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Assessment of Clinicopathological Correlation and Immunohistochemical Profile of Ovarian Tumours

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Abstract

Background: Ovarian cancer is the sixth most frequent malignancy among women and the seventh major cause of cancer death among women worldwide. The present study was conducted to assess clinicopathological correlation and immunohistochemical profile of ovarian tumours.**Material and Methods:**The study was a prospective study conducted over a period of eighteen months (2020 to 2021) at Department of Pathology, Government Medical College and Rajindra Hospital, Patiala, Punjab. Clinical history of the patient including the age, examination findings, radiological investigation, USG, CT and FNAC reports were evaluated in detail. Oophrectomy specimens were fixed in 10% neutral buffered formalin and multiple sections were taken for histopathological examination. 3-4 microns thick sections were cut and stained with Hematoxylin & Eosin (H & E). The H &E-stained slides from tumor specimens were diagnosed and histological typing was done according to WHO classification criteria. The representative sections were then subjected to IHC with antibodies against ER, PR, p53 and Ki-67.**Results:**ER expression was seen in 42% of benign serous tumors and 100% of serous cystadenocarcinoma and endometroid cystadenocarcinoma. ER expression was negative in benign mucinous, endometroid, brenner tumours and mucinous cystadenocarcinoma. PR expression was seen in 32% of benign serous tumors, 50% of borderline mucinous tumours, 67% of serous cystadenocarcinoma and 100% of endometroid cystadenocarcinoma. PR expression was negative in benign mucinous, endometroid, brenner tumors and mucinous cystadeno- carcinoma. 50% of borderline mucinous tumour, 67% of serous cystadenocarcinoma and 50% of endometroid cystadenocarcinoma expressed p-53 while none of the benign surface epithelial tumors showed p-53 positivity.**Conclusion:**Immunohistochemistry being ancillary to histopathological diagnosis, expression of ER, PR status, p-53 and Ki-67 if included in each pathology report aids in prognostication and better understanding of biological behavior which further helps to modify treatment strategies.

Keywords: Immunohistochemistry, histopathological diagnosis, ovarian tumors.

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Introduction

Ovarian cancer is the sixth most frequent malignancy among women and the seventh major cause of cancer death among women worldwide. In most of the population-based cancer

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registries in India, ovarian cancer is the third leading site of cancer among women, trailing behind cervical and breast cancer.[1]

The Age Standardized Incidence Rate (ASIR) for ovarian carcinoma in India ranged from 0.9 to 8.4 per 100,000 people per year. Studies revealed that the peak incidence is in between the age of 55-64 years. The mean annual percentage increase in ASR ranges from 0.7 to 2.4%.[2]

The risk factors associated with ovarian tumours are advancing age, positive family history, increase in the age of reproduction, higher socio-economic status and nulliparity. It has been suggested that incessant ovulation and gonadotrophin stimulation may play a role in development of ovarian cancer.[3]

Predominantly ovarian neoplasms are sporadic in nature, only about 5-10% are hereditary. These women have inherited mutations in BRCA-1 and BRCA-2, tumour suppressor genes. Following the introduction of CA-125 in 1981 as a biomarker for epithelial ovarian carcinoma, numerous other biomarkers have been emerging substantially.[4] Epidemiological evidence suggests that steroid hormones (estrogen and progesterone) and amplification of the human epidermal growth factor-2 (Her-2/neu) genes are implicated in ovarian carcinogenesis.[5] The World Health Organization (WHO) categorizes primary ovarian neoplasms according to histogenetic principles, mainly with regards to their derivation i.e. from coelomic surface epithelial cells, germ cells and mesenchyme (sex cord and stroma). In addition to primary benign and malignant neoplasms, there are also borderline tumours which represent non-invasive tumours of uncertain malignant potential.[6] The present study was conducted to assess clinicopathological correlation and immunohistochemical profile of ovarian tumours.

Materials and Methods

The study was a prospective study conducted over a period of eighteen months (2020 to 2021) at Department of Pathology, Government Medical College and Rajindra Hospital, Patiala, Punjab. The study proposal and procedures were approved by the Ethics Committee of Government Medical College, Patiala. An informed consent was taken from all the patients coming to Rajindra Hospital and Government Medical College, Patiala.

Inclusion Criteria:

All cases of ovarian tumors which were diagnosed on clinical, radiological findings and confirmed by histopathlogical findings were included in this study.

Exclusion Criteria:

Already diagnosed cases of ovarian cancers coming with relapse.

Methodology:

Details of the study protocol was explained to the subjects. An informed consent was taken from all the subjects. Clinical history of the patient including the age, examination findings, radiological investigation, USG, CT and FNAC reports were evaluated in detail. Oophrectomy specimens were fixed in 10% neutral buffered formalin and multiple sections were taken for histopathological examination. 3–4-micron thick sections were cut and stained with Hematoxylin & Eosin (H & E).

The H &E-stained slides from tumor specimens were diagnosed and histological typing was done according to WHO classification criteria. The representative sections were then subjected to IHC with antibodies against ER, PR, p53 and Ki-67.

The nuclear staining of the tumor cells was considered as a positive expression for ER and PR. Grading of nuclear ER and PR staining was performed using an immunoreactive scoring obtained by the sum of intensity of staining and the percentage of positively stained cells.

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Ki67 labelling index = Number of positive stained nucleus/100 Number of tumor cells counted.
All tumors showing p53 immunoreactivity (at least +1) were considered positive. Data thus obtained were subjected to statistical analysis. P value < 0.05 was considered significant.

Results

Table 1: Distribution of benign, borderline and malignant ovarian neoplasms

Benign		Borderline		Malignant	
No.	**%**	**No.**	**%**	**No.**	**%**
37	72.55	2	3.92	12	23.53

Table 2: Age wise distribution of benign, borderline & malignant ovarian neoplasms

Age Group (In Years)	Benign		Borderline		Malignant		Total		p-Value
	No.	%	No.	%	No.	%	No.	%	
0-20	3	50.00	0	0.00	3	50.00	6	100.00	**0.035**
21-40	27	84.38	2	6.25	3	9.38	32	100.00	
41-60	6	60.00	0	0.00	4	40.00	10	100.00	
61-80	1	33.33	0	0.00	2	66.67	3	100.00	
81-100	0	0.00	0	0.00	0	0.00	0	0.00	

Table 3: mode of presentation of ovarian tumours

Clinical features	No. of Cases	%
Abdominalmass	13	25.5
Abdominalpain	21	41.1
Abdominalmass+abdominalpain	10	19.6
Menstrualabnormality	12	23.5
Other	2	3.92

Table 4: Histological types of neoplastic lesions

Category	Subcategory	No. of Cases	%
Surface epithelial tumours	Serous cystadenoma	19	37.25
	Serous cystadenocarcinoma	3	5.88
	Borderline serous tumour	0	0.00
	Mucinous cystadenoma	6	11.76
	Borderline mucinous Tumour	2	3.92
	Mucinous cystadenocarcinoma	1	1.96
	Endometroid cystadenoma	1	1.96
	Endometrioid carcinoma	2	3.92
	Brenner Tumour	1	1.96
Sex-cord stromal tumour	Fibroma	2	3.92
	Granulosa cell Tumour	1	1.96
Germ cell tumour	Immature teratoma	2	3.92
	Mature cystic teratoma	8	15.69
	Dysgerminoma	1	1.96
Metastatic cancer	Metastatic deposits	2	3.92
Total		51	100.00

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[Table 1] shows that out of 51 cases, 37 i.e., 72.55% were diagnosed as having benign ovarian tumour; 2 cases (3.92%) were borderline tumours whereas 12 cases i.e. 23.53% had malignant lesions.

[Table 2] shows that the age of the patients ranged from 4 to 67 years. The mean age was 42.5±16.26 years. 27 (73%) out of 37 benign neoplasm were of age 21-40 years, this was followed by 6 cases (16%) in age 41-60 years & 3 (8%) in age group 0-20 years. Amongst the borderline lesions, there were 2 cases in the age group of 21-40 years. Amongst the malignant category, 4 cases (33%) were present in age group 41-60 years, followed by 3 cases each in 0-20 & 21-40 years & least 2 (17%) in 61-80 years. On statistical analysis of data, there was significant association found between age of the patient & nature of tumour, ($p < 0.05$).

[Table 3] shows that the commonest symptom with which the patients presented was abdominal pain (41.1%) followed by abdominal mass (25.5%) and abnormal bleeding per vaginum (23.5%). Both abdominal pain and abdominal mass were seen in 19.6% cases. Infertility, hirsutism were other less common presentations seen in only 3.92% cases.

[Table 4] shows that serous cystadenoma was the most common tumour comprising of 37.25% of all ovarian neoplasms followed by mature cystic teratoma comprising of 15.69%. Among thesurface epithelial tumours, mucinous cystadenoma was the second most common tumour comprising of 11.76% of all ovarian neoplasms.

Table 5: Expression of p-53 in benign, borderline and malignant surface epithelial tumours

	p-53 expression (Positive)	**p-53 expression (Negative)**	**Total**	**%age**	**p-value**
Benign (n=27)	0 (0%)	27 (100%)	27	100	
Borderline (n=2)	1 (50%)	1 (50%)	2	100	
Malignant (n=6)	4 (66.6%)	2 (33%)	6	100	<0.001

In the present study, highest p-53 immunoreactivity was seen in malignant tumours (66.6%) compared with borderline (50%) and negative immunoexpression of p-53 was noted in benign tumours (0%). On statistical analysis of data there was significant association found between p-53 expression and nature of ovarian tumour. ($p<0.001$).

Table 6: Expression of ki-67 in benign, borderline and malignant surface epithelial tumours

	Ki-67 expression (Positive)	**Ki-67 Labelling Index**	**Ki-67 Expression (Negative)**	**Total**	**%**	**p-value**
Benign (n=27)	1 (3.7%)	8.40 %	26 (96.3%)	27	100	
Borderline (n=2)	2 (100%)	24.50 %	0 (100%)	2	100	<0.001
Malignant (n=6)	5 (83.7%)	47.00 %	1 (16.6%)	6	100	

[Table 6] shows that among the malignant tumours, 5 cases (83.3%) out of 27 tumours showed Ki-67 positivity while one 1 case of benign tumour (3.70%) expressed Ki-67. Both the borderline tumours expressed Ki-67. However, the mean Ki-67 expression of malignant epithelial tumours was 47% when compared to borderline and benign tumours which had a

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mean Ki-67 Li of 24.5% and 8.4% respectively. This difference was found to be significant ($p<0.001$).

Table 7: Expression of various immunohistochemical markers in histological subtypes of surface epithelial tumours

Histological Subtypes	ER	PR	P-53	Ki-67	Ki-67 Li Mean
Benign SerousTumor(n=19)	8 (42.11%)	6 (31.58%)	0	1 (5.26%)	8.40%
Benign MucinousTumor(n=6)	0	0	0	0	0
Benign EndometrioidTumor(n=1)	0	0	0	0	0
BenignBrennerTumor(n=1)	0	0	0	0	0
Borderline Mucinous Tumors(n=2)	0	1 (50%)	1 (50%)	2 (100%)	24.50%
Serous Cystadenocarcinoma(n=3)	3 (100%)	2 (66.7%)	2 (66.7%)	3 (100%)	50.71%
Mucinous Cystadenocarcinoma(n=1)	0	0	1 (100%)	1 (100%)	35.00%
Endometrioid Carcinoma(n=2)	2 (100%)	2 (100%)	1 (50.0%)	1 (50%)	48.00%
Total (35)	13 (37.14%)	11 (31.43%)	5 (14.29%)	8 (22.86%)	
p-value	> 0.05(NS)	> 0.05(NS)	>0.05(NS)	> 0.05(NS)	> 0.05(NS)

[Table 7] shows that ER expression was seen in 42% of benign serous tumors and 100% of serous cystadenocarcinoma and endometroid cystadenocarcinoma. ER expression was negative in benign mucinous, endometroid, brenner tumours and mucinous cystadenocarcinoma. PR expression was seen in 32% of benign serous tumors, 50% of borderline mucinous tumours, 67% of serous cystadenocarcinoma and 100% of endometroid cystadenocarcinoma. PR expression was negative in benign mucinous, endometroid, brenner tumors and mucinous cystadeno- carcinoma. 50% of borderline mucinous tumour, 67% of serous cystadenocarcinoma and 50% of endometroid cystadenocarcinoma expressed p-53 while none of the benign surface epithelial tumors showed p-53 positivity.

Lowest Ki-67 expression was seen in 1 case of benign serous tumour with a Ki-67 Li of 8.40%. Serous cystadenocarcinoma had the highest Ki-67 Li of 50.71%, while mucinous cystadenocarcinoma and endometroid cystadenocarcinoma had mean Ki-67 Li of 35.0% and 48% respectively. Borderline mucinous tumor had a mean Ki-67 Li of 24.50%, intermediate between benign and malignant tumors. There was no significant relationship between histopathological type of epithelial tumours and expression of ER, PR, p-53 and ki-67 status in the present study, p value >0.05.

Discussion

A clinicopathological study of ovarian tumours was done in the Department of Pathology, Government Medical College, Patiala to know the frequency of distribution of different types of ovarian tumours and to evaluate the expression of ER, PR, p-53, and Ki-67 Li in epithelial ovarian tumours. Ovaries are common site of non-neoplastic and neoplastic lesions. Ovarian cancer is the seventh major cause of cancer death among women worldwide.[7]The diverse histopathologies in ovarian tumours reflects the different cell origins. As the symptoms are vague and manifest over time ovarian cancers are difficult to detect until they are in advanced

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stage.[8] Identification of various histological patterns of ovarian tumours is important in diagnosis, prognosis and treatment of ovarian cancers. Immuno- histochemistry is now emerging as an important tool in diagnosis of ovarian tumours.[9,10]

The present study was carried out on 51 ovarian neoplasms over a period of eighteen months. The tumours were classified as per WHO classification. The relationship of these ovarian tumors with age, menopausal status, laterality, consistency and grade of tumours was seen. ER, PR, p-53 and Ki-67 expression in primary epithelial ovarian neoplasm was studied and correlated with clinicopathological prognostic factors of ovarian tumours namely age, histological type and tumour grade.

In the present study, ovarian tumours were seen in the age range of 4 to 67 years with a peak incidence in 21-40 years range. We found that 27 (73%) out of 37 benign neoplasm were of age 21-40 years, this was followed by 6 cases (16%) in age 41-60 years & 3 (8%) in age group 0-20 years. Amongst the borderline lesions, there were 2 cases in the age group of 21-40 years. Amongst the malignant category, 4 cases (33%) were present in age group 41-60 years, followed by 3 cases each in 0-20 & 21-40 years & least 2 (17%) in 61-80 years. Kumar et al,[11] correlated various clinicopathologic variables with expression of ER, PR-A, Her-2-neu, p-53 and Ki-67 in epithelial ovarian tumours. It was observed that ER had lower expression in benign and PRA had higher expression in malignant while both ER and PRA had higher expression in serous, postmenopausal, advanced stage, Grade 3 and tumours with ascites. Her-2-neu and p-53 were negative in benign and higher in malignant, serous, Grade 3 and tumours with ascites. Ki-67 had a significant higher expression in malignant and grade 3 as compared to benign tumours.

We observed that serous cystadenoma was the most common tumour comprising of 37.25% of all ovarian neoplasms followed by mature cystic teratoma comprising of 15.69%. Among the surface epithelial tumours, mucinous cystadenoma was the second most common tumour comprising of 11.76% of all ovarian neoplasms. Highest p-53 immunoreactivity was seen in malignant tumours (66.6%) compared with borderline (50%) and negative immune-expression of p-53 was noted in benign tumours (0%). Dhatwalia et al,[12] evaluated 50 cases of ovarian tumours ; 31 benign and 19 malignant where estrogen and progesterone expression was studied by immunohistochemistry and correlated with various clinico- pathological parameters such as menopausal status, histological type, WHO grade and FIGO stage. Majority of the patients were in the age group of 18-39 years and benign tumours were more common (54%) than malignant (38%). ER expression was observed in 22 (44%) and PR expression was seen in 19 (38%) cases. Out of 50 cases, 26 (52%) were ER, PR negative, 17 (34%) were ER, PR positive, 10% were ER positive, PR negative and 4% were PR positive, ER negative. ER expression in malignant tumour was statistically significant in comparison to benign and borderline tumours.

We found that among the malignant tumours, 5 cases (83.3%) out of 27 tumours showed Ki-67 positivity while one 1 case of benign tumour (3.70%) expressed Ki-67. Both the borderline tumours expressed Ki-67. However, the mean Ki-67 expression of malignant epithelial tumours was 47% when compared to borderline and benign tumours which had a mean Ki-67 Li of 24.5% and 8.4% respectively. Mohapatra et al,[13] evaluated 52 cases of ovarian tumours diagnosed at Prathima institute of medical sciences in South India, between August 2018 to July 2020 and determined p-53 and Ki-67 antigen expression andtheir biological significance in epithelial ovarian cancer. It was seen that the mean age of diagnosis for benign was 42 years, for borderline it was 49 years and for malignant epithelial tumours it was 56 years respectively. Serous epithelial tumours (50%) was most common histological type. 89.5% of malignant tumours showed p-53 immunoreactivity while 91% of malignant tumours expressed Ki-67 immunoreactivity which was statistically significant ($p<0.05$).

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Conclusion

Authors found that that immunohistochemistry being ancillary to histopathological diagnosis, expression of ER, PR status, p-53 and Ki-67 if included in each pathology report aids in prognostication and better understanding of biological behavior which further helps to modify treatment strategies.

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