EXPRESSION OF P16 IN SURFACE EPITHELIAL OVARIAN NEOPLASM

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Abstract

Background: *Epithelial ovarian carcinoma is the major subtype of ovarian cancer, one of the most lethal gynaecological malignancies. Due to some difficulties in early detection, patients are usually diagnosed at advanced stages, and overall survival is poor. P16 is a tumour suppressor gene that regulates the cell cycle by inhibiting S phase. Studying experssion of this immuno-histochemical marker will help to diagnose and predict prognosis of ovarian epithelial tumours. The study's objective was to evaluate the expression of p16 in surface epithelial ovarian neoplasm and its association with histo-pathological grading.*

Methodology: *This cross-sectional study was carried out at the Department of Pathology, Sir Salimullah Medical College, Dhaka. A total of 46 patients diagnosed histo-pathologically as surface epithelial ovarian neoplasm were included in the study. P16 immuno-staining, as well as some demographic and clinical data, were also evaluated.*

Result*: The mean age of the patient was 48.04 ± 12.182SD years. The most common histologic subtype was serious, followed by the mucinous type. P16 was positive in 40 (86.96%) cases. A statistically significant difference in p16 expression was observed between tumour types and between tumour grades. Up-regulation of p16 expression was observed in malignant tumours more than in benign tumours. P16 expression was increased with increased grading of the malignant tumours.*

Conclusion: *P16 expression is associated with histo-pathological grading in ovarian epithelial carcinoma.*

Keywords: *p16, immunohistochemistry, ovarian tumour*

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Introduction

Ovarian cancer is one of the most common gynecologic malignancies worldwide.[1] Among ovarian cancers, surface epithelial ovarian cancer is the most common.[2] Ovarian surface epithelial tumours can originate from normal ovarian surface epithelium itself or the crypts or inclusion cysts arising from this surface epithelium.[3] Epithelial ovarian cancer is called the most lethal gynaecological malignancy.[4]

Some risk factors are associated with the increasing incidence of ovarian cancer. These include positive family history (5–10% of cases are familial), increased age of the patient, presence of BRCA1 and BRCA2 oncogenes, obesity, increased meats and saturated fats intake, and other reproductive factors. Diagnosis of ovarian carcinoma is made by clinical examination, blood tests including tumour markers (most commonly CA-125), and

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imaging like ultrasonography, CT scan, MRI, PET scan, etc. Biopsy followed by histopathology is the gold standard. Sometimes, immunohistochemistry and some other molecular diagnostic tools are also important. Several clinicopathological features are correlated with prognosis. These include histological grade, subtype, and amount of residual tumour after operation and disease state.[5]

The wide morphological variation within and between the tumour groups can result in diagnostic difficulties. Due to the absence of significant symptoms in the early stage and difficulties in early detection and diagnosis of the disease, patients have been diagnosed in the late stage, and the overall survival of patients with ovarian cancer is poor.[6] Recent advancement in immuno-histochemistry is helping to remove diagnostic difficulties.

P16 is a tumour suppressor gene located on chromosome 9p21.[7] It is a cyclin-dependent kinase inhibitor and is essential in regulating the cell cycle.[8] P16 contributes to the regulation of cell cycle progression by inhibiting the S phase. It inactivates cyclin-dependent kinases that phosphorylate Rb. Therefore it can decelerate the cell cycle. Rb phosphorylation status influences the expression of p16.[8]

In malignant tumours, p16 overexpression occurs due to a mechanism by a tendency to arrest the uncontrolled proliferation caused by failure of the Rb pathway (secondary to viral infection, mutational silencing of the Rb gene or other mechanisms).[9]

Materials and methods:

The study was a cross-sectional observational study conducted among 46 diagnosed cases of surface epithelial ovarian neoplasm in the Department of Pathology, Sir Salimullah Medical College& Mitford Hospital, Dhaka, during the period of January 2019 to December 2020. Patients of any age group and histopathologically diagnosed cases of surface epithelial ovarian neoplasm were included in this study. The slides of the cases were reevaluated, and some parameters, including tumour type and tumour grading, were assessed. Representative sections from each paraffin block were selected for immunohistochemical stain with p16. In addition, formalin-fixed paraffin-embedded tissue sections were stained with p16 antibody using the standard protocol.

Histopathological evaluation:

From each paraffin block, one section was stained with Hematoxylin and Eosin (H & E) according to the standard protocol at the department of Pathology, Sir Salimullah Medical College and Mitford Hospital, Dhaka, Bangladesh. All the cases were reviewed, and the tumours were classified on H & E stained sections according to WHO classification (2014).

Evaluation of p16:

Cells with brown-coloured nuclear or cytoplasmic staining were considered positive for p16 expression. The immunohistochemistry staining results were scored semi-quantitatively according to the per-centages of positive cells. Here 0 means 0-1%; 1+ : 2-24% ; 2+ : 25-75%; 3+ : > 75 % . The scores 1+, 2+, and 3+ were considered positive for p16.[9]

Data processing and analysis:

Statistical analyses were conducted using Statistical Package for Social Sciences version 23 for Windows. A descriptive analysis was performed for all data. The mean values were calculated for continuous variables. Intergroup comparison was made using Pearson's chi-square test. The confidence interval was set at 95%. A "p" value <0.05 was considered significant.

Ethical issue:

Ethical clearance and permission were taken from the institutional ethical committee of Sir Salimullah Medical College. All study subjects were informed about the study's nature, purpose, and implication, as well as the entire spectrum of benefits and risks. Confidentiality was maintained by using a separate locker and computer password. The study did not include any additional investigation and financial burden to the patients. Subjects were also free to withdraw themselves from the study at any time. Finally, informed written consent in Bangla was taken from the patient or the patient's guardian.

Results:

This cross-sectional study was conducted on 46 histo-pathologically diagnosed surface epithelial ovarian tumor patients. Histopathology was done by H&E followed by immunohistochemistry for p16 expression. The mean (±SD) age of the participants was 48.04(±12.182) years. The age of the study patients ranged from 18 to 70 years. Total patients were grouped according to their age with a class interval of 10 years (Table I).

Table I

Distribution of patients according to age (n=46)

Age (Years)	Frequency	Percent (%)
<20	1	2.2
21-30	3	6.5
31-40	7	15.2
41-50	14	30.4
51-60	14	30.4
>60	7	15.2
Total	46	100.0

Among the benign and malignant tumors, serous (15.22% and 63.04% respectively) was the most common histological subtype. (Table II)

Table II

Distribution of study cases according to histo-pathological diagnosis (n=46)

Tumor type		No. of patient	Percentage %
Benign	Serous	7	15.22%
	Mucinous	4	8.69%
	Brenner	1	2.17%
Malignant	Serous	29	63.04%
	Mucinous	4	8.69%
	Endometrioid	1	2.17%

Out of 46 cases, the most common type was serous adenocarcinoma followed by serous cyst adenoma. Six cases showed no p16 expression, 16 cases showed weak expression, another 18 cases showed moderate expression and 6 cases showed strong p16 expression (Table III).

Table III

Distribution of p16 expression according to histo-pathological diagnosis (n=46)

Histopathological diagnosis	P16 expression				Total
	No expression	Weak	Moderate	Strong	
Serous adenocarcinoma	1	12	11	5	29
Serous cyst adenoma	5	1	1	0	7
Mucinous cyst adenoma	0	2	1	1	4
Mucinous adenocarcinoma	0	1	3	0	4
Brenner	0	0	1	0	1
Endometrioid carcinoma	0	0	1	0	1
Total	6	16	18	6	46

The level of p16 expression was significantly different (p=0.008) in benign and malignant tumors (Table IV).

Table IV

Association of tumor type with the level of p16 expression

Tumor type	P16 expression					P
	No expression	Weak	Moderate	Strong	Total	
Benign	5	3	3	1	12	0.008
Malignant	1	13	15	5	34	
Total	6	16	18	6	46	

The mean p16 expression was 18.75±19.786 (SD) in benign cases and 39.41±23.829 (SD) in malignant cases. The difference was statistically significant (p=0.01). Table V.

Table V

Association of tumor types and p16 expression (n=46)

Tumor type	Number of cases	Mean	Standard deviation	p
Benign	12	18.75	19.786	0.01*
Malignant	34	39.41	23.829	

Table VI

Distribution of p16 expression in malignant cases according to WHO grading (n=34)

		P16 expression					p
		No expression	Weak	Moderate	Strong	Total	
Malignant	Well-differentiated	1	4	4	0	9	0.033
Ovarian tumor	Moderately differentiated	0	8	8	1	17	
	Poorly differentiated	0	1	3	4	8	
Total		1	13	15	5	34	

Most of the well-differentiated cases (4) showed weak or moderate p16 expression, in moderately differentiated cases most of the cases (8) showed weak or moderate p16 expression and in poorly differentiated cases most of the cases (4) showed strong p16 expression. This difference in p16 expression is statistically significant (p=0.033) (Table VI).

Association was observed between the grading of malignant tumors and p16 expression**.** The mean p16 expression was 28.33±16.202 (SD) in grade 1, 34.12±16.977 (SD) in grade 2, and 63.13±29.147 (SD) in grade 3 tumors. The difference was statistically significant (p<0.05), showing an increase in mean p16 expression with increasing grade of malignant surface epithelial ovarian tumors.

Discussion

This cross-sectional study was carried out to determine the expression of p16 in surface epithelial ovarian neoplasm and its correlation with tumour progression. In addition, the present study findings were discussed and compared with previously published relevant studies.

In this study, it was observed that the mean age of the participants was 48.04±12.182 (±SD) years, with ages ranging from 18 to 70 years and the highest number of patients (32%) were in the age group of 41-50 and 51-60 years (Table I). One study observed that the age range of patients with serous ovarian neoplasm was 28-70 years, and the mean age was 52.3 ± 11.4(SD) years [10]. This finding is close to the current study. In another study, the mean age of the patients was 51.6±13 (SD) years (range 19-71 years), where there were only malignant cases. In the present study, the mean age of malignant cases was 52.21±9.935(SD), which is nearly similar to this study.[11] Baloch et al. (2008) found that age was significantly higher in patients with malignant tumours compared to patients with benign tumours. In our study, the mean age of the benign, malignant groups was 36.25±10.279(SD), and 52.21±9.935(SD), respectively, and the difference was statistically significant. So our finding is similar to the previous study findings.[12]

One study observed that the maximum number of 46 patients belonged to the age group 21-30 years (32%), followed by 41 to 50 years (22%). The mean age was 38.2±7.31 (SD), ranging from 16 to 69 years. Another study report stated that the mean age of the patients in her study was 38.39±13.23 (SD) years with a range of 15-70 years.[13,14] The mean age and age range obtained by the above authors differ from the current study, possibly due to the difference in the sample size of benign and malignant cases.

In this present study, histopathological diagnosis was made according to WHO (2014) classification system, and it was observed that out of 46 cases of surface epithelial ovarian tumours, 12 were benign and 34 were malignant cases. Ali (2016) found that out of 50 studied ovarian serous tumour cases, 12 cases were benign, eight were borderline, and the remaining 30 were malignant. The number of cases correlates with the study. In contrast to the study, Ali included only serous neoplasm, but the current study included all surface epithelial ovarian neoplasm.[15]

In this study, among the 34 malignant cases, 17(50%) cases were moderately differentiated, 9(26.47%) cases were well differentiated, and 8(23.52%) cases were poorly differentiated. Yang et al. (2020) found that 43.2% of cases were well, 32.9% were moderate, and 23.9% were poorly differentiated in their study. Histologic typing revealed 36 (78%) cases of serous subtype, 8(18%) cases of mucinous subtype, and only 1(2%) case each in benign Brenner and endometrioid carcinoma subtypes in this study. But they observed out of 310 cases, 208(67.1%) were serous adenocarcinoma and 48(15.5%) cases were mucinous adenocarcinoma.[16]

Regarding the degree of p16 expression, in the present study, it was observed that out of 46 cases, 6 cases showed strong expression, 18 cases showed moderate expression, 16 cases showed weak expression, and 6 cases showed no expression. Among the benign tumours, out of 12 cases, 5 cases showed no expression, 3 cases showed weak expression, another 3 cases showed moderate expression, and 1 case showed strong expression. In malignant tumours, out of 34 cases, 5 cases showed strong expression, 14 cases showed moderate expression, another 14 cases showed weak expression, and 1 case showed no expression of p16. A statistically significant difference in p16 expression between the benign and malignant groups (p=0.033) was observed in this study. Nazlioglu et al. (2010) found p16 expression significantly different from malignant tumours to benign tumours, similar to the current study.[7] Armes et al. (2005) reported that 90% of serous adenocarcinomas were positive for p16. Dong et al. (1997) observed most of the benign neoplasm showed no p16 expression in the tumour cells, whereas only 11% of malignant cancers were p16 negative.[17,18] This result is nearly similar to the present study.

In the present study within 34 cases of malignant tumours, 97.05% of cases showed positive p16 expression and 2.94% of cases showed no p16 expression. This result is nearly similar to the study by Armes et al. (2005).[17] In the current study, among the nine well-differentiated cases, 4 cases showed weak expression, another 4 cases showed moderate expression, and 1 case showed no p16 expression. Among the 17 moderately differentiated cases, 8 showed moderate expression, another 8 showed weak expression, and one showed strong expression of p16. Among the eight poorly differentiated cases, most of the cases showed strong expression of p16. The difference was statistically significant (p=0.002) when the ANOVA test was done to observe the staining pattern of p16 in well, moderate, and poorly differentiated epithelial ovarian carcinoma.

Several studies observed high p16 expression was associated with poorly differentiated ovarian carcinoma, which is similar to the present study.[18,19,20] On the other hand, Nazlioglu et al. (2010) found no significant difference in p16 expression between different grades of serous ovarian carcinoma, which is different from the current study.[17] It may be due to differences in tumour type, the difference in the threshold level of p16 positivity, and some technical aspects.

Limitations

Our study has several limitations. First, it was done in a limited period. Second, the sample size was small. Third, staging of the tumour was not done. Finally, only one case of endometrioid carcinoma and benign Brenner tumour was included.

Conclusion with recommendation

P16 expression showed a significant difference between tumour types and the malignant

tumour grades. In between tumour types and tumour grades, up-regulation of p16 expression was observed. So it is recommended that along with H&E (Haematoxylin and Eosin) p16 is a reliable biomarker for diagnosing ovarian tumours. It can be used precisely to determine the grade of malignant ovarian neoplasm. However, a much larger study needs to be done over a longer period with inter-laboratory standardization to truly determine the value of the biomarkers as a diagnostic and prognostic tool in surface epithelial ovarian neoplasm.

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