Estrogen and Progesterone Receptor Expression and its Correlation with Various Clinicopathological Parameters in Ovarian Tumors

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Abstract

Background: This study evaluates estrogen and progesterone expressions in patients with ovarian tumors (both benign and malignant) and their correlation with various clinicopathological prognostic parameters. Receptors for estrogen and progesterone are predictive and prognostic markers of endometrial and breast cancers. However, their clinical significance in epithelial ovarian cancer is not clear due to conflicting data from only a few immunohistochemical studies available in the literature.

Methods: The present study was conducted on 60 cases of ovarian tumors, 20 benign and 40 malignant. Estrogen and progesterone expressions were studied by immuno-histochemistry and correlated with various clinicopathological parameters such as, menopausal status, histological type, WHO grade and FIGO stage.

Results: Out of 20 benign tumors the estrogen receptor was positive in 10 (50%) and progesterone receptor was positive in 14 (70%) tumors. In 40 malignant tumors, the estrogen receptor was positive in 13 (32.5%) and progesterone receptor was positive in 11 (27.5%) cases. There was statistically significant estrogen receptor expression observed in serous tumors (*P*=0.001). When compared with other clinico-pathological parameters, we noted a significant association between progesterone receptor expression and favorable prognostic parameters such as young age, benign tumors and early FIGO stage.

Conclusion: There were variable expressions of the estrogen and progesterone receptors in ovarian tumors. Progesterone receptor expression was associated with favorable prognostic factors that included younger age, benign tumor and low FIGO stage. No such association was observed with estrogen receptor expression.

Keywords: Estrogen, Progesterone, Receptor, Ovarian, Tumor

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Introduction

Cytosol estrogen and progesterone receptors are present in many organs including the breasts, endometrium, myometrium, cervix, fallopian tubes and ovaries. The ovaries are not only a source of estrogen and progesterone but they appear to be targets for these hormones.[1,2] 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 receptor are protective against ovarian cancer.[3,4] In patients with cancers of the breast and endometrium the relationship between tumor estrogen and progesterone receptor (PR) levels and prognosis is well documented. However, the clinical significance of ER and PR content in ovarian carcinomas has not been well established.[1,5]

The aim of our study was to determine the prognostic significance of ER and PR expression in various benign and malignant ovarian neoplasms by correlating with other known prognostic parameters.

Materials and Methods

The present study was conducted on 60 cases of ovarian tumors. There were 20 benign cases and 40 malignant cases included. We excluded non-neoplastic lesions. Relevant clinical details were obtained from patients' records and included age, menopausal status, FIGO stage, and follow up wherever possible. All specimens were thoroughly examined for external surface, capsular invasion, consistency of tumor (solid/cystic) and other features. Representative blocks were obtained after thorough sectioning of the tumor. Sections were prepared and stained with the hematoxylin and eosin (H & E) stain and other special stains wherever required for histopathological diagnosis, type and grade of tumor.

Immunohistochemical staining

Immunostaining for ER and PR was performed on representative sections that had an adequate area of cancer cells. The 5 μm thick sections were taken on poly-L-lysine coated slides. The tissues were deparaffinized, then rehydrated with xylene and ethanol and blocked with endogenous peroxidase with 3% H_2O_2 for 20 min. Sections were pretreated with citrate buffer at a pH of 6 in a microwave for 13 min and incubated in a protein blocking solution for 10 min. Sections were then incubated with primary monoclonal antibodies against ER and PR for 60 min followed by incubation with post primary block and polymer for 30 min. All sections were counterstained with Mayer's hematoxylin for 2 min and mounted.

Positive and negative controls were run with each batch. Positive staining of ER and PR was controlled by positively stained breast carcinoma sections; the negative control was performed on the same tissue without primary antibody.

The positive expression on the immunostained slides was interpreted as the percentage of the tumor cells that exhibited nuclear staining for the particular receptor regardless of intensity. We counted at least ten random high power fields with a minimum of 1000 cells. Sections were considered positive when more than 10% of the cells were positive for that receptor. Original H & E sections were reviewed in conjunction with the immunohistochemical stained section to obtain the final results.

Statistical analyses were performed with SPSS statistical software, version 11.5. Correlation between ER and PR expression was studied and their associations with clinicopathological parameters such as age, menopausal status, histological type, WHO grade and FIGO stage were compared using the chi square test by univariate analysis. A P-value less than 0.05 was considered statistically significant.

Results

Clinical and histological data

Patients' mean age was 43 years (range: 11-71). There were 23 (38.3%) premenopausal, 17 (28.3%) perimenopausal and 20 (33.3%) postmenopausal patients. The study comprised 20 benign and 40 malignant tumors and included 3 cases of borderline malignancy. The majority of

Table 1. Correlation of estrogen/progesterone receptor (ER/PR) expression in different types of malignant tumors (n=40).

ER/PR Expression	Surface epithelial tumors (n=32)			GCT (n=3)	SCST (n=4)	Others (n=1)
	Serous (n=23)	Mucinous (n=8)	Endometrioid (n=1)			
ER						
Positive	12 (52.8%)	1 (12.5%)	-	-	-	-
Negative	11 (47.8%)	7 (87.5%)	1 (100%)	3 (100%)	4 (100%)	1 (100%)
PR						
Positive	7 (30.4%)	1 (12.5%)	-	-	2 (50%)	1 (100%)
Negative	16 (69.6%)	7 (87.5%)	1 (100%)	3 (100%)	2 (50%)	-
ER/PR combination						
ER+/PR+	5 (21.7%)	-	-	-	-	-
ER+/PR-	7 (30.4%)	1 (12.5%)	-	-	-	-
ER-/PR+	2 (8.7%)	1 (12.5%)	-	-	2 (50%)	1 (100%)
ER-/PR-	9 (39.1%)	6 (75%)	1 (100%)	3 (100%)	2 (50%)	-
Total cases	23	8	1	3	4	1

GCT: Germ cell tumor, SCST: Sex cord stromal tumor

benign tumors (90%) were unilateral while 37.5% of malignant tumors had involvement of both ovaries. We observed a wide histomorphological spectrum of tumors in both benign and malignant categories.

Epithelial tumors constituted the largest group (78.3%) followed by sex cord stromal tumors (13.3%). Histological grading could only be performed in 29 cases of malignant surface epithelial tumors. There were 13 (45%) grade II cases, 10 (34.5%) grade I and 6 (20.5%) grade III. Staging was performed in the 40 malignant tumors; approximately half (52.5%) had early FIGO stages I and II.

Immunohistochemistry

In total, immunohistochemistry ER expression was seen in 23 (38.3%) and PR expression was seen in 25 (41.7%) cases. There was a statistically significant PR expression in 14 (70%) of the benign tumors. In malignant tumors 13 (32.5%) were ER positive and PR expression was observed in only 11 (27.5%) cases. The largest group that comprised 21 (52.5%) cases were ER and PR negative and 5 (12.5%) were ER and PR positive. All three borderline tumors were PR positive. Expressions of both receptors in various tumor types are shown in Table 1.

There was a statistically significant correlation between ER and PR expression. As seen in Table 2, a total of 70% of cases were found to be either positive or negative for both receptors (*P*=0.003).

Univariate analysis showed no correlation between ER expression and age, menopausal status, histological subtype, grade and FIGO stage. However, in a comparison between serous and non-serous malignant tumors, there was a significant ER expression with serous tumors seen in 12 of 13 positive cases (*P*=0.001). According to Table 3, PR expression was associated with younger age (<30 years), benign tumors and early FIGO stage (*P*<0.05).

We studied the combined patterns of ER and PR expression. From 60 cases, 15 (25%) expressed both ER and PR (ER+PR+), 27 (45%) were negative for both (ER-PR-) and 10 (16.7%) were only PR positive (ER-PR+). PR positive combinations significantly correlated with the low stage of benign tumors, whereas PR negative combinations were associated with high stage (Table 4).

Discussion

Worldwide, epithelial ovarian cancer (EOC) is the leading cause of death from gynecologic malignancies.[6] Malignant ovarian tumors are mostly detected at a clinically advanced stage. In addition to cytotoxic chemotherapy which has been traditionally used in the treatment of advanced cases, favorable results have also been reported after endocrine treatment. Since the

Table 2. Correlation between estrogen receptor (ER) and progesterone receptor (PR) expression.

ER/PR expression	PR+	PR-	Total
ER+	15 (25%)	8 (13.3%)	23 (38.3%)
ER-	10 (16.7%)	27 (45%)	37 (61.7%)
Total	25 (41.7%)	35 (58.3%)	60 (100%)
P=0.003			

presence of steroid hormone receptor is of prognostic significance in hormone treatment of different types of ovarian cancer, it is important to identify those tumor groups that contain steroid receptors, so that patients who may benefit from hormone therapy may be identified.[7]

Estrogen and progesterone receptors are important hormones secreted mainly by the ovaries. These hormones act through their specific receptors and have been implicated in the pathogenesis of gynecologic malignancies, including breast, endometrial, and ovarian cancers.[8-10]

Both ER and PR are well recognized as important prognostic indicators of breast and endometrial cancers. The clinical correlation of hormonal receptors in ovarian cancer is less well understood due to conflicting data, since most ER and PR data from ovarian cancers are from studies that have used the biochemical dextran-coated charcoal (DCC) method with only a few studies that have used immunohistochemistry analyses.[3,11-16]

Positive detection rates in these studies were reported in wide ranges for ER (38%-77%) and PR (26%-71%).[3,11-13,17-19] These findings depended partly on the methods used for receptors and partly on the number of cases in the study. The DCC technique yielded a higher rate of detection than those discovered via immunohistochemistry because of the presence of cytosolic ER or PR in normal ovarian stromal cells.[20]

We observed 38.3% of ER and 41.7% of PR expressions in our study. These figures agreed with a study by Siriwan et al.,[13] however they were lower than findings from other studies that used the same technique.[3,12,20] These variations could be either due to the heterogeneous study population, number of cases or different proportion of tumor histology.[13] Since ER expression was generally highest in serous carcinoma and lowest in mucinous carcinoma, the resultant higher ER expression in their study comprised 63%-77% of serous carcinoma and 2%-8% of mucinous carcinoma. The current study noted 53.3% of serous and 21.7% of mucinous carcinoma.

In our study, ER and PR expressions were significantly associated with each other. In 70% of cases either both were detected or absent. This significant correlation was observed in a few other studies.[13,21]

We studied the association of ER/PR expressions with clinicopathological factors, that included age, menopausal status, histological subtype, grade of tumor, FIGO stage, and survival data whenever follow-up data was available. We compared our results with other reports that used either DCC or immunohistochemistry because there were a limited number of studies that used the immunohistochemistry technique. Except for the possible difference in the level of positivity in the DCC method, we thought the relationship between hormonal receptors and characteristic features should not be affected by technique. However, we obtained conflicting results from various studies, even those who used the same technique. We compared two groups of patients, those <30 years and >50 years. PR expression was significantly associated with younger age, which was also observed in other studies.[5,12] A few observed higher expression of ER in the older age group.[13,18]

No significant association was seen with menopausal status in both our study and a number of other studies that attempted to correlate hormone receptor expression with menopausal status. In benign tumors there was a significantly higher PR expression (P<0.01). Only a few studies

Table 3. Clinicpathological data (n=60).

Parameters	Estrogen receptor (ER) expression		Progesterone receptor (PR) expression	
	n (%)	*P*-value	n (%)	*P*-value
Age (years)				
<30 (n=15)	6 (40)	>0.05	11 (73.3)	<0.05
>50 (n=14)	6 (42.8)		5 (35.7)	
Menopausal status				
Premenopausal (n=23)	7 (30.4)		13 (56.5)	
Perimenopausal (n=17)	6 (35.3)	>0.05	5 (29.4)	>0.05
Postmenopausal (n=20)	10 (50)		7 (35)	
Tumor type				
Benign (n=20)	10 (50)	>0.05	14 (70)	<0.01
Malignant (n=40)	13 (32.5)		11 (27.5)	
Histological type				
Serous	12/13 (92.3)	=0.001	7/11 (63.6%)	>0.05
Non-serous	1/13 (7.7)		4/11 (36.3%)	
WHO grade (n=29)				
Grade 1 (n=10)	2 (20)		3 (30)	
Grade 2 (n=13)	5 (38.5)	>0.05	2 (15.4)	>0.05
Grade 3 (n=6)	4 (66.6)		0 (0)	
FIGO stage (n=40)				
Stage I (n=17)	5 (29.4)		10 (58.8)	
Stage II (n=4)	2 (50)	>0.05	1 (25)	<0.01
Stage III (n=11)	5 (45.5)		0 (0)	
Stage IV (n=8)	1 (12.5)		0 (0)	

included benign tumors and the majority had consistent findings with our study.[14,22,23] Andrel et al. reported low PR positivity (36%) in benign tumors.[24]

In terms of tumor grade, our study found a higher percentage of grade II and III tumors that expressed ER. The association between higher grade tumor and ER expression was concordant with a few studies[13,20] and contrasted others who found ER receptors more often in grade I tumors.[25,26] PR expression in our study was more frequent in grade I tumors and altogether absent in grade III tumors, an observation that was supported by Ayadi et al.[21] However, this association of ER/PR expression and grade of tumor was not statistically significant.

FIGO stage is the only universally accepted prognostic factor for patients with ovarian carcinoma; this is a powerful prognostic predictor that most other putative prognostic factors are of little importance compared to stage.[27] In our study we have shown significantly higher PR expression in stage I tumors; all cases in stages III and IV

were PR negative. There was variable ER expression. Similarly a few other studies also reported a significant inverse correlation of higher PR concentration in early stage disease.[1,13,17,21]

We found PR positive combination patterns such as ER+/PR+ or ER-/PR+ to be positively correlated with benign tumors (*P*<0.01) and low stage. PR negative patterns such as ER+/PR- or ER-/PR- were associated with higher stage tumors (*P*=0.01). Our findings were concordant with other studies[12,13,25] that used either the DCC or immunohistochemistry techniques. Iversen et al.[25] used the DCC technique and found significantly lower survival in patients who were ER+/PR+ compared to ER-/PR-, however they did not evaluate survival of patients with ER-/PR+ expression. Munsted et al.[12] used immunohisto-chemistry and observed a positive survival influence in patients with ER-/PR+ tumors along with a significant association with other prognostic factors such as low stage, lower grade and minimal ascites at primary surgery. Siriwan et al.[13] found good prognosis in ER-/PR+ cases. They could

Table 4. Correlation of estrogen receptor (ER)/progesterone receptor (PR) combination expression.

Parameter	ER+/PR+	ER+/PR-	ER-/PR+	ER-/PR-
Age (years)				
<30 (n=15)	6 (40%)	-	4 (26.7%)	5 (33.3%)
>50 (n=14)	3 (21.4%)	3 (21.4%)	6 (42.9%)	2 (14.35%)
P-value		*P*>0.05		
Menopausal status				
Premenopausal (n=23)	7 (30.4%)	-	6 (26.1%)	10 (43.5%)
Perimenopausal (n=17)	3 (17.6%)	3 (17.6%)	2 (11.8%)	9 (53%)
Postmenopausal (n=20)	5 (25.5%)	5 (25%)	2 (10%)	8 (40%)
P-value		*P*>0.05		
Type (n=60)				
Benign (n=20)	10 (50%)	-	4 (20%)	6 (30%)
Malignant (n=40)	5 (12.5%)	8 (20%)	6 (15%)	21 (52.5%)
P-value		*P*<0.01		
Stage (n=40)				
I (n=17)	4 (23.5%)	1 (5.9%)	6 (35.3%)	6 (35.3%)
II (n=4)	1 (25%)	1 (25%)	-	2 (50%)
III (n=11)	-	5 (45.5%)	-	6 (55.5%)
IV (n=8)	-	1 (12.5%)	-	7 (87.5%)
P-value		*P*=0.01		
Grade (n=29)				
1 (n=10)	1 (10%)	1 (10%)	2 (20%)	6 (60%)
2 (n=13)	2 (15.4%)	3 (23.1%)	-	8 (61.5%)
3 (n=6)	-	4 (66.6%)	-	2 (33.3%)
P-value		*P*>0.05		

not identify a significant association of this ER-/PR+ subgroup with any favorable prognostic factor. Other studies that attempted to evaluate the prognostic role of ER and PR in combination could not demonstrate an additional benefit of ER+ or ER- expression of PR+ tumors.[5,17,26]

We could not evaluate the association of ER/PR expression with survival rates because of unavailability of follow up details in many patients.

To summarize, in the current study there was a significant association between PR and other favorable prognostic parameters such as young age, benign tumors and early FIGO stage. These could be considered potential prognostic markers. However, there were inconsistent findings of ER/PR expression and clinicopathological parameters in various studies, including ours. Hence, an absolute conclusion could not be derived. We have emphasized the need for comprehensive data from more studies that have used the same technique (immunohistochem-

istry), well-marked cut off levels for ER/PR expression and a larger sample size. Such reports would be informative and are warranted to clarify whether hormone treatment based on hormone receptor status can be an alternative treatment in ovarian carcinoma patients.

Conflicts of interest

No conflict of interest is declared.

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