Expression of estrogen receptors in epithelial ovarian carcinoma

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Background/aim
Epithelial ovarian cancer accounts for ~3% of female cancers. Steroid hormones such as estrogen and progesterone are thought to play an important role in the process of carcinogenesis of ovarian tumors. There are two subtypes of the nuclear estrogen receptor (ER-α and ER-β) encoded by separate genes. This work aimed to evaluate the expression pattern of ER-α and ER-β in epithelial ovarian carcinoma and their correlation with tumor histopathological parameters and proliferating cell nuclear antigen expression as a proliferation marker.
Materials and methods
A total of 50 cases of epithelial ovarian carcinoma were included in this study. All cases were female patients who underwent oophorectomies or subtotal or total hysterectomies with oophorectomies. Surgical specimens were sent to Pathology Department at Kasr El-Aini hospitals and to private laboratories. The cases were graded and staged according to WHO systems. The cases were stained by hemotoxylin and eosin for histopathological grading, and they were immunohistochemically stained for ER-α, ER-β, and proliferating cell nuclear antigen using streptavidin–biotin technique.
Results
In this study, 56% of cases were positively stained for ER-α. It is significantly correlated with both of the tumor histological type and proliferative state of the tumors. There was a significant inverse correlation between ER-α expression and the tumor histological grade. Approximately 62% of cases were positively stained for ER-β. There was a significant inverse correlation between ER-β positivity and both of the tumor stage and proliferative state of ovarian carcinoma cases.
Conclusion
The loss of ER-β, not ER-α, expression in ovarian tumors may be a feature of malignant transformation suggesting its potential role as tumor suppressor gene. Determination of ER subtypes may improve response to hormonal therapy using a selective ER modulator in selected cases of ovarian carcinoma.

Keywords:
estrogen receptor-α, estrogen receptor-β, immunohistochemistry, ovarian carcinoma, proliferating cell nuclear antigen

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Introduction

In USA, ovarian cancer is the main gynecological cause of death and represents the fifth cause of cancer death among US women [1]. In Egypt, ovarian carcinoma represented ~1.37% of total malignancy [2].

Ovarian cancer is diagnosed at an early stage in only 15% of cases. The 5-year survival rate becomes more than 90% when ovarian cancer is diagnosed and treated early [3].

Ovarian tumors were classified according to the cell of origin and histopathological features. Ovarian tumors of epithelial cell origin represent more than 90% of all ovarian tumors [4].

Approximately 3% of female cancers are ovarian carcinomas of epithelial origin, which are the leading cause of death from gynecologic malignancy. During the past decades, although the incidence has decreased and survival slightly improved, most tumors are diagnosed at advanced stages and the relative 5-year survival is less than 50% [4,5].

Many molecular markers were investigated in epithelial ovarian tumors by immunohistochemistry [4]. In the process of ovarian carcinogenesis, steroid hormones such as estrogen and progesterone (PR) are thought to play an important role [3].

Although the prognostic and treatment-predictive role of estrogen and PR receptors has been established in breast cancer, their role in ovarian cancer is less well defined. In small clinical trials, a clear benefit has not established in evaluating their role in hormone-blocking treatment of ovarian cancer. However, clinicians often encountered positive outcomes with this type of treatment [6].

Changes in ovarian neoplasms receptor status can be occurred and consequently, the tumors become either primary receptor negative or lose the receptors as a result of their progression [7].

Estrogen effects can be achieved on target tissues through interaction with estrogen receptors (ERs). There are two types of estrogen nuclear receptors, ER-α and ER-β, encoded by separate genes [8]. The expression of ERs in epithelial ovarian tumors was little known; however, their distribution pattern suggests that both receptors mediate different aspects of estrogen action in the ovary [9]. It was found that the loss of ER-β and/or increased ER-α/ER-β ratio may be a common step in ovarian tumor progression [10,11].

The relationships between ER-α and ER-β expression, their prognostic values in ovarian tumors, and their correlation with clinicopathological parameters were investigated in many studies [12,13].

Antiestrogen treatment has not been successful in ovarian cancer in spite of their ER expression. It was found that expression of PR is prognostically favorable, whereas the results on ER-α and ER-β are contradictory [12,14,15].

This work aimed to evaluate the expression pattern of ER-α and ER-β in epithelial ovarian carcinoma and their correlation with tumor histopathological parameters. Moreover, their expression is correlated with proliferating cell nuclear antigen (PCNA) expression as a proliferation marker to characterize the prognostic significance of these steroid receptors.

Materials and methods

Study design

A total of 50 cases of epithelial ovarian carcinoma were included in this study. The age of the patients ranged between 24 and 71 years, with a mean age of 47.5 years. All cases were female patients who underwent oophorectomies or subtotal or total hysterectomies with oophorectomies. Surgical specimens were sent to Pathology Department at Kasr El-Aini hospitals and to private laboratories.

The cases were graded according to WHO grading system [16]. Clinical staging of the cases was done according to WHO staging system [17].

Formalin-fixed and paraffin-embedded blocks from these cases were collected, and then 4-μm-thick sections were cut from each block and stained by hemotoxylin and eosin to revise the diagnosis, type, and grade of the tumor and immunohistochemical staining.

Ethical approval

The protocol was approved by the 'Ethical Committee' of the 'National Research Centre'. The agreement reference number is 09/183.

Immunohistochemical staining

Immunohistochemical staining for ER-α, ER-β, and PCNA was done using streptavidin–biotin technique. A total of 3 4-μm-thick sections from each case were deparaffinized, hydrated, and incubated in 3% hydrogen peroxide for 30 min to block the internal peroxidase activity. Antigen retrieval was done by microwave pretreatment for 10 min in 0.01 mol/l citrate buffer. For each case, one slide was incubated at 4°C overnight with anti-ER-α mouse monoclonal antibodies (clone 1D5) (Dako Corporation, Glostrup, Denmark; Microsoft corporation: Redmond, United States). The second slide was incubated with polyclonal antibody to ER-β (clone SP1) (Biogenex Corporation, Headquarters: Fremont, California, USA). The third slide was incubated with rabbit monoclonal antibody to PCNA (clone PC10) (Biogenex Corporation). These steps were followed by 30-min incubation with biotinylated horse antimouse antibody at room temperature, avidin–biotin peroxidase complex for 50 min at room temperature, and finally diamiobenzidine for 3–5 min (Universal LSAB, Rb/Mo,150 tests, clone KO673, liquid diamiobenzidine; Dako Company). The slides were counterstained with hematoxylin, dehydrated, and mounted.

Known positive breast carcinoma was used as positive control, used for both ER-α and ER-β, whereas for PCNA, a case of colon carcinoma was used, and negative control was obtained by omitting the primary antibody.

Antigen expression was studied at the Pathology Department, National Research Center using the Leica Qwin 500 Image Analyzer (LEICA Imaging Systems Ltd, Cambridge, England) which consists of Leica DM-LB microscope with JVC color video camera attached to a computer system Leica Q 500IW (LEICA Imaging Systems Ltd., Cambridge, England). Overall, 10 high power fields (x400) were

chosen randomly. Cells were judged as positive for ER-α, ER-β, and PCNA expressions when the nucleus was stained. Antigen expression was evaluated as percentage of positive stained cells.

The percentage of positive stained cells (PP) of ER-α and ER-β was estimated as follows: 0=no staining, 1=less than 10% of the cells, 2=11–50% of the cells, 3=51–80% of the cells, and 4=greater than 81% of the cells [12]. For ERs, the cases were considered positive if the percentage of positive stained cells is more than 10% [12,18].

Proliferation index (PI) was estimated as the percentage of PCNA-expressing cells. Tumors were considered low proliferative if PI is 0–10%, and high proliferative if PI is 11–100% [19].

Statistical analysis

Data were statistically described in terms of frequencies (number of cases) and percentages when appropriate. Comparisons were done using χ^2-test. Yates correction equation was used instead when the expected frequency is less than 5. All statistical calculations were done using computer programs Microsoft Excel 2007 (Microsoft Corporation, New York, USA) and SPSS (statistical package for the social sciences; SPSS Inc., Chicago, Illinois, USA) version 15 for Microsoft Windows. For a relation to be considered statistically significant, *P* value should be less than 0.05.

Results

In this study, a total of 50 cases of epithelial ovarian carcinoma have been studied. All cases are female patients who underwent oophorectomies or subtotal or total hysterectomies with oophorectomies and were referred to Pathology Department at Kasr El-Aini hospitals and in private laboratories.

The studied cases included 29 (58%) cases with endometrioid adenocarcinoma, 15 (30%) cases with papillary serous adenocarcinoma, and six (12%) mucinous-type adenocarcinoma cases.

The age of the patients ranged between 24 and 71 years, with a mean age of 47.5 years. The mean age was highest in papillary serous carcinoma cases.

According to the WHO classification 2004 grading system, 17 (36%) cases were grade I, 19 (38%) cases were grade II, and 14 (26%) cases were grade III. All mucinous cases were grade I, and 89.5% of grade II were of endometrioid type.

According to TNM staging system, 29 (58%) cases were tumor stage T1, two (4%) cases were T2, 18 (36%) cases were T3, and one case only was T4 (Table 1).

Immunohistochemical results

Proliferating cell nuclear antigen

PI was estimated as the percentage of PCNA positively stained cells. Cases were considered low proliferative if PI is 0–10%, and high proliferative if PI is 11–100%.

In the present study, four (8%) cases were of low proliferative index and 46 (92%) cases were of high proliferative index, as shown in Table 1. Figure 1 shows highly proliferative endometrioid adenocarcinoma of the ovary.

Estrogen receptor-α expression

In this study, 28 (56%) cases were positively stained for ER-α, where more than 10% of cells showed nuclear staining for ER-α.

There was a significant correlation (*P*=0.043) between ER-α and the tumor histological type, where the mucinous adenocarcinomas of the ovary are most likely to be ER-α positive (100%) followed by the papillary (60%) and then the endometrioid-type adenocarcinoma (44.8%), as shown in Table 1.

Table 1 Correlation between histologic types and clinicopathological parameters in epithelial ovarian carcinoma

	Endometrial	Papillary serous	Mucinous	Total
Grades				
Grade I	4	7	6	17
Grade II	17	2	–	19
Grade III	8	6	–	14
Total	29	15	6	50
Stage				
Stage I	17	8	4	29
Stage II	1	1	–	2
Stage III	10	6	2	18
Stage IV	1	–	–	1
Total	29	15	6	50
PI (PCNA) [*n* (%)]				
LP	2 (7)	2 (23)	–	4 (8)
HP	27 (93)	13 (78)	6 (100)	46 (92)
Total	29	15	6	50
ER-α [*n* (%)]				
<10%	16 (55)	6 (40)	–	22 (44)
>10%	13 (45)	9 (60)	6 (100)	28 (56)
Total	29	15	6	50
ER-β [*n* (%)]				
<10%	11 (38)	4 (27)	4 (67)	19 (38)
>10%	18 (62)	11 (73)	2 (33)	31 (62)
Total	29	15	6	50

ER, estrogen receptor; PCNA, proliferating cell nuclear antigen; PI, proliferation index.

There was a nonsignificant difference between ER-α expression among different age groups of all cases, with a *P* value of 0.243.

This study showed that 88% of grade I, 53% of grade II, and 21% of grade III were positively stained for ER-α, revealing a significant inverse correlation (*P*=0.001) between ER-α expression and the tumor histological grade (Table 2). Figure 2 shows ER-α positive staining of low-grade serous adenocarcinoma of the ovary.

In this study, there was a nonsignificant correlation (*P*=0.739) between ER-α positivity and the tumor histological stage (Table 3).

It was found that all cases of low proliferative index were negatively stained for ER-α, whereas 61% of high proliferative cases were positively stained for ER-α, revealing a significant correlation between ER-α expression and tumor cell proliferation (*P*=0.027; Table 4).

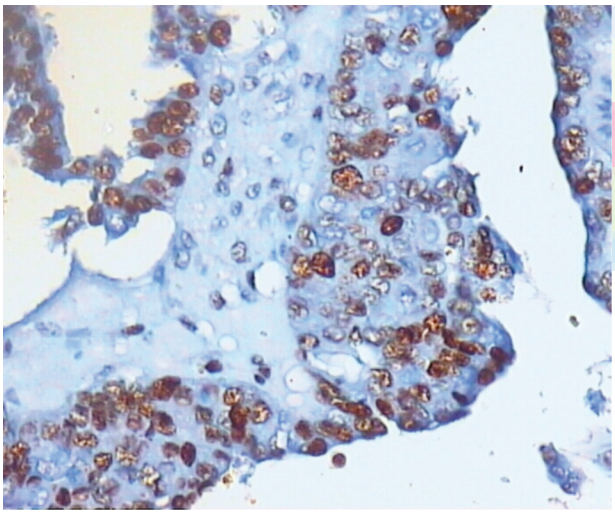
Estrogen receptor-β expression

In this study, 31 (62%) cases were positively stained for ER-β, where greater than 10% of cells showed nuclear staining for ER-β. The highest positivity was detected in papillary serous type, where 73.3% of the cases were positively stained for ER-β, revealing a nonsignificant correlation with tumor histological types, with *P* value of 0.233 (Table 1).

ER-β expression was nonsignificantly correlated with tumor histologic grade (*P*=0.471), where 70% of grade I, 53% of grade II, and 64% of grade III cases were positively stained for ER-β (Table 2). Figure 3 shows ER-β nuclear expression in low-grade mucinous adenocarcinoma of the ovary.

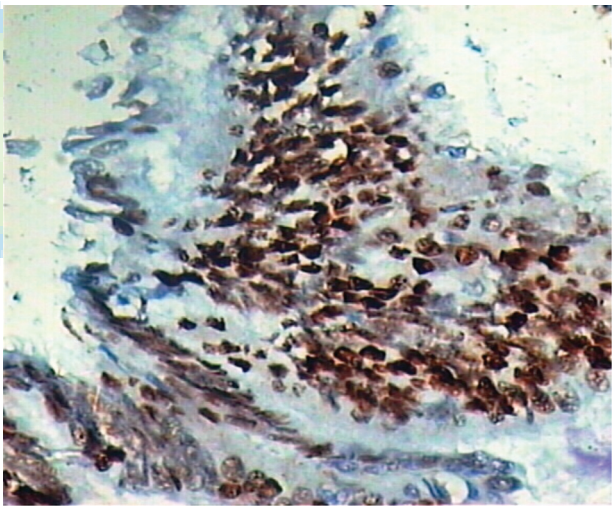
Regarding ER-β among different stages of all cases, it was found that 90% of stage I cases and 17% of high

Figure 1



Proliferating cell nuclear antigen immunohistochemical staining showing highly proliferative endometrioid adenocarcinoma of the ovary (immunohistochemistry, 200×).

Figure 2



Estrogen receptor-α-positive immunohistochemical staining of low-grade serous adenocarcinoma of the ovary (immunohistochemistry, 200×).

Table 2 Correlation between expression of estrogen receptors and histologic grade in epithelial ovarian carcinoma

	Grade I [*n* (%)]	Grade II [*n* (%)]	Grade III [*n* (%)]	Total	*P* value
ER-α					
<10%	2 (12)	9 (47)	11 (79)	22	0.001
>10%	15 (88)	10 (53)	3 (21)	28	
Total	17	19	14	50	
ER-β					
<10%	5 (30)	9 (47)	5 (36)	19	0.471
>10%	12 (70)	10 (53)	9 (64)	31	
Total	17	19	14	50	

ER, estrogen receptor.

Table 3 Correlation between expression of estrogen receptors and clinical stage in epithelial ovarian carcinoma

	Stage I [*n* (%)]	Stage II [*n* (%)]	Stage III [*n* (%)]	Stage IV [*n* (%)]	Total	*P* value
ER-α						
<10%	12 (41)	0	10 (56)	0	22	0.739
>10%	17 (59)	2 (100)	8 (44)	1 (100)	28	
Total	29	2	18	1	50	
ER-β						
<10%	3 (10)	0	15 (83)	1 (100)	19	0.035
>10%	26 (90)	2 (100)	3 (17)	0	31	
Total	29	2	18	1	50	

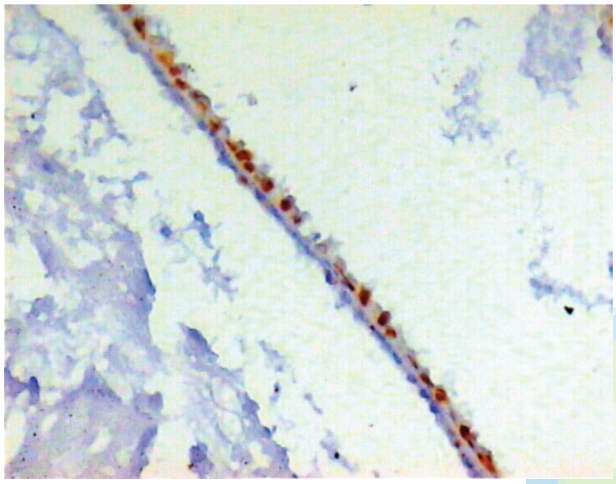
ER, estrogen receptor.

Table 4 Correlation between expression of estrogen receptors and proliferation index in epithelial ovarian carcinoma

	LP	HP	Total	*P* value
ER-α				
<10%	4 (100)	18 (39)	22	0.027
>10%	0	28 (61)	28	
Total	4	46	50	
ER-β				
<10%	0	19 (41)	19	0.039
>10%	4 (100)	27 (59)	31	
Total	4	46	50	

ER, estrogen receptor.

Figure 3



Estrogen receptor-β nuclear expression in low-grade mucinous adenocarcinoma of the ovary (immunohistochemistry, 200×).

stage (III and IV) showed positive nuclear staining, revealing a significant inverse correlation (P=0.035) between ER-β positivity and the tumor histological stage (Table 3).

It was found that ~41% of cases with high PI were negatively stained for ER-β, and all cases with low proliferation were positively stained for ER-β, revealing a significant inverse correlation between ER-β and proliferative state of ovarian carcinoma cases, with a P value of 0.039 (Table 4).

Discussion

The ovary is one of the most dynamic organs and undergoes intensive age-dependent and ovarian cycle-dependent remodeling. The equilibrium between the process of proliferation and apoptosis of ovarian cells is required for such remolding [20,21].

In western countries, ovarian cancer is the fourth common cause of death in women. Multiple gene abnormalities are associated with ovarian carcinogenesis, and they could have a predictive or prognostic relevance [3,22].

It was assumed that estrogen signaling role in ovarian cancer development and progression is less significant than for breast and endometrial cancers. Many studies have shown that normal ovaries and many malignant ovarian cancers are endocrine related and hormone dependent [18].

The importance of estrogen signaling in the development and progression of ovarian cancer has been assumed to be less significant than for breast or endometrial cancers, although clinical data, animal experiments, and receptor studies have shown that not only normal ovaries but also many malignant ovarian tumors can be considered as endocrine related and hormone dependent [23].

Previous studies have been concentrated on the classical ER, ER-α. A second ER, ER-β, was identified in 1996. The biological response to ER agonist and antiestrogens depends on the relative levels of ER-α and ER-β [24,25].

Little was known about expression levels of the ERs in ovarian epithelial tumors or in normal ovarian surface epithelium. Previous studies have shown that in ovarian cancer samples, ER-β mRNA level is decreased when compared with normal ovaries, whereas the level of ER-α mRNA is similar or slightly higher in cancer samples compared with normal biopsies [25,26].

It was shown that ER-β mRNA level is less in ovarian cancer than normal ovaries, whereas the level of ER-α mRNA is similar or slightly higher in cancer samples compared with normal biopsies [20,21].

This work aimed to evaluate the expression pattern of ER-α and ER-β in epithelial ovarian carcinoma and their correlation with tumor histopathological parameters. Moreover, their expression was correlated with PCNA expression as a proliferation marker to characterize the prognostic significance of these steroid receptors.

In this study, fifty cases of epithelial ovarian carcinoma have been studied, including 29 of endometrioid adenocarcinoma, 15 cases of papillary serous adenocarcinoma, and six cases of mucinous adenocarcinoma.

In our study, antigen expression was evaluated as the percentage of positive stained cells. ERs expression was

considered positive if the percentage of positive stained cells is more than 10% regardless of the intensity. This is in accordance with the studies by Halon *et al.* [18], Buchynska *et al.* [7], and Sharifi *et al.* [27].

Many studies have described the relationships between ER-α and ER-β expressions in ovarian tumor tissue and correlated them with clinicopathological parameters, including patients age as well as tumors histological types, tumors grading, and staging [12,13,28,29].

In this study, patients' ages revealed nonsignificant correlation with both ER-α and ER-β expressions, with a *P* value of 0.243 and 0.836, respectively. This is also agreed by the studies by Han *et al.* [13] and Yang *et al.* [30]. On the contrary, the studies of Naik *et al.* [3], Burges *et al.* [12], and Hecht *et al.* [31] revealed significant correlation between ERs and patients' age.

Regarding tumor histological types, our study showed a significant correlation (*P*=0.043) between ER-α and the tumor histological type, where all cases of mucinous-type, 60% of the papillary serous type, and 44.8% of the endometrioid-type adenocarcinomas were positively stained for ER-α. Moreover, other studies revealed a significant correlation between ER-α and the tumor histological type but highly expressed in serous adenocarcinoma [24,30,32,33]. In the study by Naik *et al.* [3], ER-α positivity was more frequently found in transitional cell carcinoma (100%) and endometrioid carcinoma (80%). On the contrary, Han *et al.* [13] and Halon *et al.* [18] revealed nonsignificant difference between ER-α expression in different histological types.

Regarding the ER-β expression in various histological types in this study, papillary serous adenocarcinomas were the highest category in expression followed by the endometrioid, and then the mucinous adenocarcinomas, with a nonsignificant correlation. This is in agreement with the studies by Han *et al.* [13], Chan *et al.* [24], and Geisler *et al.* [34] who revealed a higher expression of ER-β in endometrioid type. Lindgren *et al.* [8] study revealed significant difference of ER-β expression in different histological types.

Patient outcome is related to clinicopathological factors, the most important of which are tumor stage, residual disease after initial surgery, histological type, and tumor grade. However, different clinical outcomes can be detected in patients with similar clinicopathological characteristics. The identification of prognostic factors in ovarian cancer has been a major task during the past years [3,22].

Although most ovarian cancers express ER, antiestrogen treatment has not been successful in ovarian cancer. Several studies have assessed the prognostic value of ER-α and ER-β, but the results are contradictory [5].

In this study, ER-α expression in ovarian carcinoma samples demonstrated a significant direct association with tumor grading. This is in agreement with the results reported by Burges *et al.* [12], Yang *et al.* [30], and Tanvanich *et al.* [35]. In contrast, other studies revealed a nonsignificant correlation between ER-α expression and tumor grade [12,13,18,31,32,34].

Our study revealed decreased ER-β nuclear staining with the increase of tumor grade, with a nonsignificant correlation. This was in accordance with what was found out by Burges *et al.* [12] and Han *et al.* [13].

The prognosis of ovarian cancer is closely related to the stage at diagnosis, as determined according to the staging system developed by the International Federation of Gynecology and Obstetrics (FIGO) [13].

The study by Chan *et al.* [24] showed that patients with a higher ER-β level had better prognosis, even after regression analysis. However, ER–α level, with a similar classification as for ER-β, was not shown to be significantly correlated with survival, suggesting a different role for ER-β in ovarian carcinogenesis compared with ER-α.In our study, ER-α immunoreactivity increases with the increase in different tumor stages, with a nonsignificant correlation. Similar results were reported in previous studies [12,13,18,31]. This was in contrast to the studies by Treviño and Johnson [1], Yang *et al.* [30], and Høgdall *et al.* [32], which revealed that ER-α positive tumors were significantly associated with increasing stage, giving a *P* value of 0.0003.

In contrast to ER-α, the ER-β has been suggested as a potential tumor suppressor protein, and overexpression of ER-β in ovarian cancer cells gave rise to changes in cell cycle progression and cancer cell migration. ER-β-overexpressing cells exhibited significant reduced cell migration, and thus, enhanced ovarian cancer cell migration by loss of ER-β expression, which might contribute to the observed correlation between reduced ER-β expression and lymph node metastasis [25,36].

The present study showed that ER-β expression was significantly inversely correlated with staging. This was in accordance with Burges *et al.* [12], Chan *et al.* [24],

and Caiazza *et al.* [25] where ER-β expression was significantly higher in stage I disease compared with stage II–IV disease ($P<0.001$). However, it was unlike the results of Han *et al.* [13], which revealed nonsignificant correlation between ER-β and tumor size or lymph node metastasis.

Determination of the proliferative potential of a tumor can be achieved by immunohistochemical detection of proliferating cells [37]. PCNA is a proliferation marker helpful in predicting disease outcome in many types of malignancies including ovarian neoplasms. The role of immunostaining is now employed not only for diagnosis but also for other parameters including prognosis, microscopic tumor staging, prediction of response to therapy, and for the selection of therapeutic agents [3].

Proliferative activity and steroid hormone receptor status along with clinical and morphological characteristics of the disease have prognostic significance and may be used for the evaluation of ovarian carcinoma course. So, in our study, PCNA was used as a proliferative marker, where tumors were classified as low and high proliferating tumors according to the percentage of expressing cells.

In our study, ER-α expression was significantly correlated with tumor cell proliferation where all cases of low proliferative index were negatively stained for ER-α, whereas 39.2% of high proliferative cases were positively stained for ER-α ($P=0.072$). On the contrary, there was a significant inverse correlation between ER-β expression and proliferative state of ovarian carcinoma, where 84% of cases with low PI were positively stained for ER-β.

These data suggest that ER-β might play a protective role against ER-α mitogenic activity or that the loss of ER-β is a marker of cell dedifferentiation. ER-β antimitotic activity has recently been established in cultured breast cancer cell line. If this hypothesis is confirmed, molecules specifically activating ER-β or inducing ER-β re-expression in neoplastic cells may be beneficial for blocking tumor proliferation or invasion. Estrogen-induced gene expression especially through ER-β may also constitute a new therapeutic target [25,29].

Conclusion

Estrogen action is achieved through the regulation of target genes by two ERs, ER-α and ER-β. Loss of ER-β, but not ER-α, expression in ovarian tumors may be a feature of malignant transformation, suggesting its potential role as tumor suppressor gene. Determination of ER subtypes may improve response to hormonal therapy using a selective ER modulator in selected cases of ovarian carcinoma. Further studies are needed to determine the role of ER-β in developing and progression of ovarian carcinoma.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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