Expression of chemoresistance marker P-Glycoprotein in women with epithelial ovarian carcinoma

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Abstract

Objective:
The objective of the present study is to determine the relationship between percentage of P-glycoprotein immunoreactivity and the histologic types and clinical features of epithelial ovarian carcinoma.

Materials and methods:
Twenty-three newly diagnosed patients with primary epithelial ovarian carcinoma, were included in this study. Patients were treated with cytoreductive surgery including partial omentectomy. All patients were treated with various chemotherapy regimens. Four- to six-micrometer sections of the archival paraffin-embedded blocks were cut, deparaffinized, and stained by immunohistochemical technique using P-glycoprotein dye. Endothelial cell staining was used as the positive control of the dye. Immunostaining was categorized from 0% to 100% based on the percentage of cells stained by examining 3-4 high-power fields.

Results:
There was no significant relationship with P-glycoprotein immunoreactivity and histopathologic subtypes ($p>0.05$). P-glycoprotein immunoreactivity was not high in poor prognostic histologic types of mix and undifferentiated. There was a significant positive correlation between histologic grade and the P-glycoprotein immunoreactivity ($r=0.524$, $p=0.02$).

Conclusion:
P-glycoprotein immunoreactivity seems to have no relationship with histologic type in ovarian carcinomas and not high in poor prognostic histologic subtypes of mix and undifferentiated.

Keywords:
P-glycoprotein, immunohistochemistry, ovarian cancer

Epitelyal over karsinomu olgularında kemoresistans markiri P-Glikoprotein ekspresyonu

Özet

Amaç:
Bu çalışmanın amacı p-glikoprotein yüzde immunoreaktivitesi ile epitelyal over karsinomu histolojik tipleri ve klinik özellikleri arasındaki ilişkinin incelenmesidir.

Yöntem:
Bu çalışmaya yeni tanı konmuş 23 primer epitelyal over karsinomu olgusu dahil edilmiştir. Olgulara parsiyel omentektomiyi içeren sitoredüktif cerrahi uygulanmıştır. Tüm olgulara değişik kemoterapi rejimleri uygulanmıştır. Arşivden çıkarılan parafin bloklardan 4-6 mikrometre kalınlığında kesitler alınarak deparafinize edilmiş, ve immunohistokimyasal teknik kullanılarak P-glikoprotein boyası ile boyanmıştır. Boya için pozitif kontrol olarak endotelial hücre boyanması kullanılmıştır. İmmunboyama 3-4 büyük büyütme alanı incelenerek boyanan hücrelerin yüzdesine göre %0 ile %100 arasında değerlendirilmiştir.

Bulgular:
P-glikoprotein immunoreaktivitesi ile histopatolojik alt tipler arasında anlamlı ilişki saptanmadı ($p>0.05$). P-glikoprotein immunoreaktivitesi kötü prognostik tipler olan miks ve indiferansiye tiplerde yüksek saptanmamıştır. Histolojik derece ile p-glikoprotein immunoreaktivitesi arasında anlamlı pozitif korelasyon bulunmuştur ($r=0.524$, $p=0.02$).

Sonuç:
P-glikoprotein immunoreaktivitesi over karsinonmlarında histolojik tiple ilişkili bulunmamıştır ve kötü prognostik histolojik tipler olan miks ve indiferansiye tiplerde yüksek bulunmamıştır.

Anahtar sözcükler:
P-glikoprotein, immunohistokimya, over kanseri

Introduction

Ovarian carcinoma is the leading cause of death from gynecological malignancies (1). In ovarian carcinoma, the majority of patients are diagnosed in an advanced stage of disease and the first-line treatment in general consists of surgical debulking, followed by paclitaxel and platinum-containing chemotherapy (2,3). Meta-analysis has shown that the addition of doxorubicin to platinum-containing regimens in first-line treatment may further improve survival (4). Despite a high initial response rate, however, most patients will relapse and in these patients second-line chemotherapy with response rates of 20% is seldom curative (5).

The principal reason for treatment failure in patients with ovarian carcinoma is the presence of disease that is disseminated and resistant to therapy. Although many patients will achieve a complete remission with induction therapy, a large proportion will eventually relapse with disease that is resistant to a broad spectrum of chemotherapeutic agents. Patients with advanced epithelial ovarian cancer have 70% response rate to platinum/taxane chemotherapy, but only 20-30% of these patients are cured because the majority of these cancers become resistant to chemotherapy (6). Approximately 30% of advanced epithelial ovarian cancers are drug resistant prior to or develop resistance during first-line chemotherapy (7).

In vitro studies showed that selection of cells for resistance to one type of chemotherapeutic agent might result in cross-resistance to unrelated drugs including, anthracyclines, vinca alkaloids and taxanes. This phenomenon is called 'multidrug resistance' (8). Multidrug resistance has been related to several mechanisms among which overexpression of membrane transporter proteins such as P-glycoprotein and MRP1. P-glycoprotein is a membrane glycoprotein, encoded by the MDR1 gene that acts as an ATP-driven drug efflux pump. Overexpression of P-glycoprotein is associated with resistance to natural product drugs, including doxorubicin and taxanes (9,10).

It is important to identify the ovarian carcinoma patients who will not respond to conventional chemotherapy well and allow these women to be offered alternative treatment regimens. The objective of the present study is to determine the relationship between P-glycoprotein immunoreactivity and the histologic subtypes and clinical features of epithelial ovarian carcinoma.

Materials and Methods

Twenty-three newly diagnosed patients with primary epithelial ovarian carcinoma, treated and evaluated at the department of gynecological oncology, Ege University Faculty of Medicine between 1995 and 2000 were included in this study. Patients were treated with cytoreductive surgery including partial omentectomy. All patients were treated with various chemotherapy regimens. Primary tumors were classified according to the WHO classification using paraffin-embedded tissue (11). One section/cm tumor diameter was collected to get a good overall impression of the tumor histology. Tumors were graded into well (grade I), moderately (grade II), and poorly (grade III) as described by Sobre et al (12). The distribution of histopathologic types of cases is presented in Table 1. The demographic variables of the patients are presented in Table 2.

Four- to six-micrometer sections of the archival paraffin-embedded blocks were cut, deparaffinized, and stained by immunohistochemical technique using P-glycoprotein dye (P-glycoprotein dye, DAKO, Germany). Endothelial cell staining was used as the positive control of the dye. Immunostaining was categorized from 0% to 100% based on the percentage of cells stained by examining 3-4 high-power fields. The immunostained sections were examined by two of the authors (O.Z. and Y.E.) who were blinded to clinical parameters. At least three sections were immunostained from each paraffin block in order to determine the best field that the tumor was present. Examples of p-glycoprotein immunoreactivity were demonstrated in Figures 1-3.

Data analysis was performed using the SPSS statistical software package (SPSS Inc, Chicago, IL). The data was expressed as the mean ± standard deviation. The correlation between the percentage of P-glycoprotein positivity versus age and tumor grade was determined by using Pearson correlation analysis. Kruskal Wallis was performed to determine the differences of staining between the histologic types. Mann Whitney U test was used to compare the means. P value lower than 0.05 was accepted as significant.

Results

The chemotherapy regimens and number of cycles administered, histologic types and the P-glycoprotein immunoreactivity of the ovarian carcinoma cases were presented in Table 3. Distant metastasis was found in % (7/23) of the cases. The localization of the distant metastasis and P-glycoprotein immunoreactivity was summarized in Table 4. Omental metastasis was determine in 11 cases. The histologic types and P-glycoprotein immunoreactivity was shown in Table 5. Bilateral ovarian involvement was observed in five cases. The histologic types and P-glycoprotein immunoreactivity of bilateral ovarian carcinoma was demonstrated in Table 6. The statistical differences regarding bilaterality, distant metastasis and menopausal state and p-glycoprotein immunoreactivity were demonstrated in Table 7.

Percentage of P-glycoprotein immunoreactivity was found to be higher in patients with omental invasion (p=0.004). There was no significant relationship with P-glycoprotein

immunoreactivity and histopathologic subtypes (p>0.05). P-glycoprotein immunoreactivity was not high in poor prognostic histologic types of mix and undifferentiated. There was a significant positive correlation between histologic grade and the P-glycoprotein immunoreactivity (r=0.524, p=0.02). Table 8 demonstrates the P-glycoprotein immunoreactivity related to histopathologic types.

Discussion

Intrinsic and/or acquired chemoresistance is the major obstacle to overcome in the treatment of patients with ovarian carcinoma. MDR1 expression has been implicated as an independent poor prognostic factor in several types of cancer. Chan et al (13) determined a a strong correlation of increased levels of MDR1 expression by immunohistochemical method with relapse in pediatric soft tissue sarcomas. This study points out the potential importance of finding even small areas of P-glycoprotein expressing cells within a large tumor. Goff et al (14-15) evaluated the response of ovarian cancer to chemotherapy and the immunocytochemical expression of p53, c-erb-B-2, epidermal growth factor receptor, tumor necrosis factor alpha, P-glycoprotein, and Ki-67 (a marker of cellular proliferation). In those studies they found that Ki-67 expression correlated with response to chemotherapy, but none of the other markers were predictive of initial response.

Van der Zee et al (16) determined the prognostic value of immunostaining of P-glycoprotein in patients with advanced-stage ovarian carcinoma. Immunostaining of P-glycoprotein was performed on 89 primary tumors and 38 residual tumors after chemotherapy in patients with advanced ovarian carcinoma treated with platinum- and doxorubicin-containing chemotherapy. P-glycoprotein immunoreactivity was present in 13 (15%) cases and was not associated with any other prognostic factor or progression free or overall survival. The frequency of P-glycoprotein staining in residual tumors after chemotherapy (18 of 38 cases) was higher in comparison to untreated tumors (13 of 89 cases) (P < .001). The higher frequency of P-glycoprotein immunoreactivity in residual tumors after chemotherapy points to induction of P-glycoprotein in ovarian carcinomas by doxorubicin-containing combination chemotherapy; however, the determination of P-glycoprotein does not permit more adequate prediction of response to chemotherapy.

Arts et al (17) evaluated the expression of P-glycoprotein by immunohistochemistry of frozen tissue sections of 115 ovarian carcinoma patients and associated to clinicopathologic factors, response to chemotherapy and progression free survival. P-glycoprotein expression was observed in 20 out of 115 cases (%17). P-glycoprotein expression was neither related to response to first line chemotherapy in 59 evaluable patients nor to progression free survival in all patients. On multivariate analysis only stage and residual tumor after first laparotomy were independent prognostic factors for progression free survival.

Baekelandt et al (18) examined tumor tissue from 73 previously untreated patients with FIGO stage 3 ovarian cancer was examined with immunohistochemistry for the expression of P-glycoprotein before and after chemotherapy. Response to 4 cycles of combination chemotherapy with cisplatin and epirubicin was assessed with second look laparotomy. P-glycoprotein expression was detected in 47% of untreated cases, and correlated with unfavourable prognostic factors such as advanced age, presence of ascites and larger residual disease deposits after primary surgery. P-glycoprotein negative cases responded significantly better to chemotherapy (P< .001). In the multivariate survival analysis in contrast to other previously mentioned studies (16-17), P-glycoprotein expression was found to be an independent predictor of both overall (P = .045) and progression free (P = .006) survival.

In the present study mainly the relationship between histopathologic parameters and the P-glycoprotein positivity was investigated. There was no significant relationship with P-glycoprotein immunoreactivity and histopathologic subtypes (p>0.05). P-glycoprotein immunoreactivity was not high in poor prognostic histologic types of mix and undifferentiated. There was a significant positive correlation between histologic grade and the P-glycoprotein immunoreactivity (r=0.524, p=0.02).

In conclusion, P-glycoprotein immunoreactivity seems to have no relationship with histologic type in ovarian carcinomas and not high in poor prognostic histologic subtypes of mix and undifferentiated.

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Table 1:
Histopathologic types of patients with ovarian carcinoma

Histopathologic type	Number	Percent
Endometrioid	6	%26
Clear cell	1	%4.3
Mix type	2	%8.6
Undifferentiated	3	%13
Serous	5	%21.7
Mucinous	6	%26

Table 2:
The demographic variables of the patients with ovarian carcinoma

	Minimum	Maximum	Mean ± standard deviation
Age (years)	30	73	51 ± 11.7
Parity	0	8	3.6 ± 2.3
Gravida	0	3	1.4 ± 1.2

Table 3:
The chemotherapy regimens and number of cycles administered, histologic types and the P-glycoprotein positivity of the ovarian carcinoma cases

Case No.	Histologic type	P-glycoprotein immunoreactivity	Chemotherapy regimen	Cycles
1	Endometrioid	80%	Paclitaxel + cisplatin	12
2	Serous	0%	Cyclophosphamide + cisplatin	6
3	Serous	0%	Cyclophosphamide + cisplatin + epirubisine	4
4	Mix type	0%	Paclitaxel + cisplatin	12
5	Mucinous	20%	5-Fluoro-uracil + carboplatin	6
6	Mucinous	30%	5-Fluoro-uracil + cisplatin + epirubisine	5
7	Endometrioid	40%	Cyclophosphamide + cisplatin	6
8	Serous	60%	Cyclophosphamide + cisplatin + epirubisine	8
9	Serous	0%	Cyclophosphamide + cisplatin	6
10	Mucinous	0%	Cyclophosphamide + cisplatin	
			5-Fluoro-uracil + cisplatin	5
			5-Fluoro-uracil	6

Table 3 (continued):
The chemotherapy regimens and number of cycles administered, histologic types and the P-glycoprotein positivity of the ovarian carcinoma cases

Case No.	Histologic type	P-glycoprotein immunoreactivity	Chemotherapy regimen	Cycles
11	Mucinous	80%	5-Fluoro-uracil + cisplatin	4
12	Endometrioid	5%	Cyclophosphamide + cisplatin	6
13	Clear cell	0%	Cyclophosphamide + cisplatin	9
14	Endometrioid	20%	Cyclophosphamide + cisplatin + epirubisine	10
15	Endometrioid	80%	Cyclophosphamide + cisplatin	6
16	Mucinous	40%	5-Fluoro-uracil + cisplatin	6
17	Undifferentiated	0%	Paclitaxel + cisplatin	9
18	Mucinous	60%	Paclitaxel + cisplatin	6
19	Serous	50%	Paclitaxel + carboplatin	3
			Paclitaxel	3
20	Mix type	10%		
21	Undifferentiated	0%	Cyclophosphamide + cisplatin	10
22	Undifferentiated	0%	Cyclophosphamide + epirubisine + cisplatin	4
23	Endometrioid	40%	Paclitaxel + cisplatin	6

Table 4:
The localization of the distant metastasis and P-glycoprotein immunoreactivity in patients with ovarian carcinoma.

Case No.	Histologic type	The localization of distant metastasis	P-glycoprotein immunoreactivity
10	Mucinous	Liver parenchyma	0%
12	Endometrioid	Abdominal wall	5%
13	Clear cell	Plevral carcinomatosis	0%
14	Endometrioid	Plevral carcinomatosis	20%
		Liver parenchyma	
19	Serous	Liver parenchyma	50%
21	Undifferentiated	Supraclavicular lymph node	0%
22	Undifferentiated	Liver parenchyma	0%

Table 5:
The histologic types and P-glycoprotein immunoreactivity in ovarian carcinoma cases with omental metastasis.

Case No.	Histologic type	P-glycoprotein Immunoreactivity	Case No	Histologic type Immunoreactivity	P-glycoprotein
3	Serous	%0	13	Clear cell	%0
4	Mix	%0	17	Undifferentiated	%0
6	Mucinous	%30	20	Mix	%10
9	Serous	%0	22	Undifferentiated	%0
10	Mucinous	%0	23	Endometrioid	%40
12	Endometrioid	%5			

Table 6:
The histologic types and P-glycoprotein immunoreactivity of bilateral ovarian carcinoma

Case No.	Histologic type	P-glycoprotein immunoreactivity
10	Mucinous	%0
12	Endometrioid	%5
13	Clear cell	%0
19	Serous	%50
20	Mix	%10

Table 7:
Statistical differences regarding bilaterality, omentum metastasis, distant metastasis and menopausal state and p-glycoprotein immunoreactivity

	P-glycoprotein immunoreactivity (%)	P value
Bilateral ovarian involvement present (n=18)	30.5 ± 30.7	0.39
Bilateral ovarian involvement absent (n=5)	13.0 ± 21.1	
Distant metastasis absent (n=16)	33.7 ± 30.9	0.10
Distant metastasis present (n=7)	10.7 ± 18.8	
Premenopausal (n=12)	39.1 ± 33.7	0.08
Postmenopausal (n=11)	13.1 ± 16.4	

Table 8:
P-Glycoprotein immunoreactivity related to histologic types of ovarian carcinoma

Histopathologic type	n	P-glycoprotein positivity (%)
Endometrioid	6	44.1 ± 30.7
Mix	2	5.0 ± 7.07
Undifferentiated	3	0
Serous	5	22.0 ± 30.3
Mucinous	6	38.3 ± 28.5

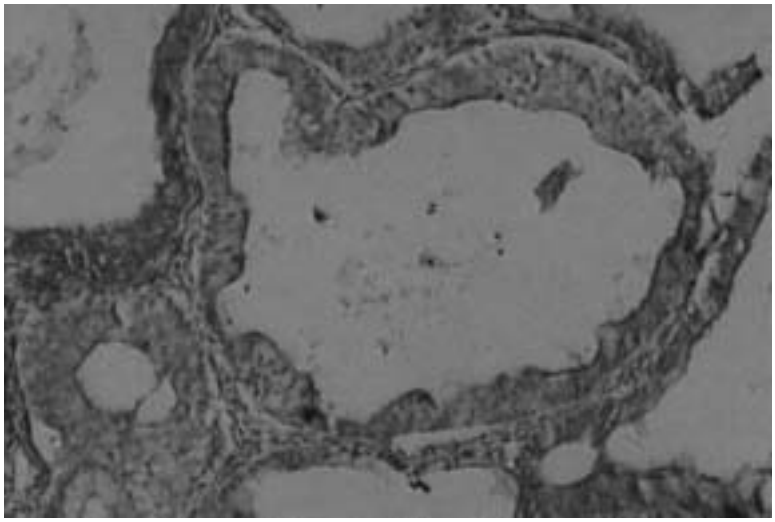

Figure 1:
P-glycoprotein immunoreactivity in a 39-year old patient with left ovarian mucinous adenocarcinoma (x 100)

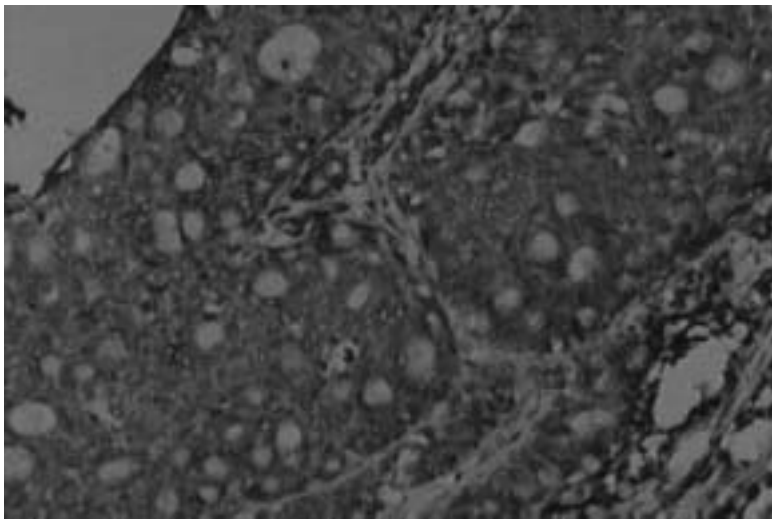

Figure 2:
P-glycoprotein immunoreactivity in a 63-year-old patient with ovarian endometrioid adenocarcinoma (x 160)

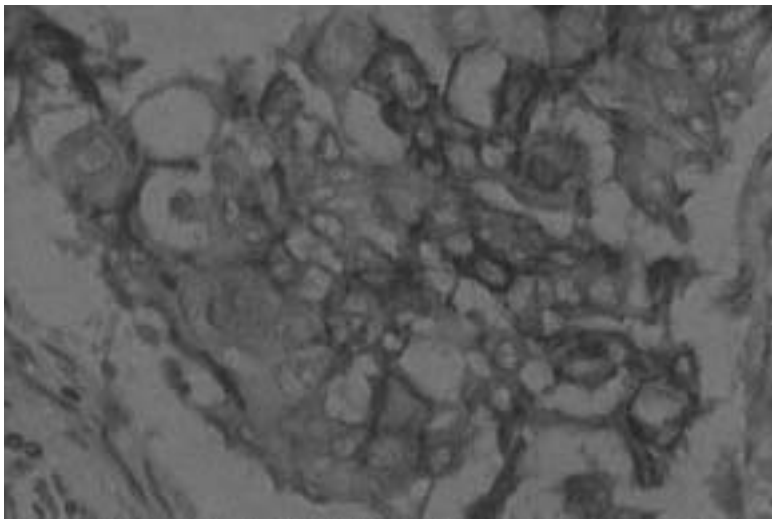

Figure 3:
P-glycoprotein immunoreactivity in 40-year-old patient with ovarian endometrioid adenocarcinoma (x 400)