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Expression of estrogen receptor and progesterone receptor in malignant epithelial ovarian carcinoma: A hospital-based study

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Abstract

Background: Ovarian cancer, one of the most lethal gynecological malignancies, remains a significant global health concern. Ovaries, being the source of estrogen and progesterone, are also the targets of these hormones. Expression of estrogen receptors (ER) and progesterone receptors (PR) is believed to have a major role in ovarian cancer. The current study aimed to evaluate the ER/PR expression in epithelial ovarian carcinomas (EOCs) and their clinico-pathological correlation.

Materials and Methods: This retrospective, observational study was conducted on 51 cases of malignant EOCs. ER/PR expression was studied by immunohistochemistry using a heat-induced epitope retrieval method and specific antibodies against ER and PR. Expression of both the hormone receptors was correlated with histopathological type, grade, FIGO stage and menopausal status of the patients.

Results: Out of the total 51 cases, serous carcinoma (70.59%) was the most common malignant ovarian carcinoma, followed by mucinous (11.775), endometrioid (9.80%), and clear cell carcinoma (7.84%). ER and PR expression was seen in 76.47% and 64.71% of cases, respectively. Expression of both the hormone receptors was seen in 54.90% of cases, while 13.72% were negative for both. A higher ER/PR expression was seen in endometrioid and serous carcinoma as compared to mucinous and clear cell carcinoma. A higher ER expression was observed in cases with higher FIGO-stage and grade of the tumor, whereas no significant correlation of the PR expression was observed for the same. ER/PR expression was also observed to be higher in pre-menopausal females.

Conclusion: ER and PR are expressed in a biologically and clinically meaningful subset of EOCs. The pattern of expression of these receptors can serve as a clue for optimizing the advanced therapeutic modalities to ensure better patient management. Future prospective studies and biomarker-driven clinical trials can translate receptor testing into robust prognostic and predictive tools for routine clinical care.

Keywords: Biomarkers, Endocrine therapy, Immunohistochemistry, Ovarian tumors, Steroid hormones

Introduction

Ovarian cancer is the most lethal of all gynecological malignancies and remains a significant global health concern.[1] It constitutes approximately 30% of all malignancies of the female genital tract, ranking third in incidence after cervical and endometrial carcinoma.[1] Owing to its insidious

onset and aggressive nature, vague symptoms, late presentation, and poor prognosis, which is largely attributed to delayed diagnosis and the absence of reliable screening methods, ovarian cancer frequently presents a diagnostic as well as therapeutic conundrum for clinicians.[2]

Among the diverse histopathological subtypes, surface epithelial ovarian cancer (EOC) accounts for approximately 90% of primary ovarian cancers and exhibits considerable heterogeneity in its morphology, molecular profile, and clinical behavior.[3,4] It comprises four major histological types namely serous, endometrioid, mucinous, and clear cell carcinoma, and based on the cytological atypia are further classified as low grade (well differentiated) or high grade (poorly differentiated).[5] Despite advances in surgery and chemotherapy, EOC has a very poor prognosis, with a 5-year relapse rate of 75% for patients diagnosed with advanced disease and a low 5-year overall survival.[6,7] Clinicopathological parameters, though important, but alone may not be sufficient in predicting prognosis of patients. This underscores the need for novel prognostic markers and targeted therapeutic strategies.

While the majority of ovarian cancers occur sporadically, advancing age, early menarche, late menopause, nulliparity, and delayed childbearing are among the well-recognized risk factors. In addition, inherited genetic mutations contribute to disease predisposition in approximately 10–15% of cases.[8] Moreover, epidemiological evidence strongly suggests that steroid hormones, particularly estrogen and progesterone, can play a pivotal role in ovarian carcinogenesis.[4]

Cytosolic estrogen receptors (ERs) and progesterone receptors (PRs) are widely distributed in various organs, including the breast, uterine endometrium, myometrium, cervix, fallopian tubes, and ovaries. While their expression is classically linked to breast and endometrial cancers, their role in EOC is still poorly understood.[8] The ovaries, besides being the primary source of estrogen and progesterone, are also direct targets for their action. Notably, around 70% of EOCs express ERs, supporting the hypothesis that estrogen promotes tumor development, whereas progesterone and its receptors are considered to exert a protective, anti-proliferative effect.[9] The expression of these hormone receptors can modulate tumor biology by influencing cellular proliferation, differentiation, and apoptosis. Moreover, the expression profiles of ERs and PRs have shown potential prognostic value and may predict tumor behavior, response to hormonal therapy, and overall clinical outcome.

Immunohistochemistry provides a reliable and reproducible method to detect ER and PR expression in tumor tissues, allowing correlation with histological subtype, grade, and clinical outcome. Studies on the pattern of ER and PR expression in EOCs can provide valuable insight into their prognostic and therapeutic implications, especially in resource-limited settings where molecular profiling may not be routinely feasible. With the above background, the present study aimed to evaluate the expression of estrogen and PRs in surface epithelial ovarian malignancies using immunohistochemistry and to correlate receptor status with histopathological characteristics. Understanding these patterns could contribute to better prognostic stratification and explore the potential role of hormonal therapy in the management of EOC.

Materials and Methods

Study design, study site, and study population

This retrospective, observational study was conducted in the department of pathology and an institutional ethics committee approval was sought before its commencement. The electronic-based medical records of the patients were revised to retrieve 51 cases of primary EOC reported during January 2015–June 2016 (18 months), covering different age groups. The diagnosis of EOC was made by macroscopic and histopathological examination of the specimens collected through surgical procedures, including total hysterectomy

with oophorectomy and ovarian cystectomy. Demographic and clinicopathological data, including age at diagnosis, self-reported age at menopause, type, grade, and FIGO stage of cancer, were obtained from patient's medical records. All the cases of malignant ovarian carcinoma reported during the aforestated period were included in the study. Cases with benign and borderline ovarian tumors and the cases under preoperative chemotherapy and/or radiotherapy were excluded from the study. Cases for which adequate tissue or paraffin blocks were not available were also excluded from the study. A trained pathologist confirmed the diagnosis and the histopathological type of the EOC after reviewing the retrieved slides.

Immunohistochemical (IHC) staining

Sections from the formalin-fixed paraffin-embedded tissue blocks of the selected cases were cut and stained by Hematoxylin and Eosin to select representative sections that had an adequate area of cancer cells for ER and PR staining. Briefly, the 3–4 μm thin sections were mounted on poly-1-lysine-coated slides. Sections were deparaffinized in xylene and rehydrated through graded ethanol series, followed by antigen retrieval using heat-induced epitope retrieval in citrate buffer (pH 6.0) for 20 min. Endogenous peroxidase activity was blocked with 3% H_2O_2 for 10 min. IHC staining was performed using monoclonal antibodies against ER (ER; clone SP1, rabbit monoclonal) and PR (PR; clone PgR636, mouse monoclonal). Slides were incubated with primary antibodies for 30 min at room temperature, followed by detection with a polymer-based horseradish peroxidase secondary system and visualization using 3,3′-diaminobenzidine chromogen. Counterstaining was done with hematoxylin, after which slides were dehydrated, cleared, and mounted. For quality assurance, each staining run included positive controls (known ER/PR-positive breast carcinoma) and negative controls (slides processed without primary antibody). Nuclear staining was considered specific, whereas cytoplasmic or membranous staining was disregarded. Hormone receptor expression was scored according to the system described by Sieh *et al*.,[10] wherein no staining was assigned a score of 0, <1% nuclear staining a score of 1, 1–50% nuclear staining a score of 2, and >50% nuclear staining a score of 3. Score 0–1 were considered as negative expression, and score 2–3 were considered as positive expression.

Statistical analysis

The study adhered to the institutional ethical guidelines and was in accordance with the Declaration of Helsinki. The data obtained were managed on an Excel spreadsheet, cleaned for errors, and analyzed. Simple descriptive statistics were used to generate frequencies, percentages, and proportions.

Results

Over a period of 18 months, a total of 51 cases of malignant EOC were identified to be included in the study, with patient ages ranging from 21 to 80 years. The maximum number of cases observed was in the age group of 41–50 years (35.29%), followed by the age group 61–70 years (27.45%) and 51–60 years (25.49%). Table 1 depicts the distribution of EOC cases among different age groups. Unilateral ovarian tumors accounted for 64.71% and were more common in comparison to bilateral tumors (35.29%). A greater proportion of cases occurred in postmenopausal women (66.67%), whereas 33.33% of cases were identified in premenopausal women.

Table 1: Distribution of the cases of malignant epithelial ovarian carcinoma among different age groups (*n*=51)

Age group (in years)	Number of cases	Percentage
21–30	01	1.96
31–40	03	5.88
41–50	18	35.29
51–60	13	25.50
61–70	14	27.45
71–80	02	3.92
Total	51	100

Based on histopathological examination, serous carcinoma (70.59%) was found to be the most common malignant ovarian carcinoma, followed by mucinous carcinoma (11.77%), endometrioid carcinoma (9.80%), and clear cell carcinoma (7.84%). Majority of the cases were noted to be of FIGO stage III (54.90%), followed by FIGO stage I (31.37%) and stage II (13.73%), while none of the tumors were found to be of stage IV. Universal grading system as proposed by Silverberg[11] was also used to grade the tumors and revealed that the maximum cases were of grade II (50.98%), followed by grade I (27.45%) and grade III (21.57%). Table 2 depicts the clinical and histopathological profiles of the patients.

Among the total 51 cases of EOC to which IHC was applied, ER positivity was seen in 39 (76.47%) cases, while 33 (64.71%) cases showed PR positivity. Expression of both ER and PR was seen in 28 (54.90%) cases, while 7 (13.72%) cases were found to be negative for ER and PR. Expression of both the hormonal receptors was observed to be higher in the cases of endometrioid carcinoma (04; 80% and 05; 100%) and serous carcinoma (32; 88.89% and 23; 63.89%) as compared to mucinous carcinoma (01; 16.67% and 04; 66.67%) and clear cell carcinoma (02; 50% and 01; 25%). Table 3 depicts the ER/PR expression seen in different histopathological subtypes of EOC. The increased ER expression was observed in cases with higher FIGO-stage and higher-grade tumor, whereas there was no significant correlation between PR expression and the FIGO-stage or the grade of the tumors. Expression of both the hormonal receptors was observed to be higher in pre-menopausal females. Table 4 depicts the ER/PR expression as per the FIGO stage, grade of the tumors, and menopausal status of the patients.

Figures 1-4 depict the histopathological types of the malignant EOCs and the ER/PR expression by IHC staining.

Table 2: Clinical and histopathological profile of the patients (*n*=51)

Various parameters	Number of cases (%)
Menopausal status	
• Pre-menopausal	17 (33.33)
• Post-menopausal	34 (66.67)
Laterality of tumors	
• Unilateral	33 (64.71)
• Bilateral	18 (35.29)
Histopathological subtype	
Serous carcinoma	36 (70.59)
• Unilateral	−19 (52.78)
• Bilateral	−17 (47.22)
Mucinous carcinoma	06 (11.77)
• Unilateral	−06 (100)
• Bilateral	−00 (00)
Clear cell carcinoma	04 (7.84)
• Unilateral	−04 (100)
• Bilateral	−00 (00)
Endometrioid carcinoma	05 (9.80)
• Unilateral	−04 (80.0)
• Bilateral	−01 (20.0)
FIGO stage	
• Stage I	16 (31.37)
• Stage II	07 (13.73)
• Stage III	28 (54.90)
• Stage IV	00 (00)
Grade of tumor	
• Grade I	14 (27.45)
• Grade II	26 (50.98)
• Grade III	11 (21.57)

Discussion

The ovary has been noted to have the largest number of tumor types in the body, which can be divided into germ cell, epithelial, sex cord stroma, and metastatic neoplasms.[12] EOC, the third most common gynecological malignancy worldwide, is one of the deadliest malignancies, with 3,13,959 new cases and over 2,00,000 deaths reported globally in the year 2020.[1] Since most of

Table 3: ER/PR expression seen in different histopathological subtypes of EOC (*n*=51)

Histopathological diagnosis	ER+/PR+ (%)	ER−/PR− (%)	ER+/PR− (%)	ER-/PR+ (%)	Total (%)
Serous carcinoma	22 (43.14)	03 (5.88)	10 (19.61)	01 (1.96)	36 (70.59)
Mucinous carcinoma	01 (1.96)	02 (3.92)	00 (00)	03 (5.88)	06 (11.77)
Clear cell carcinoma	01 (1.96)	02 (3.92)	01 (1.96)	00 (00)	04 (7.84)
Endometrioid carcinoma	04 (7.84)	00 (00)	00 (00)	01 (1.96)	05 (9.80)
Total cases	28 (54.90)	07 (13.72)	11 (21.57)	05 (9.80)	51 (100)

ER/PR: Estrogen receptors, PR: Progesterone receptors, EOC: Epithelial ovarian carcinomas

Table 4: ER/PR expression as per the FIGO stage, grade of the EOC and menopausal status of the patients (*n*=51)

Parameters	ER expression		PR expression	
	Positive (%)	Negative (%)	Positive (%)	Negative (%)
FIGO stage				
• Stage I	08 (15.69)	08 (15.69)	12 (23.53)	04 (7.84)
• Stage II	07 (13.72)	00 (00)	04 (7.84)	03 (5.88)
• Stage III	24 (47.06)	04 (7.84)	17 (33.33)	11 (21.57)
• Stage IV	00 (00)	00 (00)	00 (00)	00 (00)
Grade of tumor				
• Grade I	09 (17.65)	05 (9.80)	11 (21.57)	03 (5.88)
• Grade II	20 (39.22)	06 (11.76)	13 (25.49)	13 (25.49)
• Grade III	10 (19.61)	01 (1.96)	09 (17.65)	02 (3.92)
Menopausal status				
• Pre-menopausal	11 (21.57)	06 (11.76)	15 (29.41)	02 (3.92)
• Post-menopausal	28 (54.90)	06 (11.76)	18 (35.29)	16 (31.37)
Total	51 (100)		51 (100)	

ER/PR: Estrogen receptors, PR: Progesterone receptors, EOC: Epithelial ovarian carcinomas

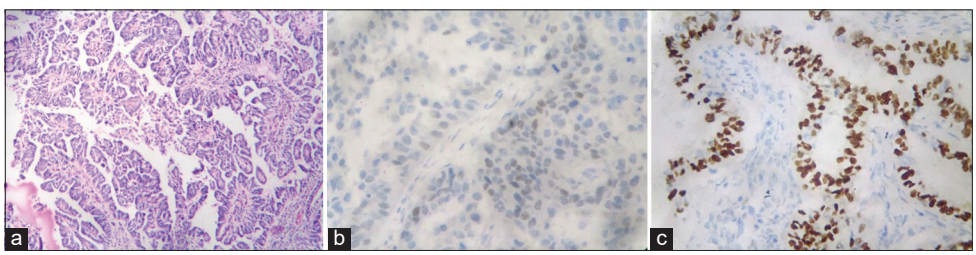

Figure 1: (a) Papillary serous cystadenocarcinoma (Hematoxylin and Eosin, ×10) displaying nuclear immunoreactivity to, (b) estrogen receptor (34% nuclear staining; score 2) and (c) progesterone receptor (94% nuclear staining; score 3) (immunohistochemical, ×40)

the patients present with an advanced stage of the disease, the available therapeutic options are limited and largely suboptimal; combined with treatment resistance, this markedly contributes to the high mortality of ovarian carcinoma.[12,13] Cisplatin-based chemotherapy although can improve the survival rate, but even in patients with a favorable response, the recurrence rate remains high.[14]

Based on their histological features, EOCs are classified, and epithelial cells are divided into benign and borderline with low malignant potential

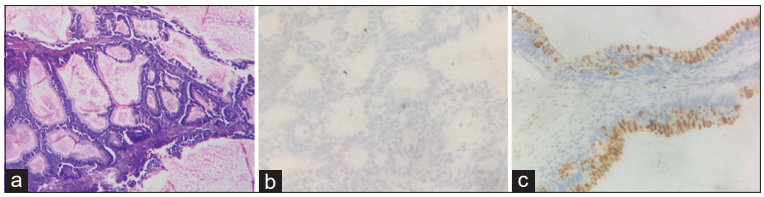

Figure 2: (a) Mucinous cystadenocarcinoma (Hematoxylin and Eosin, ×10) displaying nuclear immunoreactivity to, (b) estrogen receptor (0% nuclear staining; score 0) and (c) progesterone receptor (75% nuclear staining; score 3) (immunohistochemical, ×40)

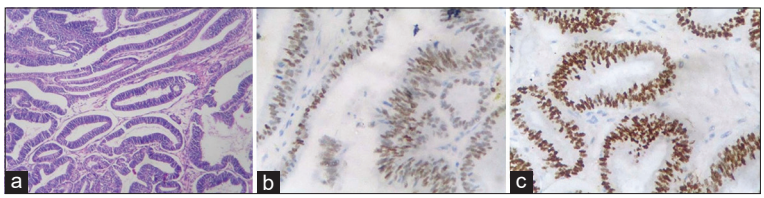

Figure 3: (a) Endometrioid carcinoma (Hematoxylin and Eosin, ×10) displaying nuclear immunoreactivity to, (b) estrogen receptor (94% nuclear staining; score 3) and (c) progesterone receptor (98% nuclear staining; score 3) (immunohistochemical, ×40)

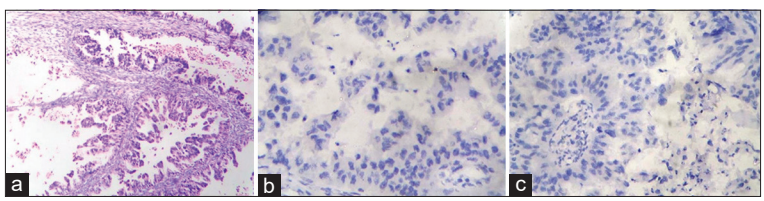

Figure 4: (a) Clear cell carcinoma (Hematoxylin and Eosin, ×10) displaying nuclear immunoreactivity to, (b) estrogen receptor (0% nuclear staining; score 0) and (c) progesterone receptor (0% nuclear staining; score 0) (immunohistochemical, ×40)

but atypical proliferation, or malignant. In the present study, among the 51 cases of malignant EOC, maximum number of cases were observed in the age group of 41–50 years (35.29%) and 61–70 years (27.45%), a finding which was in tandem with previous studies.[8,12,15] Serous (70.59%) and mucinous carcinoma (11.77%) were noted to be the most common malignant ovarian carcinoma in the present study. Our findings also revealed that unilateral ovarian tumors were more common in comparison to bilateral tumors. Moreover, 47.2% of the serous carcinoma cases were bilateral, while all mucinous carcinoma cases were unilateral. Similar findings were reported in a previous study by Finch *et al*.[16] A significant proportion of cases were noted among the postmenopausal women. These findings highlight that ovarian tumors are more likely to occur in the postmenopausal age group, underscoring the need for vigilant evaluation of adnexal masses in this population. Similar trends have been reported previously as well[8,9,13] wherein the incidence of malignant ovarian tumors was significantly higher among postmenopausal women, suggesting that menopausal status serves as an important epidemiological risk factor.

While both ER and PR are well-recognized important prognostic indicators of breast and endometrial carcinomas, their role in ovarian carcinoma is poorly understood. This is partly because most of the data on ER/PR expression in ovarian cancers is from the studies using biochemical dextran-coated charcoal method, and very few studies using IHC, which is actually

a simpler and more convenient method.[17-19] Assessment of ER and PR expression in EOC holds considerable prognostic and therapeutic significance. Receptor positivity has been associated with slower tumor progression, improved overall survival, and a more favorable clinical course.[20,21] Moreover, determining ER and PR status can inform the use of hormone-based therapies, such as aromatase inhibitors or selective ER modulators, as adjuncts to conventional chemotherapy. Evaluating these receptors also enhances understanding of the hormonal influences on ovarian tumor biology and supports a more individualized, targeted approach to patient management.[22] In the present study, we evaluated the ER/PR expression patterns among the 51 cases of malignant EOC and their correlation with histopathological type, grade, FIGO stage, and the menopausal status of the patients.

The IHC expression of ER and PR among malignant EOC cases revealed more frequent expression of ER (76.47%) than PR (64.71%). While our findings were in parallel with the previous studies by Verma *et al*.,[8] Arias-Pulido *et al*.,[17] and Naik *et al*.,[23] they were in contrast to the studies by Dhatwalia *et al*.,[13] Sylvia *et al*.[15] and Atla *et al*.,[24] who reported the higher PR expression among the cases of malignant EOC. The co-expression of ER/PR was noted in 54.90% of cases and was higher in comparison to the previous studies by Munstedt *et al*.[25] and Arias-Pulido *et al*.[17] who reported an ER/PR co-expression of 32.8% and 34.8% respectively.

The frequency of ER/PR positivity was noted to be higher in serous (88.89% and 63.89%) and endometrioid carcinoma (80% and 100%) as compared to mucinous (16.67% and 66.67%) and clear cell carcinoma (50% and 25%). Co-expression of both the hormonal receptors was noted to be highest in endometrioid carcinoma. Our study findings were in agreement with various previous studies,[10,17,26,27] wherein the ER expression was reported to be more frequent in serous carcinoma, followed by endometrioid, mucinous, and clear cell carcinoma, respectively. Similarly the studies by Dhatwalia *et al*.,[13] Sylvia *et al*.,[15] Alta *et al*.,[24] and Mohelidin *et al*.[27] reported that the frequency of PR expression was notably higher in serous carcinoma cases as compared to other histopathological types.

On correlation of tumor grade with IHC, our analysis revealed a positive correlation between tumor grade and ER expression, with significantly higher ER levels observed in grade-III tumors. A rise in ER expression was noted with increasing histological grade (grade-I: 64.29%; grade-II: 76.92% and grade-III: 90.91%). These findings were in tandem with previous studies wherein a higher ER positivity was noted with increasing histological grade of the tumor.[18,27-29] However, a study by Buchynska *et al*.[30] demonstrated that higher-grade tumors had low ER positivity. No significant relationship was found between PR expression and histological grade of the tumor, a finding which corroborated with previous studies by Verma *et al*.,[8] Naik *et al*.,[23] and Tanvanich *et al*.[31] Contrary to our findings, few studies have even reported a decrease in PR expression with increasing histological grade.[18,32]

FIGO stage is the only universally accepted prognostic factor for patients with ovarian carcinoma. With a variable ER expression, no significant correlation was noted with the FIGO stages; however, a significantly higher PR expression was noted in FIGO stage-I tumors (75%) compared to stage-II and stage-III (57.14% and 60.71%, respectively). Similar to our findings, a study by Dhatwalia *et al*.[13] and Mohelidin *et al*.[27] reported no significant correlation between ER expression and tumor FIGO stages, whereas a study by Garg *et al*.[28] revealed a significant association between PR expression and early FIGO stages. In contrast to our observations, various other studies[8,9,15,24,29,33] reported that ER/PR expression increases with increasing FIGO stages.

Menopausal status of the patients and expression of hormonal receptors were also correlated. While a rise in ER expression was noted between pre-menopausal and post-menopausal females (64.71% and 82.35%), a substantial fall in PR

expression was noted (88.24% and 52.94%) among the same. Similar to our observations, a study by Hecht *et al*.[19] noted a moderate increase in ER expression and a fall in PR expression between pre-menopausal and post-menopausal females. Studies by Kaur *et al*.[9] and Sylvia *et al*.[15] reported a higher PR expression among the patients >40 years of age.

Limitation

Our study had few limitations. First, our study was retrospective, and evaluating the association of ER/PR expression with survival rates of the patients was beyond the scope of the current study. Second, our study is limited by a smaller sample size, and the study findings being center-specific may not be representative of the whole Indian scenario and hence need to be interpreted cautiously. A future prospective study with a higher sample size and patient follow-up may provide a better insight and statistically significant results, and our study findings may surely be helpful to design the same.

Conclusion

ER and PR are expressed in a biologically and clinically meaningful subset of malignant EOCs, and their expression or co-expression suggests a better outcome compared to both ER and PR negative cases. Moreover, PR expression in particular appears to portend a more favorable prognosis. IHC analysis of ER/PR expression in high-grade serous and endometrioid carcinoma can help the primary care physicians in prognostic stratification of the patients and optimizing the available therapeutic measures. The data extrapolated from our study can serve as a template to tailor the existing clinical guidelines for effective and efficient patient management. Future clinical trials may further elucidate the role of ER/PR expression in predicting the response to endocrine therapy, identify biomarkers to determine such response, and optimize the treatment regimens accordingly.

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