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IMMUNOHISTOCHEMICAL STUDY OF P53 AND KI-67 EXPRESSION IN SURFACE EPITHELIAL TUMORS OF OVARY

Pathology

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ABSTRACT

Introduction: Ovarian cancer accounts for a significant number of deaths from female genital tract malignancies worldwide. Among ovarian neoplasm, 2/3rd of tumors are of surface epithelial origin. Most common mutated tumor suppressor gene in human malignancies is p53. Overexpression of p53 gene has been claimed to be associated with poor prognosis. Assessment of cellular proliferation marker (Ki-67 labelling index) has diagnostic and prognostic significance in ovarian neoplasm. **Material and Method:** A study was conducted at tertiary care hospital in Central Gujarat, India. Immunohistochemistry was performed on 51 cases and results were noted. **Results:** Out of 51 cases, 31 were benign, 4 were borderline and 16 were malignant. P53 expression was highest in malignant and borderline tumors. Mean Ki-67 LI was highest in malignant tumors (45%). **Conclusion:** Higher expression of Ki-67 in malignant surface epithelial ovarian tumors emphasizes its role in prognosis of tumors.

KEYWORDS

Surface epithelial tumors, p53, Ki-67 labeling index, Serous, Mucinous

INTRODUCTION:

Ovarian cancer is the 6th most common cancer in females worldwide [1]. Ovarian neoplasms are higher in developed than developing countries [2]. Ovarian cancer accounts for a total of 30% of all cancers of the female genital tract and total 3% of all carcinoma in women. The incidence of ovarian cancer ranks just after the incidence of carcinoma of the cervix and the endometrium [1]. Important etiological risk factors are increasing age, positive family history, high socio-economic classes and nulliparity [1]. Due to its lack of symptoms, ovarian cancer is diagnosed at an advanced stage when the cancer has already spread to secondary sites. Surface epithelial tumors are classified based on morphological features (serous, mucinous, endometrioid, clear cell, transitional) and are then further subclassified as benign, borderline or malignant [3]. Serous carcinoma is the most common type of ovarian cancer [4]. The distinction between borderline and malignant tumors is the biggest dilemma which is important for prognostic significance [4].

Identification of biological prognostic markers would be of helpful to identify patient with poor prognosis and to improve treatment modalities. Molecular markers which are associated with pathogenesis and prognosis of ovarian neoplasm having been investigated since several years [3]. One of the most studied markers is overexpression of p53. P53 gene is located on the short arm of chromosome 17. P53 has been called as "Guardian of the genome" as it is associated with DNA repair, cell cycle arrest and apoptosis. The pattern of p53 abnormalities is most consistent with spontaneous mutation rather than the activity of chemical carcinogens [5]. Mutant p53 proteins have prolonged half-life, accumulate in the nucleus, and can be detected by immuno histochemistry [6].

Similarly, Ki-67 is a proliferation marker helpful in predicting disease outcome in ovarian neoplasms. The determination of cell proliferation (Ki-67 labeling index) has diagnostic and prognostic significance as it is important to decide clinical behaviour and aggressiveness of ovarian tumors [7]. High expression of Ki-67 antigen is associated with tumor aggression, vascular invasion, tumor metastasis and poor prognosis [8].

The purpose of this study was to evaluate expression of p53 and also Ki-67 antigen in benign, borderline and malignant tumors for better understanding of its association with histopathological parameters.

MATERIAL AND METHOD:

A 2- year retrospective and 10 months prospective study was conducted at Department of Pathology in a tertiary care hospital in Central Gujarat in Western India. A total of 51 cases were taken for study during this period. All cases were reviewed and representative sections from pathological area were selected and corresponding blocks were processed with hematoxylin and eosin stain to make diagnosis of neoplasm.

Most representative sections for immunohistochemistry were selected and 3 µ thick sections from each tumor blocks were obtained on poly-L-lysine coated slides. Sections were deparaffinized on hot plate and antigen retrieval procedure was performed by TRIS-EDTA based antigen retrieval solution using microwave method. Sections were thoroughly washed with phosphate buffer in between every step.

Peroxidase blocking was done followed by application of p53 (Biogenex ready to use Mouse Monoclonal antibody D07) and Ki-67 (Biogenex ready to use Mouse Monoclonal antibody MKI76/2462) monoclonal antibodies. Sections were incubated for 1 hour at room temperature and in next step secondary antibody was added. After incubation for 30 minutes, freshly prepared DAB (Diaminobenzidine) was added to sections for 10 minutes and then sections were counterstained with hematoxylin.

Interpretation:

Positive staining was observed as brown, granular nuclear staining. P53 immunoreactivity was based on percentage of tumor cells showing positive nuclear staining, scored as follows [9]:

- None (<5%)
- Weak (+, 5-25%)
- Moderate (++, 25-75%)
- Intense (+++, >75%)

Based on intensity of positive reaction in tumor cells, staining intensity was graded and scored as [1]:

- None (No brownish color seen using 40X magnification)
- +1- Weak (brownish color seen using 40X magnification)
- +2- Moderate (brownish color seen using 10X magnification)
- +3- Strong (brownish color seen using 4X magnification)

The percentage of immunopositive cells for Ki-67 referred to as labeling index (LI). Ki-67 expression was quantitatively assessed and regarded as negative (Ki-67 LI < 1%) and positive (Ki-67 LI >1%). Scoring was done as follows [10]:

- 0 - Score 0
- 1-10% - Score 1
- 11-50% - Score 2
- 51-100% - Score 3

To express final score out of 100, sum of 25 microscopic fields were taken and obtained score was expressed as % expression.

Statistical analysis:
The statistical analysis was performed using MedCalc software version 20.022.

RESULTS:
In present study, we identified 31 benign (60.78%), 4 borderline (7.84%) and 16 malignant (31.37%) ovarian tumors. The age of patients ranged from 20 to 76 years with mean age of 46 years. The most common histological type was serous epithelial tumors (54.90%) followed by mucinous tumors (37.25%). Serous tumors were more common in both benign (45.16%) and malignant (68.8%) tumor groups. Majority of tumors were unilateral (48 cases-94.11%). On gross examination, most of the tumors were cystic (64.70%). Among 51 cases in present study, 48 cases (94.11%) showed positive p53 immunoexpression. P53 immunoreaction became positive in 100% of malignant and borderline tumor, 90.32% of benign tumors; and higher positivity found in high grade serous cystadenocarcinoma (100%).

Among 48 cases with positive p53 expression, 22.9% showed +3 IHC score (≥75%), 31.3% showed +2 IHC score (25-75%) followed by 45.8% showed +1 IHC score (5-25%). +3 IHC score was observed mainly in malignant tumors. Majority of which are high grade serous carcinoma (10/11=90.9%). +2 IHC score was observed in 6 malignant tumors (2 cases each of mucinous and serous cystadenocarcinoma, 1 case each of transitional and endometrioid carcinoma), 6 benign tumors (4 cases of benign serous cystadenoma and 1 case each of benign brenner tumor and benign serous cystadenofibroma) and 3 borderline tumors (2 cases of mucinous borderline tumor and 1 case of serous borderline tumor). 22 cases showing +1 IHC score were benign tumors (12 cases of benign mucinous cystadenoma, 9 cases of benign serous cystadenoma and 1 case of benign seromucinous cystadenoma). Intensity of p53 immunostaining in malignant tumors was as per Table-1.

Table-1: Intensity of p53 in malignant surface epithelial tumors of ovary

Intensity of staining	Serous cystadenocarcinoma	Mucinous cystadenocarcinoma	Endometrioid carcinoma	Transitional carcinoma
None	0	0	0	0
+1	1(9.1%)	1(33.3%)	0	1(100%)
+2	4(36.4%)	1(33.3%)	1(100%)	0
+3	6(54.5%)	1(33.3%)	0	0

Positive Ki-67 immunoexpression was observed in 44 cases of surface epithelial tumors. Among 44 cases with positive Ki-67 immunoexpression, 25 (80.64%) cases were benign, 3(75%) cases were borderline and 16(100%) cases were malignant tumors. The mean Ki67 labelling index was highest in malignant tumor (45%) followed by borderline (8%) and benign tumors (7%). Highest Ki-67 labelling index i.e., 88% was found in high grade serous cystadenocarcinoma and least Ki-67 LI i.e., 1% was found in benign serous cystadenoma. Out of 11 cases of high-grade serous cystadenocarcinoma, 7 cases showed Ki-67 LI above 50%, 3 cases showed Ki-67 LI between 11-50% and 1 case showed Ki-67 LI <11%. Out of 11 cases of benign mucinous tumors, 8 cases showed Ki-67 LI between 1-10% and 3 cases showed Ki-67 LI between 11-50%. Among 3 cases of malignant mucinous tumors, 2 cases showed Ki-67 LI between 11-50%.

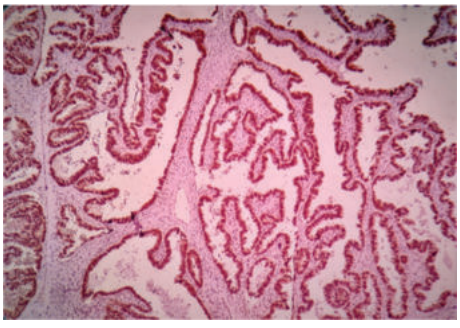

Figure-1: p53 expression in 92% of cells in serous papillary carcinoma with strong intensity of staining (IHC p53, 10x)

DISCUSSION:
Ovaries are common site for non-neoplastic and neoplastic lesions. Mean age of presentation of surface epithelial tumors was 46 years. Comparable results were observed by Mohapatra I et al. [7], Singh A et al. [11] and Singh M et al [1]. In present study, peak incidence of surface epithelial tumors was seen in 4th decade which was similar to Mohapatra I et al. [7] and Singh A et al. [11] study.

The most common histological type was serous epithelial tumors (54.90%) followed by mucinous tumors (37.25%) which were similar to study conducted by Sheronica Laishram et al. [12], Choudhary P et al. [4] and Singh M et al [1]. Benign ovarian tumors constituted 60.7% of all cases followed by malignant tumors (31.3%) and borderline tumors (7.8%) which was in concordance with Choudhary P et al. [4] and Singh A et al. [11] study. Among all the malignant tumors, serous cystadenocarcinoma was the most commonly observed tumor (68.7%) in present study which was similar as observed by Choudhary P et al. (69.7%) [4].

In present study, 94.11% of all cases were positive for p53 immunoexpression. Similar higher positivity for p53 was reported by Verma R et al. [2] and Ayadi L et al [13]. We found 100% of malignant tumors revealed positive p53 immunoexpression. Singh A et al. [11], Mohapatra I et al. [7] and Qasim et al. [3] also observed majority of malignant tumors revealing p53 immunoexpression. In contrast to other study, present study showed higher positivity for p53 immunoexpression among benign tumors (90.32%). P53 immunoexpression in surface epithelial tumors of different studies was as per table-2.

Table-2: Comparison of p53 positivity in surface epithelial ovarian tumors in relation to other studies

Author	Benign	Borderline	Malignant
Singh A et.al [11]	21.05%	50%	71.9%
Mohapatra I et.al [7]	14.3%	75%	89.5%
Singh M et.al [1]	NA	NA	43.33%
Sylvia MT et.al [15]	-	20%	57.6%
Choudhary P et.al [4]	NA	83.33%	78.8%
Verma R et.al [2]	NA	NA	16.7%
Qasim et.al [3]	35%	20%	90%
Abbasi et.al [16]	14.9%	50%	55.6%
Present study	90.32%	100%	100%

Frequency of p53 positive immunoreactivity in the serous tumors analyzed in the present study was 92.8%, the comparative analysis with other studies showed variable results ranging from 15-78%. The possible reasons for this variation among studies may be attributed to numbers of samples analyzed, interobserver variability and properties of different antibodies. In the present study, we have studied positive p53 immunoexpression was higher in patients with age ≥40 years which was in concordance with study conducted by Qasim et al. [3] and Singh A et al [11].

In present study, we observed highest mean Ki-67 labelling index in malignant tumors (45%) which was similar to study done by Monisha Choudhary et al. (49%) [14] and sylvia MT et al. (48%) [15]. Score +3 of Ki-67 labelling index was observed only an in malignant tumor which was in concordance with results observed in Verma et al. study. Higher expression of Ki-67 in malignant tumors indicates high proliferation rate and aggressive nature of malignant tumors.

Mean Ki-67 was high in serous tumor (29%) as compared to mucinous tumors (13%) analyzed in present study which was in tune with study conducted by Singh M et al. [1] and Sheronica Laishram et al [12].

CONCLUSION:
Immunohistochemical expression of p53 was more commonly seen in borderline and malignant surface epithelial tumors of ovary which explained its importance in pathogenesis and disease progression towards more invasive forms. Immunohistochemical marker Ki-67 overexpressed in malignant tumors which indicates their role in oncogenesis and aggressiveness of tumors. Maximal expression of p53 and Ki-67 emphasize their significance in disease progression and therapeutic modalities of ovarian surface epithelial tumors.

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