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Research Article

Clinicopathological and IHC study (estrogen receptors, progesterone receptor, HER2/NEU) in malignant ovarian tumors

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ABSTRACT

Background: Ovarian cancer is the second most common gynaecologic malignancy, most common cause of gynaecologic cancer death and has worst prognosis among all gynecological malignancies. The clinical significance of ER and PR content in ovarian carcinomas has not been well established in the literature.
Methods: A prospective study was conducted over a period of 2 years (2013-2015) in the department of pathology, Andhra Medical College, Visakhapatnam, Andhra Pradesh, India. The study includes 42 cases of ovarian cancers. Representative 3-4µm sections were taken and IHC was performed with specific antibodies.
Results: The mean age at presentation was 39.5 years, majority of the ovarian carcinomas occurred in the age group of third and fifth decade (20/42). The commonest clinical presentation was mass per abdomen. The commonest histological type was malignant surface epithelial tumors (25/42, 59.55%) of which serous cystadenocarcinioma was the predominant tumor followed by germ cell tumors (9/42, 21.42%). Ascites was associated with higher grade and higher stage of tumors. Majority of the ovarian carcinomas were of grade 2 (57.14%) and stage 3 (35.7%). ER was positive in (9/42) 21.42%, PR was positive in (10/42) 23.8% and Her2/neu was equivocal in (3/42) 7.14% of ovarian carcinomas. ER, PR and Her2 showed similar expression, with higher expression in cases of advanced disease.
Conclusions: The expression of steroid hormonal receptors in ovarian cancers paves way for antihormonal therapy/ targeted therapy and this requires more number of studies with larger sample size.

Keywords: Ovary, Carcinoma, Steroid receptors, Her2/Neu

INTRODUCTION

Ovarian cancer is the second most common gynecologic malignancy, the most common cause of gynecologic cancer death, and the fifth leading cause of cancer death in women in developed countries.[1] In India, ovarian cancer is the third leading site of cancer among women, trailing behind cervix and breast cancer. Each year, over 22,000 women are diagnosed worldwide with epithelial ovarian cancer and 15,000 die of it.[2] In patients with cancers of the breast and endometrium, the relationship between tumor estrogen and progesterone receptor (PR) expression and prognosis is well documented. Estrogen is considered a primary culprit in the development of ovarian cancer as 70% of ovarian cancers express estrogen receptors (ERs), whereas progesterone and its receptors are protective against ovarian cancer.[3,4] However, the clinical significance of ER and PR content in ovarian carcinomas has not been well established due to conflicting data from only a few immunohistochemical studies available in the literature.

The present study was taken up to understand the disease morbidity and to know the role of steroid receptors in the pathogenesis of ovarian cancers.

The main aims and objectives was to study the various clinicopathological parameters in ovarian cancer and to evaluate the expression of estrogen and progesterone receptors and Her2/neu in patients with ovarian cancer and to correlate the results of immunohistochemistry with various clinicopathological parameters.

METHODS

This is a prospective study conducted for a period of 2 years from August 2013 to July 2015, in the Department of Pathology, Andhra Medical College, Visakhapatnam, India. A total number of 42 cases of ovarian cancers received to the department were included in the study. All non-neoplastic, benign and borderline lesions were excluded. All the relevant clinical details were obtained.

Tissue was subjected to routine processing and sections were stained with hematoxylin and eosin (H&E). The histopathological sections were diagnosed based on WHO classification. 3-4 micron sections were taken from paraffin embedded tissue blocks for immunohistochemistry. Data was analysed statistically using Chi-square test. Positive and negative controls were run with each batch. Positive staining was controlled by positively stained breast carcinoma sections; the negative control was performed on the same tissue without primary antibody. ER and PR are nuclear markers and a positive staining is defined when more than 10% of cells take up the nuclear stain of any intensity. HER2/neu is a cell membrane marker.

ER, PR staining was quantified by using Allred score. All the slides were quantified by giving proportional score based on the percentage of cells showing nuclear stain and intensity score based on intensity of staining.

Immunohistochemical assessment of HER2/neu over-expression was graded as per ASCO (American society of clinical oncology)/CAP (college of American Pathologists) 2013 guidelines.

Staging of all the ovarian carcinomas was done according to FIGO (International federation of gynaecology and obstetrics) staging and histological grading was done as per Silverberg's universal grading system.

RESULTS

The commonest clinical presentation in the present study was mass per abdomen (32 cases) followed by pain abdomen (24 cases). Majority of the ovarian cancers (20 cases, 47.6%) were found in the third and fifth decade of life (Table 1). Majority of the surface epithelial tumors were of age group above 50 years (44%) and majority of germ cell tumors were below 20years. Half of the metastatic tumors belong to third decade. In the present study, surface epithelial tumors (59.5%) were the commonest followed by germ cell tumors (21.42%) (Table 2). Majority of the ovarian cancers were of stage 3 (35.7%) and grade 2 (57.14%) (Table 3). Ascites was associated with ovarian cancer in 42.8% of cases (18 cases) of which majority are of higher grade and higher stage with a "P" value of less than 0.05 which is statistically significant. In the present study, CA-125 levels were available for only 25 cases. Median CA-125 levels were highest in serous (600 IU/ml) and lowest in mucinous carcinoma (60.9 IU/ml). Median CA-125 levels were highest in grade 3 and stage 3 ovarian cancers. ER showed positivity in 9 cases i.e. 21.42% and PR showed positivity in 10 cases i.e. 23.8% of cases. Her2 was weak positive/equivocal in 3 cases i.e. 7.14% (Table 4). In the present study, all the 9 ER positive tumors were seen in age group above 40 years and were predominantly of higher stage and higher grade. Association of ER with grade and stage was statistically significant. PR also showed positivity predominantly in age group above 40 years and also in higher grade tumors. Association of Her2/neu with high histological grade was statistically significant (Table 5).

Table 1: Age distribution in the ovarian cancers.

Age group (years)	No. of cases (total, n=42)	Percentage
<20yrs	7	16.6
21-30	7	16.6
31-40	10	23.8
41-50	8	19.04
>50	10	23.8

Table 2: Relative frequency of different ovarian cancers.

Histological type	No. of cases (n=42)	Percentage
Surface epithelial tumors	25	59.5
Serous cystadenocarcinoma	13	52
Mucinous cystadenocarcinoma	7	28
Endometrioid carcinoma	3	12
Clear cell carcinoma	1	4
Malignant brenner	1	4
Germ cell tumors	9	21.42
Dysgerminoma	3	33.33
Yolk sac tumor	3	33.33
Mixed germ cell tumor	3	33.33
Sex cord stromal tumors	4	9.54
Granulosa cell tumor	4	9.54
Metastatic / krukenberg tumor	4	9.54

Table 3: Correlation of histological type of ovarian carcinoma with stage (FIGO) and grade of tumor (total no. of cases=42).

Histological type	Figo stage				Grade (Silverberg's universal grading)		
	I	II	III	IV	I	II	III
Surface epithelial tumors							
Serous cystadenocarcinoma (n=13)	2	3	7	1	2	9	2
Mucinous cystadenocarcinoma (n=7)	3	2	1	1	2	5	0
Endometrioid carcinoma (n=3)	0	0	2	1	2	1	0
Clear cell carcinoma (n=1)	0	0	0	1	0	1	0
Malignant Brenner (n=1)	0	0	0	1	0	0	1
Germ cell tumors							
Dysgerminoma (n=3)	0	3	0	0	0	1	2
Yolksac tumor (n=3)	0	2	1	0	1	2	0
Mixed germ cell tumors (n=3)	0	2	1	0	1	1	1
Sex cord stromal tumors							
Granulosa cell tumor (n=4)	1	0	3	0	1	3	0
Metastatic tumor / krukenberg tumor	4	0	0	0	1	1	2
Total	10 (23.8%)	12 (28.5%)	15 (35.7%)	5 (11.9%)	10 (23.8%)	24 (57.14%)	8 (19.04%)

Table 4: Correlation of ER, PR,Her2/neu positive cases with histopathological type of ovarian carcinoma (total cases=42).

ER	Serous n=13	Mucinous n =7	Endometroid n = 3	Clear cell n = 1	Malign Brenner (n= 1)	GCT n =9	SCST n= 4	Metastatic n=4	Total
Positive	6	0	2	0	1	0	0	0	9 (21.42%)
Negative	7	7	1	1	0	9	4	4	33
PR									
Positive	3	0	2	0	0	2	1	2	10 (23.8%)
Negative	10	7	1	1	1	7	3	2	32
Her2									
Positive	1	1	0	1	0	0	0	0	3 (7.14%)
Negative	12	6	3	0	1	9	4	4	39

GCT = germ cell tumors; SCST = sex cord stromal tumors.

DISCUSSION

In the present study , majority of the ovarian cancers were found in the third and fifth decade of life i.e. 10 cases each, which together accounts for 47.6% of cases. The mean age at presentation was 39.5 years which correlated with Ruchikagarg et al.[5] The youngest patient in our study was of 14 years and the oldest patient was 77 years old. In our study, mass per abdomen was the predominant clinical presentation followed by pain abdomen. In the study conducted by RuchikaGarg et al the commonest presentation was abdominal distension followed by pain abdomen. Four patients were nulliparous and all four had tumor in advanced stage. Family history of ovarian carcinoma was obtained in one case which was a mixed germ cell tumor (yolksac tumor with dysgerminoma).

Malignant surface epithelial tumors were the commonest histological type comprising of 59.5% of all the ovarian carcinomas and serous cystadeno carcinomas being the predominant. Germ cell tumors were the next most common in the present study accounting for 21.42% (9 cases) of all the cancers including 3 cases each of dysgerminoma, yolk sac tumor and mixed germ cell tumors while Shilpa et al had only 3 cases of GCT's.[6] The mixed germ cell tumors in the present study comprising of one case of dysgerminoma with choriocarcinoma, one case of yolk sac tumor with embryonal carcinoma and one case of yolk sac tumor with dysgerminoma.

Metastatic tumors / Krukenberg tumors accounted for 9.52% (4 cases) of ovarian cancers in the present study. Three out of four cases were known to have primary in the stomach. The present study we

encountered an interesting case of 35 years female who presented with pain abdomen, vomiting's and a palpable lump in the epigastric region. CT and MRI scan revealed concentric wall thickening of body of stomach and pylorus with bilateral solid ovarian neoplasms. It was a case of simultaneous presentation of gastric carcinoma and bilateral ovarian carcinoma.

Table 5: Correlation of ER, PR, HER2/NEU with clinical parameters in ovarian cancer.

	ER (n=9)	PR (n=10)	Her2 (n=3)
Age			
<40 years	0	3	1
>40 years	9	7	2
P- value	>0.05	>0.05	>0.05
Stage II			
I	1	2	1
II	0	3	0
III	4	4	2
IV	4	1	0
P- value	<0.05	>0.05	>0.05
Grade			
I	2	3	0
II	6	4	3
III	1	3	0
P- value	<0.05	>0.05	<0.05
CA – 125	1434 IU/ml	172.5 IU/ml	--
ASCITES	5	4	1

In the present study, majority of the tumors were found to be of stage 3, accounting for 35.7% (15/42 cases). MT Sylvia et al found 46% of their cases in stage 1 and 33% in stage 3 while Shilpa et al found majority of their carcinomas in early FIGO stages (i.e. stage 1 and 2).[6,7] Majority of the ovarian cancers were found to be of grade 2, which accounts for 57.14% (24 cases) followed by 23.8% (10 cases) of grade 1 and 19.04% (8 cases) of grade 3 tumors. According to Shilpa et al and MT Sylvia et al who followed WHO grading, majority of their cases of ovarian carcinomas were of grade 2 accounting for 45% and 49% respectively in these studies.[6,7]

CA-125 levels were available for 25 cases. With a cutoff point of 35 IU/ml for detecting malignant ovarian tumors, sensitivity was 84% as four out of 25 cases of malignant ovarian tumors had CA-125 levels below 35 IU/ml resulting in false negatives. These cases which had normal CA-125 levels were predominantly early stage tumors. Zorn et al have showed that 7.6% of patients with malignant tumors have normal levels.[8] Median CA-125 levels were raised in all the histological types but were found to be highest in serous cystadeno carcinoma (>600 IU/ml) and malignant Brenner (>600 IU/ml) and lowest in mucinous cystadeno carcinoma (60.9 IU/ml).

Median CA-125 levels were significantly elevated among all grades of the tumors but highest in grade 3 tumors. Similarly median CA-125 levels were highest in stage 3 and stage 4 tumors. Median CA-125 levels were significantly increased in high grade and advanced tumors. These findings of the present study are in concordance with MT Sylvia et al.[7]

ER expression was seen in 9 cases accounting for 21.42%. ER showed highest expression in serous cystadeno carcinomas accounting for 66.6% of all ER positive tumors (Figure 1). ER was positive in 2 cases of endometrioid carcinoma accounting for 22.2% of all ER positive tumors (Figure 2) and single case (100%) of malignant Brenner. ER was negative in all mucinous cystadeno carcinomas, clear cell carcinoma, germ cell tumors, sex cord stromal tumors and metastatic tumors. ER showed higher expression in age group above 40 years, serous carcinomas, higher grade and stage of the tumors and were associated with ascites in majority and had significantly high CA-125 levels thereby suggesting the association of ER expression with advanced disease tumor. The ER positivity in the study was less compared to that of Shilpa et al (32.5%) and MT Sylvia et al (33%). On correlating ER positive tumors with stage and grade, there was statistical significance with a p-value <0.05. Our findings of the study are in concordance with MT Sylvia et al which also reported similar findings for ER expression.

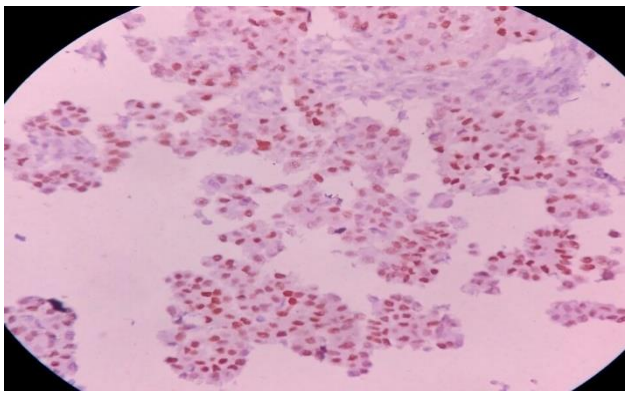

Figure 1: Photomicrograph of serous cystadeno carcinoma showing ER positivity (100x).

PR showed positivity in 10 cases accounting for 23.8%. PR was positive in 3 cases of serous cystadenocarcinoma, 2 cases of endometrioid carcinoma accounting for 66.6% of all endometrioid tumors (Figure 3), 2 cases of germ cell tumors (Figure 4), 2 cases of metastatic tumors/Krukenberg tumors (Figure 5) accounting for 50% and 1 case of sex cord stromal tumor accounting for 25% (Figure 6). PR did not show any expression in mucinous cystadeno-carcinomas, clear cell carcinoma and malignant Brenner tumor. PR expression in the

present study was less compared to the other studies in the literature. MT Sylvia et al (63.6%) reported higher expression for PR while Shilpa et al (27.5%) reported lower PR expression in their study, but still both the studies had higher PR positivity than our study. Association of PR with advanced disease in the present study correlated with MT Sylvia et al., but it is in contrast to Shilpa et al and Hecht et al who reported PR positivity in tumors with good prognostic factors.[9]

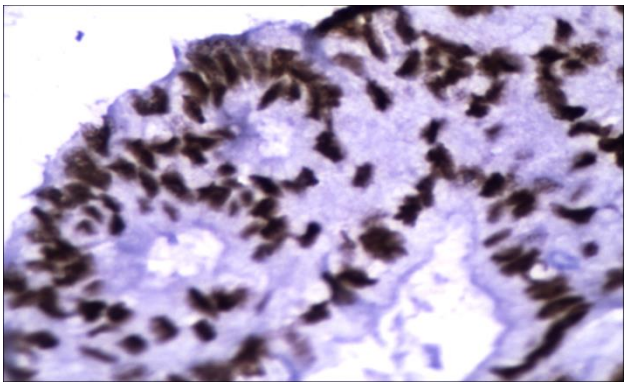

Figure 2: Photomicrograph of endometrioid carcinoma showing ER positivity(400X).

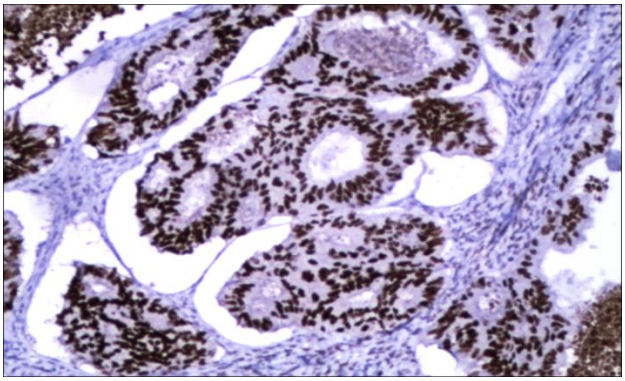

Figure 3: Photomicrograph of Endometrioid carcinoma showing positive immunostaining with PR(100X).

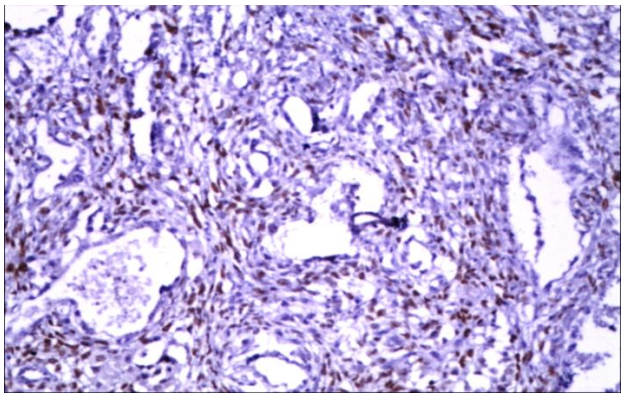

Figure 4: Photomicrograph of yolk sac tumor showing positive immunostaining for PR (100X).

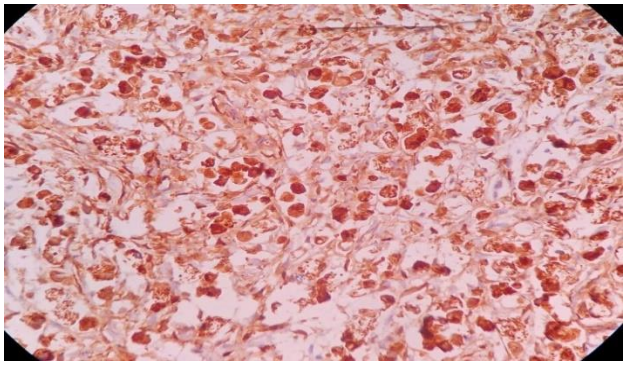

Figure 5: Photomicrograph of Krukenberg tumor showing positive immunostaining for PR.

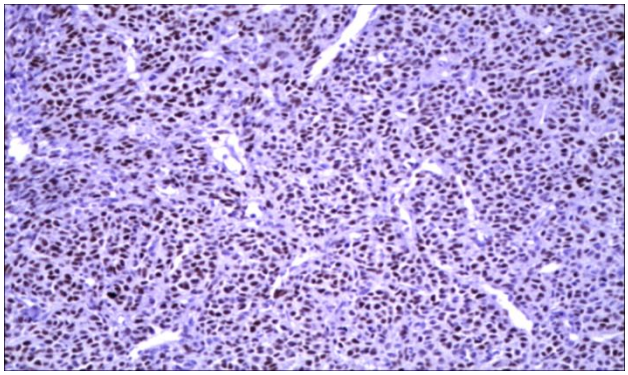

Figure 6: Photomicrograph of granulosa cell tumor showing positive immunostaining for PR (100X).

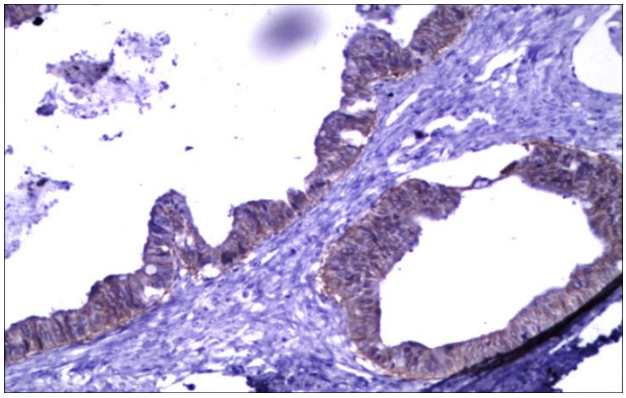

Figure 7: Photomicrograph of mucinous cystadeno carcinoma showing weak positive immunostaining for Her2 (100x).

In the present study, Her2/neu was weakly positive/ equivocal in only 3 cases accounting for 7.14% which includes one case each of serous cystadenocarcinoma, mucinous cystadenocarcinoma and clear cell carcinoma (Figure 7). Thus, Her2 expression was seen only in surface epithelial carcinomas. Her2/neu positivity was very less compared to other studies. MT Sylvia et al reported an expression of 21% which is similar to the study done by Mahdi MA with 21.05% positivity.[10] In our study, two of the three cases were above 40 years of age,

two cases were of stage 3 and all the three cases were histologically grade 2. Ascites was associated with only one case among these Her2 positive tumors. Her2 was negative in all grade 1 tumors. The association of Her2 with higher grade, stage and ascites suggest an aggressive tumor type and advanced stage of disease. Her2 expression in high grade tumors was statistically significant with p-value <0.05.

The expression of steroid hormonal receptors in ovarian cancers paves way for antihormonal therapy/targeted therapy. The role of hormone therapy in the treatment of ovarian cancer is not clear and data on their efficacy and safety in recurrent ovarian cancer have been accumulated through phase II clinical studies. Most of the studies were conducted in platinum-resistant recurrent ovarian cancer, and although complete response rates were not high, reported adverse events were low. If administered to patients who are positive for hormone receptors, hormone therapy may become a viable option for the treatment of recurrent ovarian cancer.

CONCLUSION

The expression of steroid receptors paves way for anti hormonal therapy. CA125 levels reflect the tumor bulk and the median levels were higher in tumors with adverse prognostic factors. However, there were inconsistent findings of ER/PR expression and clinicopathological parameters in various studies. Hence, an absolute conclusion could not be derived. We emphasize the need for comprehensive data from more studies that used the same technique (immunohistochemistry), well-marked cut off levels for ER/PR expression and a larger sample size. Such reports would be more informative and are warranted to clarify whether hormone therapy based on hormone receptor status can be an alternative treatment in ovarian carcinoma patients.

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