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IMMUNOHISTOCHEMICAL EXPRESSION OF P53 AND P16 IN SEROUS NEOPLASMS OF OVARY

Histopathology

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ABSTRACT

Introduction:- The morphological aspects and histological grades of serous ovarian carcinomas provide important diagnostic information, but immunohistochemistry is an important tool that is used in differential diagnosis and in the evaluation of molecular features of serous ovarian neoplasms. **Objectives:-** 1.Histomorphological study and categorization of serous neoplasms of ovary. 2. Expression of p16 and p53 in various subtypes and histological grades. **Materials And Methods:-** A cross sectional study was done using seventy two samples diagnosed as High Grade and Low Grade Serous ovarian carcinomas. Molecular expression of p53 and p16 was studied by immunohistochemical examination. Statistical analysis was done by Chi-square test and Fisher Exact Test using Statistical Package for the Social Science(SPSS) version 25.0. **Results:-** In the present study, most of the patients were in the 5th decade at the time of presentation (44.44%), followed by 4th decade (26.39%). p53 showed positivity in 82.46% cases of High grade and 13.33% cases of Low grade Serous Tumours. 61.40% cases of High grade and 6.67% cases of Low Grade Serous tumours were positive for p16. **Conclusion:-** Therefore, it can be concluded that p53 staining is a robust marker of HGSC. HGSCs mostly exhibited diffuse and strong expression of p16 in comparison to LGSCs.

KEYWORDS

Immunohistochemistry, Ovary and Serous carcinoma.

INTRODUCTION

Ovarian cancer is among the top five cancers that occur in Indian women. A study period from 2001 to 2006, showed the incidence rates (standardized for age) were from 0.9 to 8.4 per 100,000 people.[1,2] The incidence of occurrence of ovarian cancer in India is lower than the rates in the western world. Owing to poor awareness, long relatively asymptomatic latency period, and inadequate diagnostic infrastructure, most women present late in advanced stages of the disease,[3] which results in poor overall survival.

Ovarian carcinomas have conventionally been classified based on the histology of the primary cell type.[4] This is the method which is currently followed in the World Health Organization's classification of ovarian carcinoma.[5]

A binary pathway for development of ovarian serous tumors has been proposed which is based on the grade of tumor. According to that, high-grade ovarian serous carcinoma (HOSC) seems to directly arise as cancer from the ovarian surface epithelium or at the fimbrial end of the fallopian tubes or from the epithelium of the cortical inclusion cysts.[6,7] These tumors do not have a definitive precursor lesion and are strongly associated with mutations of the p53 tumor suppressor gene.[6,7,8,9] Low-grade ovarian serous carcinoma (LOSC), has been seen to evolve from a benign cystadenoma through the stage of a borderline serous tumor in a sequential stepwise fashion. The LOSC have more mutations in B-RAF and K-RAS genes than in p53 gene than the HOSC.[10,11] Thus, a different set of genes and entirely different pathways are involved in the different grades of ovarian serous carcinoma, and it does not seem to be a simple step-wise gradient as previously thought.

High-grade serous ovarian carcinomas (HGSOCs) comprise the majority of serous ovarian carcinomas (SOCs), as they account for 80%–90% of these tumours and are the most aggressive [12,13,14]. The morphological aspects of SOCs and their histological grades provide important diagnostic information, but immunohistochemistry (IHC) is an important tool that is used in differential diagnosis and in the evaluation of molecular features, which further aids in the characterization of morphology and clinical behaviour[15].

Serous ovarian carcinoma is divided into two major groups: (1) low-grade carcinoma and (2) high-grade carcinoma. This distinction is made based on the degree of nuclear atypia and correlates with patient survival. Low-grade carcinomas may arise in association with serous borderline tumors, while high-grade carcinomas arise from in situ lesions in the fallopian tube fimbriae or from serous inclusion cysts within the ovary.[16]

Regardless of their origin, studies have shown that low and high-grade serous carcinoma have distinct mutational profiles, as follows[16]:

Low-grade tumors arising in serous borderline tumors have mutations in the KRAS, BRAF, or ERBB2 oncogenes, and usually have wild-type TP53 genes.

High-grade tumors have a high frequency of TP53 mutations and lack mutations in either KRAS or BRAF. Almost all ovarian carcinomas arising in women with BRCA1 or BRCA2 mutations are high-grade serous carcinomas with TP53 mutations.

OBJECTIVES:-

1. Histomorphological study and categorization of serous neoplasms of ovary.
2. Expression of p16 and p53 in various subtypes and histological grades of serous ovarian neoplasms.

MATERIALS AND METHODS:-

This study was conducted on a group of patients admitted in Department of Gynaecology and Obstetrics, R G Kar Medical College & Hospital, Kolkata & Department of Pathology, R G Kar Medical College & Hospital, Kolkata. Approval from an institutional review board was obtained at the initiation of the study. All patients were provided with signed informed written consent.

Inclusion Criteria:

The specimens of adnexal masses sent to the Department of Pathology, by unilateral or bilateral salphingo oophorectomy with or without hysterectomy.

Exclusion Criteria:

1) History of trauma
2) Any history of previous chemo or radiotherapy for other type of cancer
3) History of neo-adjuvant chemotherapy or radiotherapy in ovarian neoplasm specimens.

Ultra thin sections (3-4microns) are obtained by microtome from formalin fixed paraffin embedded blocks. After floatation they were picked on poly-L-lysine coated slides, dried, deparaffinized and rehydrated in descending grades of alcohol.

Then heat induced epitope retrieval (HIER) was done with Tris Hydroxymethyl Aminomethane(TRIS) buffer by using microwave method(EMPARTA, pH 9.0). TRIS Buffer(EMPARTA, pH 7.2) was used for washing. Endogenous peroxidase activity was blocked using PolyExcel Peroxidase Block (PATHNSITU) incubation with primary antibody (Monoclonal antibody against anti-human p53 protein and

anti-p16) was done at 37°C for 60 minutes. For visualisation of result, serial incubation for 30 minutes was done each was done with PolyExcel Target Binder(PATHNSITU); Poly Horse Radish Peroxidase (HRP) (PolyExcel Stunn DAB Detection System, PATHNSITU) and chromogen (PolyExcel Stunn Dia DAB Buffer and PolyExcel Stunn Diaminobenzidine(DAB) Chromogen, PATHNSITU). The sections were then counterstained with Harris Hematoxylin and mounted.

Positive control: Cases of Invasive Breast Carcinoma, known positive for p53. Cases of cervical cancer, known positive for p16.

Negative control: Supplied by vendor. Expression of p53 and p16 were done by semiquantitative method using light microscopy.

IHC Interpretation Of P53 Immunostaining[23]

Intensity of stain	Interpretation
No nuclear staining of tumour cells(complete absence)	Negative
Nuclear staining of >1% and <70% of tumour cells	Focal Positive
Nuclear staining of >70% of tumour cells	Diffuse Positive

IHC Interpretation Of P16 Immunostaining[23]

Cytoplasmic and nuclear p16 staining were described as follows:
Cases in which >90% of cells were stained were considered positive, and cases in which <90% of the cells were stained were considered negative.

Statistical Analysis:-

The data was collected and analysed by Chi-square test and Fisher Exact Test using SPSS , version 25.0.

RESULTS

In our study, maximum number of cases were in 5th decade (44.44%), followed by 4th decade (26.39%). The youngest and oldest member of the study population were one patient of 34 years old and one of 75 years old respectively. Mean age of presentation was 54.02 years, median 55 years, and standard deviation is 9.13. **Table 1** reveals that the High Grade Serous Tumours showed the peak with 34.8% cases occuring in the age group of 51-60 years and the Low Grade Serous tumours showed the peak with 8.33% cases occuring in the age group of 31-40 years. Thus, it could be stated that low grade tumours were clustered two decades earlier than the high grade tumours.

Table 1 : Distribution of the type of tumour according to the age of the patient(n=72)

Age (Years)	HGSOC	LGSOC	Total
31-40 Row percentage Column percentage	2 25% 3.5%	6 75% 40%	8 100% 11.1%
41-50 Row percentage Column percentage	17 77.3% 29.7%	5 22.7% 33.3%	22 100% 30.6%
51-60 Row percentage Column percentage	25 92.6% 43.9%	2 7.4% 13.3%	27 100% 37.5%
61-70 Row percentage Column percentage	12 92.3% 21.1%	1 7.7% 6.7%	13 100% 18.1%
71-80 Row percentage Column percentage	1 50% 1.8%	1 50% 6.7%	2 100% 2.7%
Total Row percentage Column percentage	57 79.2% 100%	15 20.8% 100%	72 100% 100%
CHI SQUARE STATISTIC VALUE	DEGREES OF FREEDOM	P VALUE	IMPRESSION
19.623	4	0.000593	SIGNIFICANT

Table 2 shows that 43.06% (31/72) cases of the serous tumours were less than 10 cm in maximum dimension and 56.94% (41/72) cases were ≥ 10 cm in maximum dimension. Data analysis showed that p value is 0.5593, so it is an insignificant study.

Table 2: 2X2 Contingency Table showing Association between histological grade and Maximum dimension of tumours

Grade	Maximum Dimension (<10 cm)	Maximum Dimension (>10 cm)	Total
HGSOC Row percentage Column percentage	26 45.6% 83.9%	31 54.4% 75.6%	57 100% 79.2%
LGSOC Row percentage Column percentage	5 33.3% 16.1%	10 66.7% 24.4%	15 100% 20.8%
Total Row percentage Column percentage	31 43.1% 100%	41 56.9% 100%	72 100% 100%
CHI SQUARE STATISTIC VALUE	DEGREES OF FREEDOM	Asymptomatic Significance (2-sided)	IMPRESSION
0.7305	1	0.392732	NOT SIGNIFICANT
FISHER'S EXACT TEST		Exact Significance (2-sided)	IMPRESSION
		0.5593	NOT SIGNIFICANT

Table 3 shows the distribution of ovarian tumours on the basis of the intactness of the capsule. We found that 58.3% (42/72) cases of serous tumours had intact ovarian capsule.

In the present study, on cut section of the tumours were of three types: solid- cystic, solid-cystic with papillary projections and solid-cystic(necrotic). **Table 4** shows that 72.2% cases were solid-cystic, 15.3% cases were solid-cystic with papillary projections and 12.5% cases were solid-cystic with necrotic component. 81.8% of the cases with papillary projections were later diagnosed as High grade serous carcinoma.

Table 3: 2x2 Contingency table showing association between type of tumours and intactness of the capsule

Type	Intact capsule	Capsular breach	Total
HGSOC Row percentage Column percentage	28 49.1% 66.7%	29 50.9% 96.7%	57 100% 79.2%
LGSOC Row percentage Column percentage	14 93.3% 33.3%	1 6.7% 3.3%	15 100% 20.8%
Total Row percentage Column percentage	42 58.3% 100%	30 41.7% 100%	72 100% 100%
CHI SQUARE STATISTIC VALUE	DEGREES OF FREEDOM	Asymptomatic Significance (2-sided)	IMPRESSION
9.5495	1	0.002	SIGNIFICANT
FISHER'S EXACT TEST		Exact Significance(2-sided)	IMPRESSION
		0.0024	SIGNIFICANT

Table 4: 2x2 Contingency table showing association between type of tumours and gross appearance

Grade	Solid-Cystic	Solid-Cystic with Papillary projections	Solid-Cystic (Necrotic)
HGSOC	40	9	8
LGSOC	12	2	1

Chi-Square Test of Table 4

Pearson Chi-Square	Value	Degree of freedom	Asymptomatic Significance(2-Sided)
	0.7214	2	0.69714

Table 5 shows that 82.46% cases (47/57) of High grade and 13.33% cases (2/15) Low grade Serous Tumours were positive for p53. Data analysis shows that pvalue is < 0.00001, so it is a highly significant study. **Table 6** shows that 61.40% cases(35/57) of High grade and 6.67% cases (1/15) of Low Grade Serous tumours were positive for p16.

Table 5: 2x2 Contingency table showing association between

Histological Grade and p53 staining

Grade	P53 positive	P53 negative	Total
HGSOC	47	10	57
Row percentage	82.5%	17.5%	100%
Column percentage	95.9%	43.5%	79.2%
LGSOC	2	13	15
Row percentage	13.3%	86.7%	100%
Column percentage	4.1%	56.5%	20.8%
Total	49	23	72
Row percentage	68.1%	31.9%	100%
Column percentage	100%	100%	100%
CHI SQUARE STATISTIC VALUE	DEGREES OF FREEDOM	Asymptomatic Significance (2-sided)	IMPRESSION
26.0986	1	0.00001	SIGNIFICANT
FISHER'S EXACT TEST		Exact Significance(2-sided)	IMPRESSION
		0.00001	SIGNIFICANT

Table 6: 2x2 Contingency table showing association between Histological Grade and p16 staining

Grade	P16 positive	P16 negative	Total
HGSOC	35	22	57
Row percentage	61.4%	38.6%	100%
Column percentage	97.2%	61.1%	79.2%
LGSOC	1	14	15
Row percentage	6.7%	93.3%	100%
Column percentage	2.8%	38.9%	20.8%
Total	36	36	72
Row percentage	50%	50%	100%
Column percentage	100%	100%	100%

DISCUSSION

In the present study, most of the patients were in the 5th decade at the time of presentation (44.44%), followed by 4th decade (26.39%). The mean age at presentation was 54.02 years with standard deviation 9.13 and median 55 years. The High Grade Serous Tumours showed the peak with 34.8% cases occuring in the age group of 51-60 years and the Low Grade Serous tumours showed the peak with 8.33% cases occuring in the age group of 31-40 years. Thus, it could be stated that low grade tumours were clustered two decades earlier than the high grade tumours.

Our study results corroborated with **Pooja S. Naik et al**(2015)[17], **Sylvia et al** (2012)[18], but slightly differed from **Nalini Modepalli et al**(2016)[19] where they described that the mean age of diagnosis of surface epithelial tumours was 42.4 years and the age varied from 14-76 years. Small sample size and limited study duration might be the reason behind this disparity.

43.06% (31/72) cases of the serous tumours were less than 10 cm in maximum dimension and 56.94% (41/72) cases were $\geq$ 10 cm in maximum dimension. Data analysis showed that p value is 0.5593, so it is an insignificant study.

Hence, our study showed that there was no relation between type and size of the tumour. This finding was similar to the study conducted by **Purti Agrawal et al** (2015)[21] where they described that the size of the tumor was not related to the nature of the tumor.

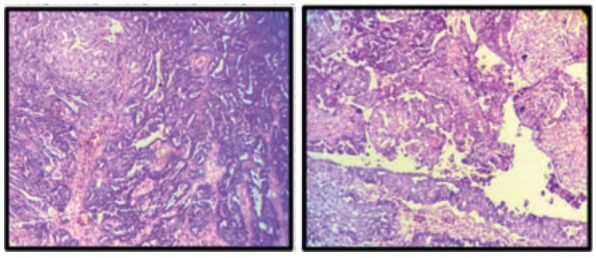
We found that 58.3% (42/72) cases of serous tumours had intact ovarian capsule.

In the present study, on cut section of the tumours were of three types: solid- cystic, solid-cystic with papillary projections and solid-cystic(necrotic). 72.2% cases were solid-cystic, 15.3% cases were solid-cystic with papillary projections and 12.5% cases were solid-cystic with necrotic component. 81.8% of the cases with papillary projections were later diagnosed as High grade serous carcinoma.

Our study results were similar to the study by **Seung Eun Jung et al** (2002)[22], where they concluded that epithelial tumors are primarily cystic and, when malignant, are associated with varying proportions of a solid component and papillary projections are a distinctive feature of epithelial tumors.

After the clinical details and the macroscopic study, histopathological examination was done.

As per this study, Low Grade Serous Carcinoma (LGSC) constituted 20.83% cases (15/72) and High Grade Serous Carcinoma (HGSC) constituted 79.17% (57/72) cases.



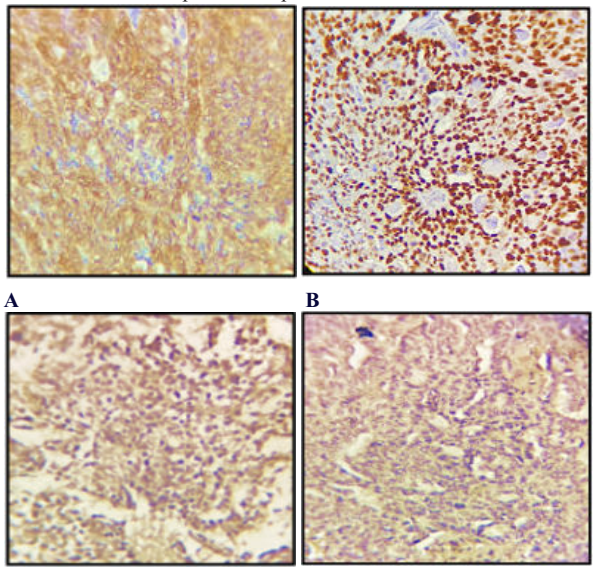
A B

Fig.1 a.Photomicrograph showing High grade Serous Carcinoma, ovary (H&E, Mag:X100), b.Photomicrograph showing Low grade Serous Carcinoma,ovary (H&E,Mag:X100)

This type of distribution of cases can be attributed to the nature of sample selection in our study. Hence this study suffered from hospital study biases.

Further, immunohistochemistry for p53 and p16 was done for all the ovarian tumours.

82.46% cases (47/57) of High grade and 13.33% cases (2/15) Low grade Serous Tumours were positive for p53. Data analysis shows that pvalue is < 0.00001, so it is a highly significant study. 61.40% cases(35/57) of High grade and 6.67% cases (1/15) of Low Grade Serous tumours were positive for p16.



A B C D

Fig.2 a.Photomicrograph showing High Grade Serous Carcinoma (p53, Mag:X400), b.Photomicrograph showing Low Grade Serous Carcinoma (p53, Mag:X400), c.Photomicrograph showing High Grade Serous Carcinoma (p16, Mag:X400),d.Photomicrograph showing Low Grade Serous Carcinoma (p16, Mag:X400)

The above results were similar to the study by **Luis Felipe Sallum et al**(2018)[23] where they stated that p53 expression was diffuse in 68.2% and was completely absent (null type) in 30.6% of women with HGSOCs (totalling 98.8% of cases). p16-positive IHC expression (i.e., ≥90% expression) was observed in 58.5% of women with HGSOC.

These findings were concordant with the study by **V.Manu et al**(2018)[24] .In their study, they found p53 staining to be a robust marker of HOSC and HOSCs mostly exhibited diffuse and strong expression of p16 in comparison to LOSCs.

Our study findings were similar to the study conducted by **O'Neill CJ et al**(2007)[25] which stated that the increased expression of p16 in high-grade serous carcinoma compared with low-grade serous carcinoma

and serous borderline tumour is in keeping with a different underlying pathogenesis.

These findings were concordant with the study by **O'Neill CJ, Deavers MT, Malpica A, Foster H, McCluggage WG**(2005)[26] which stated there was a statistically significant higher expression of p53, MIB1, BCL2, HER-2/neu, and C-KIT in high-grade compared with low-grade OSC.

However the study had few limitations: 1)Study on a larger cohort of patients could have provided statistical results more representative of the epidemiology of the disease in the population. 2)The follow up of the patients was outside the preview of the study due to the restricted time period. 3)All the cases in the study were selected after a radiological evidence of ovarian tumour was indicated. Hence, the study suffered from biases like Berkesonian bias and other types of biases in a hospital based study. 4)Conduction of ancillary investigations like BRCA mutation study was constrained by finance and minimal infrastructural facilities.

CONCLUSION

The IHC p53 and p16 and the morphological classification are closely matched, and the p53 and p16 seems to be a good marker for the differentiation of LGSOC and HGSOC. In the evaluation of a new case of SOC, the initial assessment should be performed according to WHO morphologic criteria. In doubtful cases, IHC should be performed. Thus, p53 and p16 expression have an important role in the routine differential diagnosis of ovarian carcinoma.

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