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TO STUDY THE EXPRESSION OF Ki-67 AND p53 IN EPITHELIAL OVARIAN TUMORS

Histopathology

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ABSTRACT

Context: Surface epithelial tumors of the ovary account for approximately two-thirds of all ovarian neoplasms, and their malignant forms represent about 90% of ovarian cancers. Epithelial ovarian cancer is the most lethal gynecological malignancy. Due to its lack of symptoms, this disease is diagnosed at an advanced stage when the cancer has already spread to secondary sites. Accordingly, reliable markers that are independent and complementary to clinical parameters are needed for a better management of these patients. Mutations in the p53 gene and subsequent gene product have been related to most cancer types. Uncontrolled proliferation is a driver for cancer and is one of the hallmarks of this disease. **Aim:** To evaluate the proliferative activity by using immunohistochemical expression of Ki-67 and p53 and its correlation with histological subtype and staging in ovarian tumours. **Settings And Designs:** A Prospective and retrospective hospital based study. **Materials And Methods:** There were 100 cases of ovarian tumors encountered over the period of 18 months from January 2020 to June 2021, Out of total 100 cases of ovarian tumors, 69 cases of epitheial ovarian tumors were selected for immunohistochemical expression of Ki-67 and p53. Samples were received and reported in Department of Pathology Gandhi Medical College Bhopal, Madhya Pradesh. **Statistical Analysis:** To assess the expression of Ki-67 and p53 and their correlation were compared using Chi-Square test. P-value less than 0.05 was considered significant. **Results:** A Total of 69 cases of Epithelial ovarian tumors were included in this study predominated by serous epithelial tumors (36 cases /69 cases) followed by mucinous epithelial tumors. Most cases were benign (42 cases/69cases), 8 cases were borderline and 19 cases were malignant. Higher immunoexpression of p53 and Ki 67 were seen in patients with ovarian tumor aged more than 40 years. Statically highly significant correlation was found in the immunoexpression of both p53 and Ki 67 marker between benign, borderline and malignant surface epithelial tumors (P value < 0.05). **Conclusion:** Immunohistochemistry is a good screening method that can be used to predict malignant versus proliferative tumors. Both the markers showed higher expression among malignant tumors, while among borderline tumor variable expression were seen, although it was significantly different from benign tumors.

KEYWORDS

P53, Ki 67 immunohistochemical markers and ovarian tumors

INTRODUCTION-

Surface epithelial tumors of the ovary account for approximately two-thirds of all ovarian neoplasms, and their malignant forms represent about 90% of ovarian cancers[1]. Epithelial ovarian cancer is the most lethal gynecological malignancy. Due to its lack of symptoms, this disease is diagnosed at an advanced stage when the cancer has already spread to secondary sites. Accordingly, reliable markers that are independent and complementary to clinical parameters are needed for a better management of these patients. For several years, efforts to identify prognostic factors have focused on molecular markers, with a large number having been investigated[2].

The determination of cell proliferation has been reported to be of diagnostic and prognostic significance as it plays an important role in clinical behavior and aggressiveness of ovarian tumors.[3] Innumerable numbers of molecular markers have been investigated by immunohistochemistry. A central transcriptional mediator p53 when mutated and a nuclear non-histone protein Ki-67 over expression has been claimed to be a marker of poor prognosis in epithelial ovarian tumors.[4] Ki-67 has been correlated with the clinical stage of tumors and may be a promising factor for targeted molecular therapies.[5]

The aim of the study, to assess the expression of p53 and Ki 67 proteins in surface ovarian tumor in an attempt to investigate their involvement in ovarian carcinogenesis and to find out whether a correlation exists between overexpression of these markers and histopathological parameters.

MATERIALS AND METHODS-

This was a retrospective and prospective study The study was conducted in Department Of Pathology, Gandhi Medical College & Associated Hospitals, Bhopal, Madhya Pradesh from January 2020 to June 2021, a total of 69 formalin fixed, paraffin-embedded surface epithelial tumors of ovary specimens were studied , out of which 36 cases were of serous epithelial and 23 cases were of mucinous epithelial tumors. Sections were prepared for IHC staining for both p53 and Ki 67 markers.

For all cases 4µm histological sections were deparaffinized in xylene and rehydrated in descending dilutions of ethanol and were mounted on positively charged glass slides for immunohistochemical staining and subjected to antigen retrieval. Only nuclear p53 and Ki 67 expressions were accepted as specific reactions.

Evaluation of paraffin IHC was performed by three reviewers. 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 [6].

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 [7].

Positive cells determined by counting 1000 cells in at least 10 HPF were measured for each case Luminita *et al* [18].

Non-surface ovarian epithelial tumor has been excluded from the study. The institutional ethical committee approved the study.

RESULTS-

The mean age of diagnosis for benign, borderline and malignant epithelial tumors was 31 years, 46 years, 52 years respectively with a range of 19 to 74 years. Most common histological type was serous epithelial tumors (52%) followed by mucinous tumors. Benign ovarian tumors constituted 60% of all cases followed by malignant tumors (27%) and borderline tumors (11%). Highest p53 immunoreactivity was seen in malignant tumors (100%) compared with borderline (75%) and benign tumors (19%). This association of p53 over expression with biological tumor behavior was found to be statistically significant ($p<0.05$).

From the table: 1 it is evident that Benign epithelial tumor constituted 60.8% of total cases, followed by malignant tumors constituted 27.3%.

Table 1: Distribution Of Cases According To Histological Type Of EOT

Type	Benign	Borderline	Malignant	Total
Serous	26 (37.6%)	4(5.7%)	16 (23.1%)	46 (66.6%)
Mucinous	16(23.1%)	4 (5.7%)	3 (4.3%)	23 (33.3%)
Total	42(60.8%)	8 (11.5%)	19(27.3%)	69(100%)

Table 2: Distribution Of p53 Immunoreactivity In Various Histological Subtypes Of EOT.

Histological subtype	P53				Total
	Negative	1+	2+	3+	
Benign	34 (80.9%)	8 (19%)	0 (0%)	0 (0%)	42 (100%)
Borderline	2 (25%)	4 (50%)	0 (0%)	2 (25%)	8 (100%)
Malignant	0 (0%)	3 (15.7%)	10 (52.6%)	6 (31.5%)	19 (100%)
Total	36 (52.1%)	15 (21.7%)	10(14.4%)	8 (11.5%)	69 (100%)

Table.2: Showed highest p53 expression were seen in malignant epithelial ovarian tumors, followed by borderline epithelial ovarian tumors. Most of the benign epithelial ovarian tumors were negative for p53.

Table 3: Histological Type Of Tumor And p53 Immunoreactivity.

P53 immunoreactivity			
	Negative	Positive	Total
Serous	21 (30.4%)	25 (36.2.4%)	46 (66.6%)
Mucinous	15(21.7%)	8 (11.5%)	23 (33.3%)
Total	15 (21.7%)	8 (11.5%)	69 (100.0%)

Tbale.3: Shows p53 immunoexpression among Serous and Mucinous epithelial ovarian tumors

Table 4: Distribution Of Ki-67 Over Expression In Various Histological Subtypes Of EOT

Histological subtypes	Ki-67 LI				Total
	Negative	1+	2+	3+	
Benign	32 (90.5%)	8 (4.8%)	2 (4.8%)	0 (0%)	42 (100%)
Borderline	2(25%)	2 (25%)	2 (25%)	2 (25%)	8 (100%)
Malignant	0 (0%)	6 (31.5%)	8 (42.1%)	5 (26.3%)	19 (100%)
Total	34 (49.2%)	16(23.1%)	12 (17.3%)	7(10.1%)	69 (100%)

Table.4: Showed highest Ki-67 expression were seen in malignant epithelial ovarian tumors followed by in borderline epithelial ovarian tumors. Most of the benign epithelial ovarian tumors were negative for ki-67.

Table.5: Histological Type Of Tumor And Ki-67 Immunoreactivity.

Ki-67 LI			
	Negative	Positive	Total
Serous	22 (21.2%)	24 (21.2%)	46 (42.3%)
Mucinous	12 (11.5%)	11 (23.1%)	23 (34.6%)
Total	34 (40.3%)	35 (59.7%)	59 (100%)

Table.5: Shows Ki-67 immunoexpression among Serous and Mucinous epithelial ovarian tumors.

In the present study, Ki-67 was expressed on 50.7% of epithelial tumors of ovary. Highest Ki-67 LI [2+ to 3+] was significantly associated with malignant epithelial tumors 68.4% (13 out of 19) while majority of the cases 76.1% (32 out of 42) with negative immune expression belonged to benign histological subtype. This was found to be statistically significant (p value<0.05).

Table 5 described the Ki-67 expression in different types of epithelial tumors and no significant relationship between histopathological type of epithelial tumors and Ki-67 over expression was observed in this study (p value>0.05).

As per Table.6 Statistically significant correlation was observed between immune expression of both p53 and Ki-67 markers for diagnosis of epithelial tumors (p value <0.05); as with high p53 over expression, there was a tendency towards a higher expression of Ki-67.

Table.6 Correlation Between p53 And Ki-67 Immunoexpression For EOT

Tumor	Ki-67 score	Benign	Borderline	Malignant	P-53 score	Benign	Borderline	Malignant
Serous	Score 0	20	2	0	Score 0	21	0	0
	Score 1	6	2	6	Score 1	5	4	3
	Score 2	0	0	5	Score 3	0	0	9
	Score 3	0	0	5	Score 4	0	0	4
	Total	26	4	16	Total	26	4	16
Mucinous	Score 0	12	0	0	Score 0	13	2	0
	Score 1	2	0	0	Score 1	3	0	0
	Score 2	2	2	3	Score 2	0	0	1
	Score 3	0	2	0	Score 3	0	2	2
	Total	16	4	3	Total	16	4	3

As per Tbale.6: Among serous ovarian neoplasm, most of benign cases were negative, while borderline serous tumors expressed weak positivity. Higher expression were seen among malignant cases for both the markers. Similarly most of benign mucinous ovarian tumor were negative and higher expression were seen among malignant cases for both the marker.

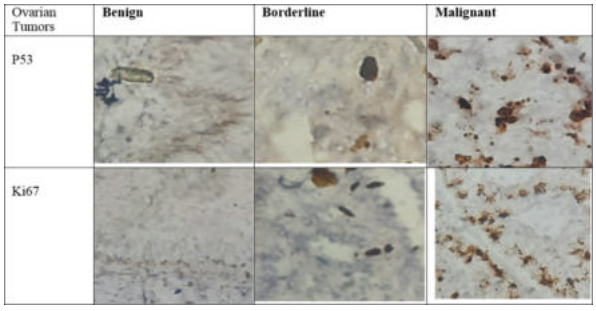

Fig I-Showing p53 and Ki67 IHC staining among Benign, Borderline and Malignant Epithelial Tumors of ovary(From left to right showing 1+, 2+ and 3+ positivity)

DISCUSSION-

The mean age of presentation of benign, borderline and malignant epithelial tumors of ovary was 21 years, 46 years and 51 years respectively. This can be explained by the fact that accumulation of somatic mutations in cancer driver genes with increasing age is associated with emergence of malignant neoplasms.[10] Comparable results were observed by Lumina et al and QasimYA et al.[9,4] Systematic review of 145 articles by Momenimovahed et al[11]. concluded that epithelial ovarian cancer is an age- related disease, and is considered mainly a postmenopausal disease. Increased incidence of this cancer is more pronounced in women over 65 years of age, with a median age at diagnosis being 50-79 years[11]

Most common histological type was serous epithelial tumors (66.6%) followed by mucinous tumors (33.3%). Benign ovarian tumors constituted 60% of all cases followed by malignant tumors (27.3%) and borderline tumors (11.5%). Serous tumor was more common in both benign and malignant group which was similar as observed by Laishram S et al.[12]

Tumor-suppressor p53 gene is located on the short arm of chromosome 17. This acts by suppressing abnormal cell growth at the beginning of the S-phase of the cell cycle.[13] The mutation and/or over expression of the p53 are the most frequent anomalies described in human cancers.[14] Studies have shown a correlation between the mutation/over expression of p53 and the patient prognosis with different types of tumors like breast cancer, rectal, intestinal cancer, lung cancer and also ovarian cancer.[15] The two main methods for studying p53 are sequence analysis and immunohistochemical staining.

In our study, highest p53 immunoreactivity was seen in malignant

tumors (100%) compared with borderline (75%) and benign tumors (19.0%). This association of p53 over expression with biological tumor behaviour was found to be statistically significant ($p<0.05$).

The tumor proliferative fraction of ovarian carcinomas has been investigated immunohistochemically by means of antibodies that recognize the nuclear antigen Ki-67 expressed in proliferating cells. This is known to predict disease outcome in many human malignancies.[16] Gursan N et al. noticed a significant relationship between high Ki-67 immunoexpression in ovarian neoplasms and disease-free survival.[17]

In the present study, Ki-67 was expressed on 50.7% of epithelial tumors of ovary. Highest Ki-67 LI [2+ to 3+] was significantly associated with malignant epithelial tumors 68.4% (13 out of 19) while majority of the cases 76.1% (32 out of 42) with negative immunoexpression belonged to benign histological subtype. This was found to be statistically significant (p value<0.05). Gursan et al[17]. demonstrated that the mean Ki-67 LI in benign tumors was 14.9%; in borderline tumors 22.8%; in malignant tumors 42.8% respectively.[17] When compared with the benign tumors, Ki-67 LI was found to be significantly higher in the malignant counterpart. KI-67 over expression is associated with aggressive cytomorphology of the tumor, vascular invasion, tumor metastasis and poor prognosis.[18]

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