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# Spectrum Of IHC P16 Expression In Epithelial Ovarian Tumors And Its Correlation With Histological Grading

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## ABSTRACT

**Introduction**- Neoplastic ovarian disorders originate from three main cell types: (1) müllerian epithelium, (2) germ cells, and (3) sex cord–stromal cells. The WHO classifies ovarian tumors, with most arising from müllerian epithelium, based on differentiation and proliferation. The three major histologic types are serous, mucinous, and endometrioid tumors. **Aim:** To study the spectrum of IHC p16 expression in epithelial ovarian tumors and its correlation with histological grading.

**Methodology:** This cross-sectional study was conducted in a tertiary care medical centre in western Uttar Pradesh.It includes ovarian tumors received in department of pathology, diagnosed as surface epithelial tumors during time frame of 18 months. IHC p16 analysis was done and on the basis of staining expression, were categorised into three classes: Absent, heterogeneous or normal and block or strong positivity. The correlation between p16 expression patterns and tumor histological grade was statistically analyzed.

**Results:** A total of 35 cases were analyzed, with the majority being serous tumors (77%), followed by mucinous tumors (20%) and one Brenner tumor. Among benign tumors, 83.3% exhibited heterogeneous p16 expression, whereas all high-grade carcinomas (100%) demonstrated strong p16 positivity. Low-grade carcinomas primarily showed heterogeneous expression, while borderline tumors displayed variable p16 staining. The correlation between p16 expression and tumor grading was statistically significant (p-value = 0.0033), suggesting a strong association between p16 expression and high-grade malignancies.

**Conclusion:** This study highlights the diagnostic and prognostic significance of IHC p16 expression in epithelial ovarian tumors. The findings suggest that p16 overexpression is a hallmark of high-grade carcinomas, while benign and low-grade tumors predominantly retain normal p16 expression. These results reinforce the role of p16 as a valuable IHC marker for tumor grading and prognostic assessment in ovarian carcinoma.

## 1. INTRODUCTION

Ovarian cancer is the third most common cancer among Indian women and eighth overall, constituting 3.44% of all cancer cases, as per Globocan 2018[1].

Majority of ovarian neoplasms (90%) of them are surface epithelial tumors with serous tumors topping the list.Ovarian cancer mostly present at advanced stage because of relatively long asymptomatic phase resulting in poor overall survival[2].

Traditionally neoplastic disorders of ovary can be grouped according to their origin from each of the three main ovarian cell types: (1) müllerian epithelium, (2) germ cells, and (3) sex cord–stromal cells.Most primary ovarian neoplasms arise from müllerian epithelium and classification is based on both differentiation and extent of proliferation of the epithelium.There are three major histologic types- serous, mucinous, and endometrioid tumors[2].

About 80% of the ovarian tumors are benign, and occur mostly in young women between 20 and 45 years whereas malignant tumors are more common in women between 45 and 65 years of age.Recent studies have highlighted different pathways of pathogenesis of epithelial tumors depending on their grading into low and high grade. Accordingly epithelial ovarian carcinomas is now divided into two major groups: Type I tumors progress from benign tumors through borderline tumors that give rise to low grade carcinomas. Type II tumors arise from fallopian tube epithelium via intraepithelial precursors and

demonstrate high grade nuclear features of serous type and correlates with patient survival. At molecular level different set of genes and entirely different set of pathways seem to play a role with Low grade carcinomas having mutations in KRAS,BRAF and ERBB2 oncogenes and High grade carcinomas high frequency of p53 mutations. In the context of serous tumors they are termed as LOSC and HOSC[2].

Immunohistochemistry has occupied a key position in tumor diagnosis and prognosis.The tumor supressor gene p16 plays a key role in controlling the cell cycle. Under normal conditions cells express variable amounts of p16 protein that can be detected by IHC in both nuclear and cytoplasmic localizations (heterogeneous p16 expression)[3]. Two types of abnormal p16 expression patterns can be noted i) absent ii) overexpressed. Overexpression is also termed as block expression by the Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions (LAST)[4].

Previous research integrating all histotypes has demonstrated that either p16 overexpression or total absence is linked to adverse outcomes[5]. According to recent histotype-specific research, in two cohorts of 334 and 115 women with HGSOC, normal heterogeneous p16 expression was likewise substantially linked to extended progression-free and overall survival (OS) [6].

The G1/S transition state of the cell cycle is reflected in the pattern of p16 expression. In contrast to absent or block expression, which reflects abnormalities of the G1/S cell cycle checkpoint complex linked to unfavorable outcomes, heterogeneous p16 expression is thought to reflect the normal G1/S transition status, which in ovarian tumors is associated with a favorable outcome[7].

### *RATIONALE OF THE STUDY*

Various studies done on epithelial ovarian tumors have shown varied results while correlating expression of p16 by IHC with various subtypes and grading of ovarian epithelial tumors. This study was therefore undertaken to analyse whether abnormal p16 expression is associated with any particular histological subtype and its grade on histopathological examination in epithelial ovarian tumors.

## 2. AIMS AND OBJECTIVES

To study the spectrum of IHC p16 expression in epithelial ovarian tumors and its correlation with histological grading.

## 3. MATERIAL AND METHODS

This is a cross sectional study conducted in pathology department of a tertiary care centre in western Uttar Pradesh. All the ovarian tumors received and diagnosed on histological examination as surface epithelium tumor were included(January 2021-December 2024).In case of epithelial ovarian tumor being diagnosed on histomorphology, classification of tumor was done as per WHO 2020 protocol.They were then graded benign, borderline and malignant tumors. Malignant tumors were further graded as low and high grade depending on nuclear size and mitotic activity ( high grade - showing greater than 3 fold variation in nuclear size and mitotic activity of >12/10 hpf)[8].

Following this all cases were subjected to IHC p16 and pattern of expression was noted as below -

Absent – negative staining in tumor cells (0% positive cells)

Heterogenous/normal expression – variable nuclear and cytoplasmic expression (1-50% positive nuclei)

Block/strong expression – diffuse staining of tumor cells in nuclear and/or cytoplasmic compartment with at least moderate intensity (51-100% positive nuclei)

Pattern of p16 expression was then correlated with histological subtype of epithelial tumor and with its grade benign, borderline and low grade or high grade epithelial cancer.

Statistical Analysis-All the data analysed was entered into a Microsoft word excel sheet each line representing epithelial ovarian tumors. The continuous or quantitative data analysis is presented as mean +/- SD (min-max) and the results of categorical data has been processed in percentages (%). SPSS software version 26 was used for descriptive statistics.

## 4. RESULTS

We took into consideration 35 ovarian epithelial tumors. The age group of the patients ranged from 15 to 72 years (Table-1). Maximum patients (25%) belonged to 5$^{th}$ decade.We encountered only one case below 20 years, whereas >70 years, comprised of 8.5% of the study population.Our findings highlighted that epithelial ovarian tumors most commonly presented in middle aged females with a notable decline in incidence in both younger and older age groups.

**Table1 : Age distribution of cases**

| Age Group (Years) | Number of Cases (n) | Percentage (%) |
|---|---|---|
| <20 | 1 | 2.8% |
| 21-30 | 6 | 17.1% |
| 31-40 | 8 | 22.8% |
| 41-50 | 9 | 25.7% |
| 51-60 | 4 | 11.4% |
| 61-70 | 4 | 11.4% |
| >70 | 3 | 8.5% |
| Total | 35 | 100% |

Most of the epithelial tumors showed serous differentiation (77%), mucinous tumors accounted for 20% of total. Apart from these two types only other epithelial tumor in our study was one case of benign Brenner tumor.

**In our study benign epithelial tumors constituted** largest proportion, accounting for **68.6%** of the total cases. Borderline and Low-grade carcinoma each contributed 5.7% of the total. **High-grade carcinoma formed 20%** of the total. Altogether malignant category formed 1/4$^{th}$ of samples i.e. 25.7%.

**Three types of pattern of expression of p16 IHC was noted – (Table2)**

| IHC p16 | Benign | Borderline | Low grade carcinoma | High grade carcinoma | Total |
|---|---|---|---|---|---|
| Absent | 2 ( Serous) | 1(Serous) | 0 | 0 | 3 (8.5%) |
| Heterogenous | 20 (15 Serous,5Mucinous) | 0 | 2 (Serous) | 0 | 22(63%) |
| Block positive | 2 1Brenner,1Serous | 1(mucinous) | 0 | 7 (6-Serous) 1-Mucinous | 10(28.5%) |
| Total | 24 | 2 | 2 | 7 | 35 |

1) Absent or negative tumor expression for IHC p16 was seen in 8.5% of cases (3/35), all the three cases were of serous nature with two benign and one borderline subtypes. Absent/negative staining was not observed in malignant cases.

2) Heterogenous pattern or normal pattern of staining was observed in 63% (22/35) . Majority-91% of such cases belonged to benign tumors and only minority (9%) were falling into low grade carcinoma. Again none of the high grade tumors expressed heterogenous pattern of IHC p16 staining.

Heterogenous expression in the category of benign tumors was majorly serous type (15/20-75%) and 25% were benign mucinous tumors. Two cases of low grade serous carcinomas encountered, both showed heterogenous pattern of expression with less than 50% of nuclei showing positivity.

3) Strong/Block positivity – This type of expression, where more than 50% of tumor cell nuclei showed strong staining could be observed in 28.5%(10/35) of cases. On further analysis of such cases, about 70% of them displayed high grade nuclear features. 20% of such cases were of benign nature and 10% borderline.

In our study we encountered seven high grade carcinomas. All of them i.e. 100% of them showed block or strong p16 expression on IHC. Majority of them (6/7-85.7%) were high grade serous carcinomas and one of high grade mucinous carcinoma.

None of the low grade malignancy revealed strong/block positivity.

Of the two borderline epithelial tumors, one showed strong positivity and had mucinous differentiation and the other was negative for p16 staining with serous differentiation.

In benign tumor category ( 2/24-8.3%) of them were found to express p16 strongly. One of them was Brenner and showed

urothelial differentiation and other was serous in nature.

## 5. DISCUSSION

In our study, age of the patient ranged from 15 to 72 years with mean of 50 years. In the study conducted by Prem et al [9] age ranged from 30-70 years. In their study, pre/perimenopausal group accounted for 67% of cases, in our case this age group accounted for 37% of cases. In study conducted by Mudi et al [10] the age range was 18–72 years with mean of 48 years, so our findings are in consensus with them. Maximum number of our patients belonged to 5$^{th}$ decade (25.7%) followed by those in 4th decade indicating that majority of surface epithelial tumors present in middle age women

**Table 3 : Association of p16 positivity with histological differentiation**

| WHO category | Absent | Heterogenous | Strong | Total |
|---|---|---|---|---|
| Serous tumors | 3 (2benign, 1 borderline) | 17 ( 15benign, 2low grade) | 7 (6 HGSC,1benign) | 27 |
| Mucinous | 0 | 5(benign) | 2(1borderline,1high grade) | 7 |
| Others (Brenner) | 0 | 0 | 1(benign) | 1 |
| Total | 3 | 22 | 10 | 35 |

Benign tumors accounted for 68.6% of total and malignant tumors 25.7%. Prem et al encountered 50% of benign and 23% malignant, so our findings were corroborating with their findings.

In the study published by Mudi et al, in which they evaluated 46 cases of surface epithelial tumors for p16 expression – they observed that 26% of them were benign and 74% were malignant, in their study they came across more malignant tumors, therefore our findings were contrary to them in this regard. Serous tumors in their study accounted for 79%, and mucinous tumors-17% ,which is close to what we also observed that serous tumors accounted for 77% and mucinous 20%.

With respect to benign cases, the present study observed that in 8.3% there was absence of expression, 83.3% heterogeneous expression, and 8.3% revealed strong expression. These results contrast sharply with Prem et al, who reported an 81% absence of expression, 15.6% heterogeneous expression, and 23.7% strong expression. Similarly, Manu et al[11] found a much higher absence rate of 30%, with 70% of cases exhibiting heterogeneous expression and no strong expression.

The significant disparity between the our study and Prem et al. may be attributed to slight variation in IHC grading technique and difference in sample sizes or population demographics.

The higher heterogeneous expression in benign tumors in the present study suggest that most of the benign tumors do not have alteration in G1/S pathway and therefore express normal p16 on IHC.

In cases of high-grade cancer, which predominantly were of serous differentiation (HSOC), notably none of them had absent or heterogenous expression. All of them revealed strong/block positivity for IHC p16. These findings contrast significantly with those reported by Prem et al., who observed a small proportion (1%) of cases that exhibited no expression, 56% of cases displayed heterogeneous expression and strong expression was seen in 73% cases. This disparity may be because they have not divided malignant tumors into low and high grade.

According to Rambau et al [12] findings, in which they studied 7492 cases of ovarian epithelial malignancy and graded the malignancy into low grade and high grade like us. In the subset of High grade serous carcinomas, 6.2% of cases lacked any expression, 37.5% exhibited heterogeneous expression, and 56.3% showed strong expression. In our case we reported strong expression in 100% of high grade carcinomas of which almost all were serous except one case which showed mucinous differentiation. In the subset of low grade cancers of which accounted for two of our cases – both revealed heterogenous or normal expression (100%), Rambau et al observed heterogenous expression in 81% of low grade serous cancers, but a small subset did express both absent and strong positivity, but since majority of low grade cancers expressed heterogenous expression and high grade cancers, majority revealed strong expression on IHC p16 in their and our studies, both are in concordance.

Another study conducted by Manu et al, serous ovarian tumors was also in concordance with our findings as none of their low grade serous cancer had strong expression and all of them had heterogenous (weak+moderate) expression i.e. 100% as our finding. They also observed strong p16 expression in 65% of high grade serous cancers, which is lower than that observed by us (100%).

Since all our high grade carcinomas expressed block or strong positivity on p16 immunostaining, which act as a surrogate for abnormal retinoblastoma pathway activation in contrast to borderline and low grade cancers that in majority expressed

heterogenous staining pattern, it is safe to infer that pathogenic pathways for both low grade and high grade epithelial carcinomas in ovary is different with mutations in RB1, CCNE1, CCND1 playing a central role.

## 6. CONCLUSION

Our study highlights the strong correlation between p16 overexpression and high grade epithelial carcinomas. IHC p16 expression can be used as potential diagnostic marker, particularly in cases where histological features are ambiguous.

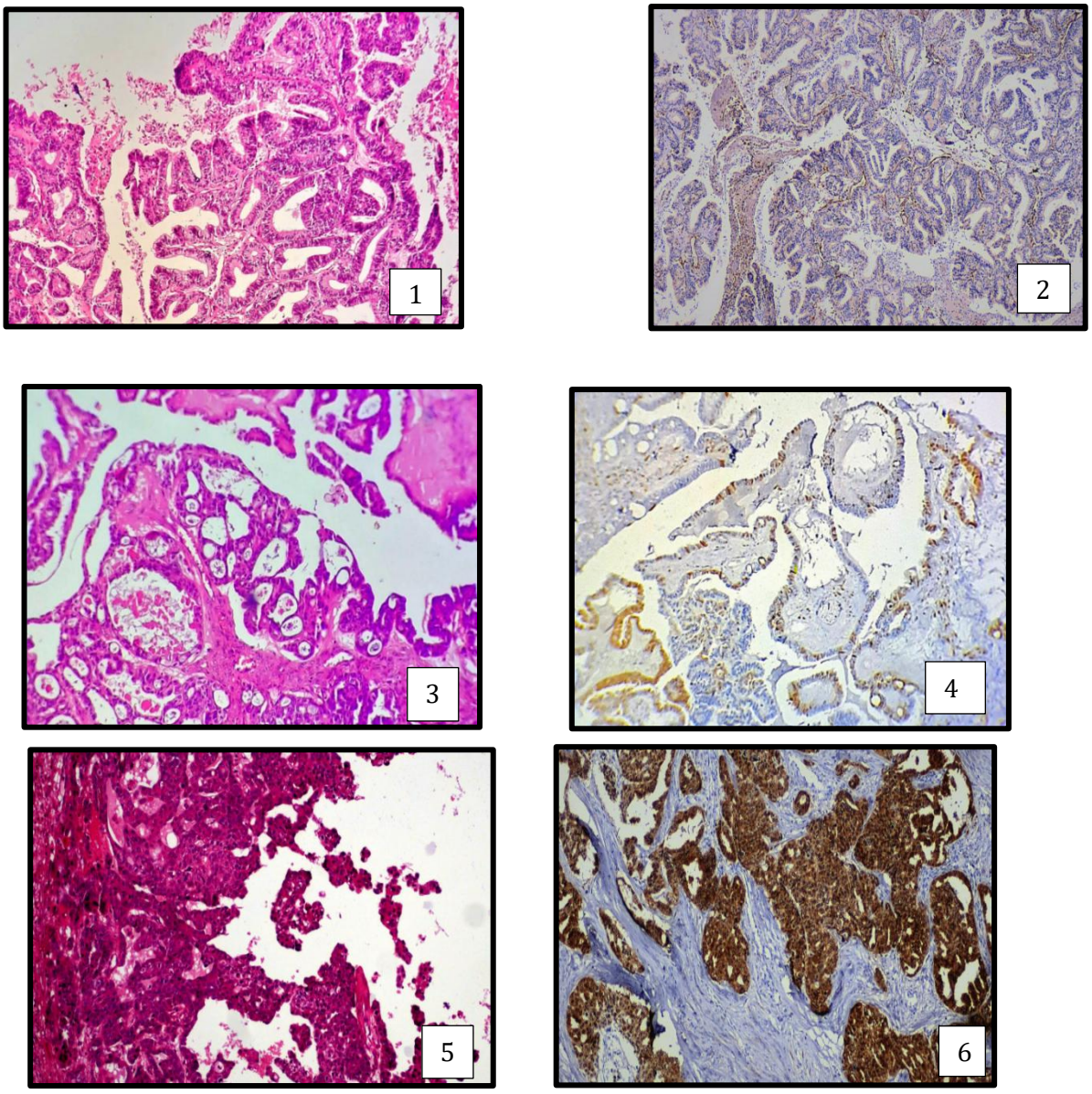


**Fig 1** H&E Stained Section at 10X of Serous Borderline Tumor

**Fig 2:** IHC p16 at 10X - Serous Borderline Tumor showing Absent expression

**Fig 3**: H&E Stained Section at 10X of Low grade serous carcinoma

**Fig 4**: IHC p16 at 10X - Low grade serous carcinoma showing heterogenous expression

**Fig 5:** H&E Stained Section at 10X of High grade serous carcinoma

**Fig 6**: IHC p16 at 10X - High grade serous carcinoma showing block positivity

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