, moderate or strong (0, + 1, + 2, + 3)

- 0 for none (no brownish colour seen using X 40 magnification).
- + 1 for weak (brownish colour seen using x 40)
- + 2 for moderate (brownish colour seen using x 10 magnification)
- +3 for strong staining (brownish colour seen using x 4 magnification).

The expression of primary tumour proliferation related to Ki-67 antigen was immunohistochemically evaluated by monoclonal MIB-1 antibody (Novocastraleica Biosystems, New Castle, United Kingdom). On microwave, oven processed formalin fixed paraffin embedded tissue and citrate PH6 as antigen, retrieval development was performed with DAB (3, 3- diaminobenzidine dihydrochloride) solution, which was applied for 3 - 5 minutes. Nuclei were stained using Mayer's Haematoxylin. We considered a positive reaction only in the presence of immunostained nuclei (in brown shades). For Ki-67 scoring the most immunopositive area of the tumour was selected avoiding foci of inflammation. The number of immunopositive nuclei was counted in 1000 tumour cells in at least 10 HPF (x 40).[5] The percentage of immunopositive cells is referred to as labelling index (LI) / Proliferative index. The average of three counts of Ki-67 immunopositive cells over the same slide was taken and expressed as the percentage of Ki-67 immunopositive cells in the tumour.[6] Ki-67 expression was quantitatively assessed and regarded as negative (if Ki-67 LI < 1 %) and positive (if Ki-67 LI $\geq$ 1 %).[7]

Statistical Analysis

We used chi-square test for testing association of p53 expression in different malignant SEOT. Degree of freedom taken was 2.

RESULTS

Histopathological Results (H & E Staining)

In our study, we identified 30 malignant and 70 benign tumours. Benign tumours were represented by serous cyst-adenomas, mucinous cystadenomas and endometroid cystadenofibromas. From the entire group of benign tumour from our study, 60 % were serous cystadenoma, 30 % mucinous cystadenoma and 10 % were of endometroid cystadenofibromas.

Benign tumours were most common in the age group of 31 - 40 years. Out of 42 Serous cystadenoma cases, 36 cases (85.71 %) were seen in the age group of 21 - 50 years with a mean age of 35.5 years. Mucinous cystadenoma had total 21 cases out of which 14 cases [66.66 %] were seen in the age group of 31 - 50 years with a mean age of 40.5 years. Endometroid cystadenofibromas cases were total 7 in number. Benign tumours were easily differentiated from malignant tumours on histopathological slides. So, p53 immunohistochemistry was not performed on benign tumours and Ki-67 immunohistochemistry was performed in

10 random benign tumours out of 70 benign tumours in study. None of borderline malignant potential case was observed. Serous malignant tumours were the most frequent malignant lesion in our study. 18 cases (60 %) out of total 30 malignant lesions were of serous variety. There were 12 mucinous cystadenocarcinoma cases. (Fig. 1).

Maximum malignant lesions (14 cases (46.6 %)) were reported in the age group of 41 – 50 years followed by 33.33 % in age group of 31 – 40 years and 16.66 % in age group of 51 – 60 years. Of 30 malignant lesions, 29 cases (96.66 %) were present in the age group of 31 - 60 years.

p53 Immunoexpression

p53 immunohistochemical staining was applied to all 30 malignant tumours including low grade and high grade serous cystadenocarcinoma; and mucinous cystadenocarcinoma. p53 immunoreactions became positive in more than 50 % cases of serous cystadenocarcinoma; and higher positivity was found in high grade serous carcinoma (HGSC) (88.8 %). p53 immunoreactions were positive only in 25 % cases of mucinous cystadenocarcinoma. (Table 1)

The chi-square test of independence was performed to examine the relation between p53 expression and different malignant SEOT. The relationship between these variables were significant, X^2 (2, N = 30) = 10.88, P = .0043. p53 was more likely to be expressed in high grade serous cystadenocarcinoma (HGSC) and less likely to be expressed in low grade serous cystadenocarcinoma (LGSC) and mucinous cystadenocarcinoma. (Table 2)

The chi-square statistic was 10.8824. The P value was .004334. The result is significant at P < .05. Nuclear staining was generally intense and moderate; and was limited only to neoplastic cells without intervening the stromal nuclei. (Figure 2)

Ki-67 Immunoexpression

Ki-67 positive stained sections were observed in 18 cases of serous cystadenocarcinoma, 12 cases of mucinous cystadenocarcinoma and 10 cases of random benign SEOT.

We noticed a strong diffuse pattern in high grade serous cystadenocarcinomas (Fig 3) of which 3 cases showed Ki-67 LI between 0 - 25, 14 cases showed Ki-67 LI between 26 - 50 and 1 case showed Ki-67 LI above 50. Highest Ki-67 LI i.e. 52 % was found in high grade serous cystadenocarcinoma. Out of 12 cases of mucinous cystadenocarcinoma, 4 cases (33.33 %) showed Ki-67 LI between 0 - 25 and 8 cases (66.66 %) showed Ki-67 LI between 26 - 50. (Fig 4) Among all, highest Ki-67 LI was found to be 42 % and least Ki-67 LI was 18 % (Table 3). None of the benign SEOT out of 10 random benign cases, stained positive for Ki-67.

Sl. No.	Diagnosis	Total Cases	Positive p53 cases	Percentage of Positive p53 Cases
1	High grade serous cystadenocarcinoma	9	8	88.88
2	Low grade serous cystadenocarcinoma	9	2	22.22
3	Mucinous cystadenocarcinoma	12	3	25

Table 1. Immunohistochemistry - p53

	Positive Cases	Negative Cases	Row Totals
HGSC	8 (3.90) [4.31]	1 (5.10) [3.30]	9
LGSC	2 (3.90) [0.93]	7 (5.10) [0.71]	9
Mucinous cystadenocarcinoma	3 (5.20) [0.93]	9 (6.80) [0.71]	12
Column totals	13	17	30 (Grand Total)

Table 2. Chi-Square Table of p53 Expression

Sl. No.	HPE Diagnosis	Median Ki–67 (LI)
1	Low grade serous cystadenocarcinoma	24 %
2	High grade serous cystadenocarcinoma	45 %
3	Mucinous cystadenocarcinoma	29 %

Table 3. Immunohistochemistry - Ki–67 Labelling Index

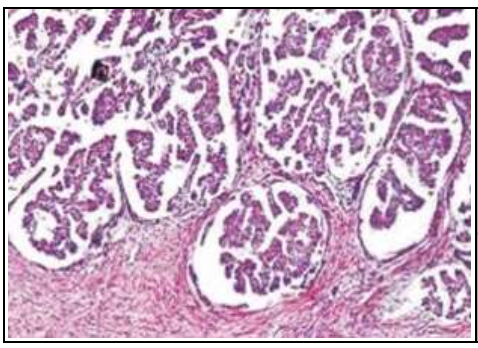

Figure 1. Serous Cystadeno-carcinoma

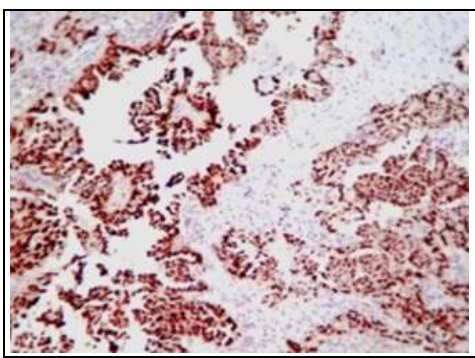

Figure 2. High Grade Serous Cystadeno-carcinoma - Diffuse p53 Nuclear Staining (10x)

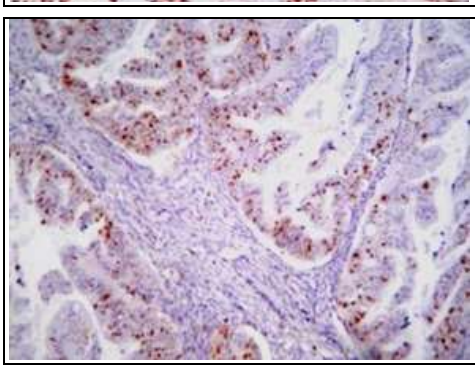

Figure 3. Serous Cystadeno-carcinoma – Ki-67 LI – 42 % (40x)

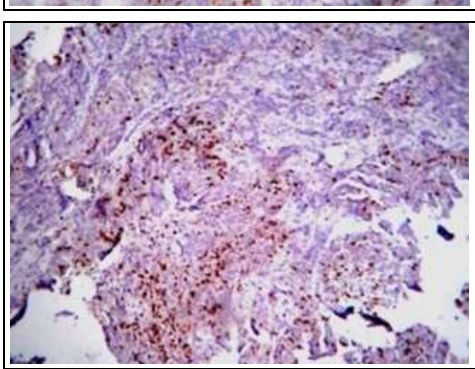

Figure 4. Mucinous Cystadeno-carcinoma Ki-67 LI – 36 % (40x)

DISCUSSION

Ovarian cancer is an extremely heterogeneous disease which poses a challenge to its early diagnosis.[8] Benign

tumours were the most common tumours present in our study. It is similar to studies conducted in the past.[9]

Assessment of Ki-67 Immunoexpression

Our study was conducted to evaluate the biological significance of Ki-67 antigen expression in benign and malignant ovarian tumour and correlate it with histological type and grade.

In our study we observed that only malignant tumours were positive for Ki-67 expression (Ki-67 LI > 1 %). Benign tumours had no Ki-67 expression (Ki-67 LI < 1 %). Similarly, in the study conducted by Verma et al.[10] positive Ki-67 expression was seen in the malignant tumours.

The higher expression of Ki - 67 in malignant tumour could be explained by the fact that it is a nuclear protein present in proliferating cells and its expression is indicative of high proliferation rate and aggressiveness of malignant tumour cell as compared to benign tumour.[11]

In our study, Ki-67 LI was higher in serous cystadenocarcinoma than mucinous cystadenocarcinoma. Similarly, in the study of Mahadevappa et al.[12] the mean Ki-67 LI of serous adenocarcinoma was 65.03 $\pm$ 21.67, which was higher than that of mucinous carcinoma 60.24 $\pm$ 21.91.

In our present study, the median Ki-67 LI of HGSC was 45 % and Ki-67 LI of LGSC was 24 % which signifies that there is higher Ki-67 expression in HGSC as compared to LGSC. Similar results were obtained in the study of Kobel et al. and Mahadevappa et al. In Kobel et al.[13] the median Ki-67 LI of LGSC was 2.5 % and that of HGSC was 22.4 %. In Mahadevappa et al., the median Ki-67 LI of LGSC was 37.96 % and that of HGSC was 65.34 %.

Assessment of p53 Immunoexpression

In our study, we observed that p53 positivity was found in 88.8 % high grade serous adenocarcinoma. Similarly, Amanullah et al.[14] found that 94 % of HGSC overexpressed p53. Also Kobel et al.[15] showed p53 positivity in 99 % of HGSC. (Table 4)

Sl. No.	Diagnosis	Present Study	Nayak et al.[9]	Kobel et al.[15]	Morita et al.[16]	Amanullah et al.[14]	Ndukwe et al.[17]
1	High grade serous cystadenocarcinoma	88.88	100	99	63	94	85.2
2	Low grade serous cystadenocarcinoma	22.22	40	-		0	26.1
3	Mucinous cystadenocarcinoma	25	100	-	22	0	35.7

Table 4. Comparison of Percentage of p53 Positive Cases in Different Studies

p53 positivity in low grade serous adenocarcinoma was only 22.22 % in our study. The value of p53 expression in HGSC and LGSC was statistically significant. In 2014, WHO recommended the use of aberrant p53 staining pattern to distinguish between HGSC and LGSC.[18]

Out of 12 mucinous cystadenocarcinomas, p53 positivity was found only in 3 cases, which suggest insignificance of p53 profiling in the same.

CONCLUSIONS

Cellular proliferation plays a significant role in the clinical behaviour and aggressiveness of ovarian tumour. Ki-67 is a cost effective immunohistochemical marker. Since, it is only expressed in malignant SEOT, it easily differentiates between benign and malignant SEOT. Therefore, use of Ki-67 immunoexpression in routine histopathological report of SEOT will help in better understanding of the biological behaviour of the tumour and can also help in modifying treatment strategies.

p53 positivity is statistically higher in HGSC than LGSC. It helps to differentiate between HGSC from LGSC, and between borderline and malignant tumours. It will also help in correct classification of morphologically confused EOT.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
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