A Study on P16 Expression in Surface Epithelial Tumours of Ovary

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ABSTRACT

BACKGROUND

Ovarian cancer is the 6th most common cancer in women worldwide.¹ According to Indian Cancer registries, ovaries are the third leading sites of cancer among women.² 2/3rd of all ovarian neoplasms are surface epithelial tumours and the majority of endometrioid and clear cell carcinomas and some serous tumours show a poor prognosis. p16, a protein encoded by the gene, CDKN2A, located in chromosome 9p21.3, plays a major role in cell cycle regulation by hindering its progression through phase G1 to S. Many studies have found a significant prognostic implication of p16 on surface epithelial tumours and thereby identify such tumours and initiate early therapy. Our study was conducted to find out any significant association of p16 expression in ovarian surface epithelial tumours.

METHODS

This was a cross-sectional study, done in the Department of Pathology, Government Medical College, Thiruvananthapuram from March 2018 to March 2019. 118 cases were selected, IHC analysis was done and the data were analysed using statistical software SPSS version 20.0.

RESULTS

118 cases of ovarian surface epithelial tumours were chosen for the study of which 60 cases were benign, 28 were malignant and 20 were borderline tumours. Strong p16 expression was found in 50 % of malignant tumours and 81.6 % of benign tumours had weak expression. This was found to be statistically significant ($P=0.000$). Also, a comparison of p16 expression among the various subtypes of surface epithelial tumours was found to be statistically significant ($P < 0.01$).

CONCLUSIONS

The present study found a positive correlation between p16 expression in tumour cells and histopathological grades of surface epithelial ovarian tumours, with the highest expression noted in malignant tumours. A significant relation was observed between p16 expression and the subtypes of surface epithelial tumours with endometrioid and clear cell carcinomas showing the highest expression.

KEY WORDS

Surface Epithelial Tumours, Immunohistochemistry, p16 Expression

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BACKGROUND

Ovarian cancer is the 6th most common cancer in women worldwide and the 7th most common cause of cancer-related deaths.^{1,2,3} According to Indian Cancer registries, ovaries are the third leading sites of cancer among women, after cervix and breast.² 90 % of all the ovarian cancers and 2/3rd of all ovarian neoplasms are surface epithelial tumours with serous tumours at the top of the list, closely followed by mucinous and endometrioid tumours.^{4,5,6} Among ovarian neoplasms, 80 % are benign and are frequently seen in the age group of 20-45 years whereas borderline tumours are seen at an older age.^{7,8,9} Premenopausal and perimenopausal females form the predominant ones to develop malignant epithelial tumours.^{10,11}

Across the globe many mutational studies on surface epithelial ovarian tumours are now going on, to establish a proper genetic classification for determining adequate treatment protocols and proper prognostic assessment. The major ones being studied are p53, KRAS, ERBB2, BRAF & PTEN.^{12,13} p16 is yet another protein studied in this context. p16, also known as p16^{INK4a} or MTS1 (multiple tumour suppressor 1) is a protein encoded by gene CDKN2A in humans and is located in chromosome 9p21.3, its major role is in cell cycle regulation by hindering the progression of the cell cycle through phase G1 to S.^{14,15}

Many studies have found a significant prognostic implication of p16 on surface epithelial tumours. p16 along with p53 is involved in the regulation of the cell cycle and hence considered by many authors as indicators of tumour aggressiveness and therefore the prognosis.^{16,17} Normal p16 expression is heterogeneous (both nuclear and cytoplasmic) and an overexpression or block positivity is considered to be abnormal.¹⁸

A significant association with tumour grade and p16 expression, i.e. stronger p16 expression in malignant tumours compared to borderline and benign tumours has been found.^{15,17}

Thus, those epithelial tumours that show an abnormal p16 expression are said to have a poor prognosis.¹⁹ Our purpose was to study the expression of p16 in various histopathological grades of surface epithelial tumours of the ovary and also study the difference in p16 expression in various subtypes of surface epithelial tumours.

METHODS

This was a cross-sectional study conducted in the Department of Pathology, Government medical college Thiruvananthapuram, from March 2018 to March 2019 after obtaining human ethics committee clearance.

This was a record-based study and all specimens that were histopathologically diagnosed as surface epithelial ovarian neoplasms were included.

Sample Size

Was 118 calculated by using the formula

$$4pq/d^2$$

$$\{p=45.7\%, q=100-p (54.3), d = 20\% \text{ of } p = 9.14\}^{(19)}$$

Sampling Method

The first 118 consecutive specimens received during the period of study were included.

Study Tools

Formalin-fixed, paraffin-embedded blocks of all diagnosed cases of surface epithelial neoplasms of the ovary.

IHC marker p16

Paraffin-embedded, tissue blocks of the cases were selected, 3-5 micron thick sections were taken and stained with p16 IHC marker and the slides were examined for their expression. The p16 expression was scored based on the nuclear and cytoplasmic positivity as follows:

Immunohistochemical Scores²⁰

0 (0 % positive cells) negative

1 (1–10 % positive nuclei) - weak

2 (11–50 % positive nuclei) - intermediate

3 (51–100 % positive nuclei) - strong

Data Analysis

Categorical and quantitative variables were expressed as frequency (percentage). The chi-square test was used to find an association between categorical parameters. $P < 0.05$ was considered the threshold for statistical significance. Statistical analysis was performed by using a statistical software package SPSS, version 20.0

RESULTS

Age Distribution

The age group of patients chosen for our study ranged from 30 – 70 years, of which 67 % of patients were in the pre/perimenopausal age group.

Distribution of the Sample According to Histopathological Subtype

Among the 118 cases of surface epithelial tumours studied, 60 cases were benign, 28 were malignant and 20 were borderline tumours. The majority of these (46 cases) were serous tumours, closely followed by mucinous (40 cases) and endometrioid tumours (40 cases) and only 6 clear cell carcinomas and 3 each of Brenner and seromucinous tumours were obtained.

Association of p16 Score and Histopathological Grades of Surface Epithelial Tumours

Strong p16 expression was found in 50 % of malignant tumours and 81.6 % of benign tumours had weak expression. This association was found to be statistically significant ($P=0.000$)

p16 Score	Histopathological Grade						Total	Tau - b	P-Value
	Benign		Borderline		Malignant				
	No.	%	No.	%	No.	%			
Weak/Negative	49	81.66	10	16.67	1	1.67	60	0.62	0.000
Intermediate	5	15.63	9	28.13	18	56.26	32		
Strong/Diffuse	6	23.07	1	3.84	19	73.07	26		
Total	60	50.85	20	16.95	38	32.20	118		
Table 1. Association of p16 Score and Histopathological Grades of Surface Epithelial Tumours									

Table 1. Association of p16 Score and Histopathological Grades of Surface Epithelial Tumours

Figure A. Serous Cystadenoma, B. Mucinous Cystadenoma, C. Serous Carcinoma, D. Mucinous Carcinoma, E. Clear Cell Carcinoma, F. Endometrioid Carcinoma

Figure A. Weak p16 – Serous Cystadenoma, B. Intermediate p16 – Borderline Serous Tumour, C, D, E Strong p16 – Serous Carcinoma, Clear Cell Carcinoma, Endometrioid Carcinoma respectively, F. Weak p16 – Mucinous Carcinoma

Comparison of Histopathological Subtypes and Their p16 Scores

A significant association was found between p16 expression and the histopathological subtypes ($P < 0.01$). 80 % of endometrioid tumours and 66.7 % of clear cell tumours showed strong p16 expression. 67.5 % of mucinous tumours and 100 % of the Brenner tumours showed weak p16 expression.

Histopathological Sub Type	Weak/Negative		Intermediate		Strong/Diffuse		χ^2	P-Value
	Count	%	Count	%	Count	%		
Serous	28	60.9	12	26.1	6	13.0	67.6	$p < 0.01$
Mucinous	27	67.5	13	32.5	0	0.0		
Endometrioid	0	0.0	4	20.0	16	80.0		
Clear cell	1	16.7	1	16.7	4	66.7		
Brenner	3	100.0	0	0.0	0	0.0		
Seromucinous	1	33.3	2	66.7	0	0.0		

#Chi-square test

Table 2. Comparison of Histopathological Subtypes Based on p16 Score

DISCUSSION

In our study, we attempted to find out the difference in p16 immunohistochemical expression in various grades and subtypes of surface epithelial tumours of the ovary. Out of a total of 118 cases studied in 1 year period, the majority of patients (67 %) were in the pre/perimenopausal age group. Among the 118 cases of surface epithelial tumours studied, 60 cases were benign, 28 were malignant and 20 were borderline tumours. The majority of these (46 cases) were serous tumours, closely followed by mucinous (40 cases) and endometrioid tumours (40 cases) and only 6 clear cell carcinomas and 3 each of Brenner and seromucinous tumours were studied. Strong p16 expression was found in 50 % of malignant tumours and 81.6 % of benign tumours had a weak expression which is similar to a study done by Marinas et al.²¹

In the study by Cabral et al., they found that the difference in p16 expression with different grades of the tumour was statistically significant ($P=0.000$) which is comparable to our study ($P=0.000$).²⁰ In our study, we found that the difference in expression of p16 among the various histopathological subtypes of surface epithelial tumours was statistically significant ($P < 0.01$). This was comparable with a study by Cabral et al.²⁰ ($P=0.034$) and Rambau et al. ($P=0.0001$)¹⁸ in contrast the study by Nazgliou et al. found no such significant relation.¹⁷

CONCLUSIONS

The present study found a positive correlation between p16 expression in tumour cells and histopathological grades of surface epithelial ovarian tumours, with the strongest expression noted in the malignant tumours. Also, a significant relation was observed between p16 expression and the histopathological subtypes of surface epithelial tumours with endometrioid and clear cell carcinomas showing the strongest expression. However, further studies are required to unravel the association of p16 with patient survival and to establish its prognostic utility in surface epithelial tumours. As the attempts to develop p16 targeted therapy are underway in different parts of the world, such a significant relationship may aid in the early identification of such patients, their further management and hence improvement in their overall survival.

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

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REFERENCES

- [1] Boyle P, Levin B. World Cancer report 2008. Cancer Control 2008.
- [2] Parkin DM, Bray F, Ferlay J, et al. Global Cancer Statistics, 2002. CA Cancer J Clin 2005;55(2):74-108.

- [3] Basu P, De P, Mandal S, et al. Study of 'patterns of care' of ovarian cancer patients in a specialized cancer institute in Kolkata, eastern India. *Indian J Cancer* 2009;46(1):28-33.
- [4] Walker RA. World Health Organization Classification of Tumours. Pathology and genetics of tumours of the breast and female genital organs. *Histopathology* 2005;46(2):229.
- [5] Clement PB, Young HR. Ovarian surface epithelial-stromal. In: Mills SE, Carter D, Greenson JK, eds. *Sternberg's diagnostic surgical pathology*. 5th edn. tumours. Lippincott Williams & Wilkins 2010:2272-306.
- [6] Kanthikar SN, Dravid NV, Deore PN, et al. Clinico-histopathological analysis of neoplastic and non-neoplastic lesions of the ovary: a 3-year prospective study in Dhule, north Maharashtra, India. *J Clin Diagn Res* 2014;8(8):FC04-7.
- [7] McCluggage WG. Morphological subtypes of ovarian carcinoma: a review with emphasis on new developments and pathogenesis. *Pathology* 2011;43(5):420-32.
- [8] Naik P, Deshmukh S, Khandeparkar SGS, et al. Epithelial ovarian tumors: clinicopathological correlation and immunohistochemical study. *J Midlife Health* 2015;6(4):178-83.
- [9] Prat J. Ovarian carcinomas: five distinct diseases with different origins, genetic alterations, and clinicopathological features. *Virchows Arch* 2012;460(3):237-49.
- [10] Modepalli N, Venugopal SB. Clinicopathological study of surface epithelial tumours of the ovary: an institutional study. *J Clin Diagnostic Res* 2016;10(10):EC01-4.
- [11] Swamy GG, Satyanarayana N. Clinicopathological analysis of ovarian tumors--a study on five years samples. *Nepal Med Coll J* 2010;12(4):221-3.
- [12] Kurman RJ, Visvanathan K, Roden R, et al. Early detection and treatment of ovarian cancer: shifting from early stage to minimal volume of disease based on a new model of carcinogenesis. *Am J Obstet Gynecol* 2008;198(4):351-6.
- [13] Makhija P, Singh N. Surface epithelial tumours of the ovary. In: Farghaly SA. *Advances in diagnosis and management of ovarian cancer*. Springer 2014:59-86.
- [14] Stone S, Jiang P, Dayananth P, et al. Complex structure and regulation of the P16 (MTS1) locus. *Cancer Res* 1995;55(14):2988-94.
- [15] Romagosa C, Simonetti S, López-Vicente L, et al. P16Ink4a overexpression in cancer: a tumor suppressor gene associated with senescence and high-grade tumors. *Oncogene* 2011;30(18):2087-97.
- [16] Kudoh K, Ichikawa Y, Yoshida S, et al. Inactivation of p16/CDKN2 and p15/MTS2 is associated with prognosis and response to chemotherapy in ovarian cancer. *Int J Cancer* 2002;99(4):579-82.
- [17] Nazlioglu HO, Ercan I, Bilgin T, et al. Expression of p16 in serous ovarian neoplasms. *Eur J Gynaecol Oncol* 2010;31(3):312-4.
- [18] Rambau PF, Vierkant RA, Intermaggio MP, et al. Association of p16 expression with prognosis varies across ovarian carcinoma histotypes: an Ovarian Tumor Tissue Analysis consortium study. *J Pathol Clin Res*. 2018;4(4):250-61.
- [19] Khouja MH, Baekelandt M, Nesland JM, et al. The clinical importance of Ki-67, p16, p14, and p57 expression in patients with advanced ovarian carcinoma. *Int J Gynecol Pathol* 2007;26(4):418-25.
- [20] Cabral VD, Cerski MR, Sa Brito IT, et al. P14 expression differences in ovarian benign, borderline and malignant epithelial tumors. *J Ovarian Res* 2016;9(1):69.
- [21] Marinaş MC, Mogoş DG, Simionescu CE, et al. The study of p53 and p16 immunoexpression in serous borderline and malignant ovarian tumors. *Rom J Morphol Embryol* 2012;53(4):1021-5.