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The impact of p16 and HER2 expression on survival in patients with ovarian carcinoma

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The purpose of this study was to investigate the impact of p16 and HER2 expression on survival in patients with ovarian carcinoma.

This descriptive-analytic, cross-sectional study, was conducted on 47 paraffin blocks of epithelial ovarian tumors. Suitable slides were prepared to evaluate HER2 and p16 by immunohistochemistry using HercepTest kit (DAKO) and p16INK4A kit (DAKO, code 5334). Clinical information and pathology data were extracted from patients' medical and pathology records. Data entry and analysis was done by SPSS (version 16) software. Chi-square test, Mann-Whitney test, t-test, Kruskal-Wallis test, log rank test and Kaplan-Meier method were used.

The mean age of the patients was 51.6 years (range 19-71 years). The most common histological type of epithelial ovarian cancer was serous adenocarcinoma (68.1%). P16 expression was detected in 34% of epithelial ovarian tumors. P16 expression was significantly associated with stage of disease ($P = 0.04$) and overall survival ($P = 0.001$), but HER2 expression was not associated with overall survival, stage of disease and tumor histological type.

Expression of p16 may be used as a prognostic factor of overall survival and stage of disease, while HER2 expression may not be used as a prognostic factor of overall survival.

Key words: p16, HER2/neu, immunohistochemistry, epithelial ovarian tumors

Ovarian cancer is the eighth common cancer and the seventh cause of death in women [1]. In Iran, ovarian cancer is the 8th most frequent for incidence [2]. The life-time risk of developing ovarian cancer is estimated to be 1-1.5%. The prevalence of histological types of epithelial ovarian tumors has been reported as follows: serous (75%), mucinous (20%), endometrioid (2%), clear cell (<1%) and undifferentiated carcinomas (<1%) [3]. Since symptoms of ovarian cancer in early stages of the disease, are non-specific, most patients are diagnosed in advanced stages [4]. Several biological factors are involved in the disease progression. P16 protein as a tumor-suppressor, plays an important role in cell cycle regulation [5]. Previous studies have demonstrated that high expression of p16 was associated with poor differentiation, poor prognosis, and advanced stage and grade in patients [6, 7].

HER2/neu overexpression has been detected in approximately 30% of breast cancers [8], 25–30% of ovarian cancers [9], 35–45% of pancreatic carcinomas [10, 11], and 30–80% of esophageal adenocarcinoma [12, 13], and squamous cell carcinoma [14-16]. Some previous studies have demonstrated that the expression of HER2 is not an important prognostic factor in advanced epithelial ovarian cancer, while the other studies have demonstrated that the overexpression of HER2 is a major prognostic factor in epithelial ovarian cancer [17, 18].

In the present study, we examined the expression of HER2 and p16 by immunohistochemistry (IHC) in epithelial ovarian tumors and their correlation with clinicopathologic variables. The aim of the current study was to investigate the impact of p16 and HER2 expression on survival in patients with ovarian carcinoma.

Materials and methods

This descriptive-analytic, cross-sectional study, was conducted on 50 paraffin blocks of epithelial ovarian tumors obtained from the Pathology Department of Ghaem Hospital of Mashhad University of Medical Sciences during 2007 to 2013. Specimens (consisted of 43 malignant tumors (32 serous adenocarcinomas, 7 mucinous adenocarcinomas, 2 endometrioid and 2 clear cell carcinomas), 3 benign tumors (1 mucinous and 2 serous cystadenomas) and 4 serous borderline tumors) were selected by the simple sampling method. Benign tumors were excluded from our study. This study was approved by the ethics committee of Mashhad University of Medical Sciences. The suitable block selection criteria include: 1) the maximum tumoral tissue; 2) the minimum extent of necrosis. HercepTest kit (DAKO) which is an immuno-histochemical semi-quantitative assay and P16INK4A kit (DAKO, code 5334) were used to determine the expression of HER2 and p16, respectively. The DAKO HercepTest was conducted exactly according to the manufacturer's instructions. Then the results were divided into two general groups: positive and negative staining. The HER2-positive staining cases were divided into; two groups based on cytoplasmic and membrane staining, three groups of mild, moderate and severe staining based on staining intensity and also four groups of negative (0-10%), 1+ (11-50%), 2+ (51-75%), and 3+ (76-100%) according to the percentage of cells staining positive based on the distribution of cell membrane staining (Figure 1). The presence or absence of nuclear and cytoplasmic staining was considered for p16 and the expression of p16 was considered as positive (Figure 2). Clinical information and pathology data such as age, histological type, and tumor stage were extracted from patients' medical and pathology records. All data were analyzed by SPSS (version 16) software. Chi-square test, Mann-Whitney test, t-test, Kruskal-Wallis test, log rank test and Kaplan-Meier method were used. A p-value less than 0.05 was considered significant.

Results

The mean age of the patients was 51.6±13 (SD) years (range 19-71 years). The patients were divided into four age groups: <19, 20-40, 41-60 and 61-80 years. The majority of patients were in the age range of 41-60 years. Among 47 cases, the most common histological type was serous adenocarcinoma (68.1%, 32 cases), and followed by mucinous adenocarcinoma (14.9%, 7 cases), serous borderline tumor (8.5%, 4 cases), endometrioid carcinoma (4.3%, 2 cases) and clear cell carcinoma (4.3%, 2 cases). 2.1% of patients had stage I disease, 27.7% had stage II disease, 63.8% had stage III disease and 6.4% had stage IV disease. Median follow-up time in patients with ovarian tumors was 27.7 months (range, 6-60 months). During follow-up, 28 patients died. The 24- and 60-month disease-free survival probabilities were 42% and 17% and overall survival probabilities were 70% and 20%, respectively. P16 was positive in 46.9% of serous adenocarcinomas and 25% of serous borderline tumors, while it was negative in all mucinous adenocarcinomas, endometrioid and clear cell carcinomas. Overall, p16 expression was detected in 34% of epithelial ovarian tumors. The relation between p16 expression and stage of disease has been shown in Table 1. P16-positive cases had a significantly higher stage of disease than p16-negative cases ($P = 0.04$). The relation between p16 expression and disease-free survival and overall survival probabilities (at 24 and 60 months) have been shown in Table 2. P16-positive cases had a significantly lower overall survival probability than p16-negative cases ($P = 0.001$). Furthermore, the disease-free survival probability in p16-positive cases was

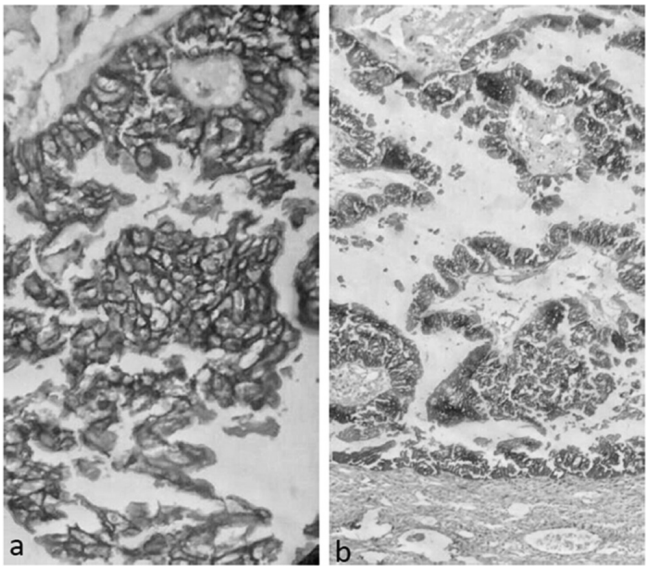

Figure 1. HER2 immunohistochemistry: (a) score 3+, ×40, serous adenocarcinoma. (b) score 3+, ×4, serous adenocarcinoma.

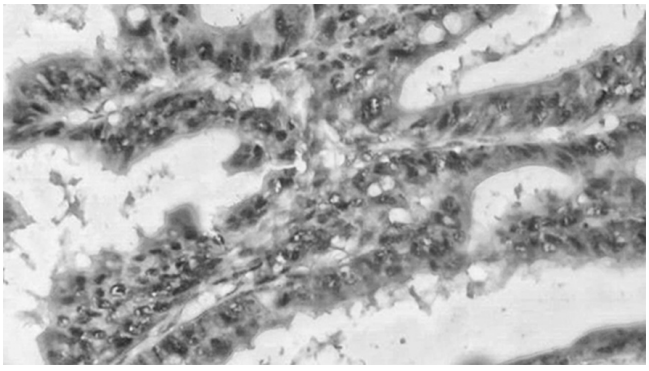

Figure 2. P16 immunohistochemistry, nuclear and cytoplasmic, ×40, serous adenocarcinoma

Table 1. The relation between p16 expression and stage of tumor (p-value=0.04)

	p16 expression		
Stage	Positive	Negative	Total
I	0 (0.0%)	1 (3.2%)	1
II	2 (12.5%)	11 (35.5%)	13
III	13 (81.3%)	17 (54.8%)	30
IV	1 (6.3%)	2 (6.5%)	3
Total	16	31	47

Table 2. The relation between p16 expression and disease-free survival and overall survival probabilities

Follow-up time	Overall survival probability		Disease-free survival probability		p-value
(months)	p16 positive	p16 negative	p16 positive	p16 negative	
24	42%	86%	33%	47%	0.001
60	8%	17%	5%	22%	0.25

Table 3. The relation between cytoplasmic HER2 expression and histological type of tumor (p-value =0.55)

	Cytoplasmic HER2 expression		
Histological type	Positive	Negative	Total
serous adenocarcinoma	10 (31.3%)	22 (68.8%)	32
mucinous adenocarcinoma	1 (14.3%)	6 (85.7%)	7
endometrioid carcinoma	1 (50.0%)	1 (50.0%)	2
clear cell carcinoma	0 (0.0%)	2 (100.0%)	2
serous borderline tumor	0 (0.0%)	4 (100.0%)	4
Total	12	35	47

Table 4. The relation between cytoplasmic HER2 expression and stage of tumor (p-value=0.25)

	Cytoplasmic HER2 expression		
Stage	Positive	Negative	Total
I	0 (0.0%)	1 (2.9%)	1
II	2 (16.7%)	11 (31.4%)	13
III	9 (75.0%)	21 (60.0%)	30
IV	1 (8.3%)	2 (5.7%)	3
Total	12	35	47

Table 5. The relation between HER2 membrane expression intensity and stage of tumor (p-value=0.70)

	HER2 membrane expression intensity**				
Stage	0	1+	2+	3+	Total
I	1 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1
II	6 (46.2%)	4 (30.8%)	3 (23.1%)	0 (0.0%)	13
III	13 (43.3%)	7 (23.3%)	7 (23.3%)	3 (10.0%)	30
IV	2 (66.7%)	0 (0.0%)	1 (33.3%)	0 (0.0%)	3
Total	22	11	11	3	47

**0, no staining or membrane staining in fewer than 10% of tumor cells (negative); 1+, faint or barely perceptible incomplete membrane staining in more than 10% of tumor cells (negative); 2+, weak to moderately complete membrane staining in more than 10% of tumor cells (weakly positive); 3+, strong, complete membrane staining in more than 10% of tumor cells (strongly positive).

lower than p16-negative cases, but the difference was not statistically significant (P = 0.25). The relation between cytoplasmic HER2 expression and histological type of tumor has been shown in Table 3**.** Cytoplasmic HER2 was expressed in 50% of endometrioid carcinomas, 31.3% of serous adenocarcinomas and 14.3% of mucinous adenocarcinomas, while the cytoplasmic HER2 expression was negative in 100% of clear cell and borderline tumors, that was not statistically significant (P = 0.55). The relation between cytoplasmic HER2 expression and stage of disease has been shown in Table 4. There was not a significant relationship between cytoplasmic HER2 expression and stage of disease (P = 0.25). Overall survival probability (at 24 and 60 months) in cytoplasmic HER2-positive cases was higher than negative cases (P = 0.16), whereas the disease-free survival probability in cytoplasmic HER2-positive cases was lower than negative cases (P = 0.47). There was not a significant relationship between cytoplasmic HER2 expression and overall and disease-free survival. Our results showed that the rate of membrane expression of HER2 was 29.8% (score 2+ and 3+ considered as positive). Of the 47 cases, 3 (6.4%) were 3+, 11 (23.4%) were 2+, 11 (23.4%) were 1+ and 22 (46.8%) were 0. HER2 membrane staining intensity (score 2+ and 3+) in borderline tumors, endometrioid carcinomas and serous adenocarcinomas was 75%, 50% and 31.3%, respectively, while it was negative (score 0 and 1+) in all clear cell carcinomas and mucinous adenocarcinomas, that was not statistically significant (P = 0.05). The relation between HER2 membrane expression intensity and stage of disease has been shown in Table 5. The most HER2 membrane expression intensity was observed in stage III, that was not statistically significant (P = 0.70).

The relation between HER2 membrane staining distribution and histological type of tumor has been shown in Table 6. The most HER2 membrane staining distribution (positive staining in > 50% of cells) was observed in serous borderline tumor (75%), endometrioid carcinoma (50%) and serous adenocarcinoma (31.3%), while it was in the range of 0-10% (negative) in clear-cell carcinoma, that was not statistically significant (P = 0.06). Overall survival probability (at 24 and 60 months) in membrane HER2-positive cases was lower than negative cases (P = 0.07), furthermore the disease-free survival probability (at 24 and 60 months) in membrane HER2-positive cases was also lower than negative cases, that was not statistically significant (P = 0.64) (Table 7).

Table 6. The relation between HER2 membrane staining distribution and histological type of tumor (p-value=0.06)

	HER2 membrane staining distribution				
Histological type	0-10%	11-50%	51-75%	76-100%	Total
serous adenocarcinoma	14 (43.8%)	8 (25.0%)	6 (18.8%)	4 (12.5%)	32
mucinous adenocarcinoma	5 (71.4%)	1 (14.3%)	1 (14.3%)	0 (0.0%)	7
endometrioid carcinoma	1 (50.0%)	0 (0.0%)	1 (50.0%)	0 (0.0%)	2
clear cell carcinoma	2 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2
serous borderline tumor	0 (0.0%)	1 (25.0%)	1 (25.0%)	2 (50.0%)	4
Total	22	10	9	6	47

Discussion

The purpose of this study was to investigate the impact of p16 and HER2 expression on survival in patients with ovarian carcinoma. In previous studies, high expression of p16 was associated with poor differentiation, poor prognosis, and advanced stage and grade in patients [6, 7]. The expression of p16 in ovarian carcinoma has been reported different in various studies. In our study, p16 expression was detected in 34% of epithelial ovarian tumors. P16 was positive in 46.9% of serous adenocarcinomas and 25% of serous borderline tumors, while it was negative in all mucinous adenocarcinomas, endometrioid and clear cell carcinomas. In the study by Khouja *et al*, high level of p16 was expressed in 31% of advanced ovarian carcinoma [7]. In the study by Dong *et al*, only 11% of malignant ovarian tumors were p16 negative and the benign ovarian tumors showed no expression of p16 [6]. In our study, p16-positive cases had a significantly higher stage of disease than p16-negative cases. Dong *et al.* (1997) studied 190 epithelial ovarian tumors, and demonstrated that high number of p16-positive tumor cells was associated with advanced stage [6]. In our study, p16-positive cases had a significantly lower overall survival probability (at 24 and 60 months) than p16-negative cases. The disease-free survival probability (at 24 and 60 months) in p16-positive cases was lower than p16-negative cases. A significant influence of p16 expression on overall survival was not confirmed by Milde-Langosch *et al* [19].

HER2/neu overexpression has been reported in several human cancers, such as breast, ovary, stomach, esophagus, endometrium, pancreas, colon, bladder and lung [20-28]. In ovarian cancer, the percentage of HER2-positive tumors has been reported varied in various studies [29-36]. In epithelial ovarian cancer, the prognostic value of HER2/neu overexpression remains controversial [37-41]. Rubin *et al.* had reported that the expression of HER2 is not an important prognostic factor in advanced epithelial ovarian cancer [18], while Coronado *et al.* demonstrated that the overexpression of HER2/neu is a major prognostic factor in epithelial ovarian cancer and it is an independent prognostic factor for overall survival [17]. In Verri *et al.*' s study no significant correlation was detected between HER2 membrane staining intensity and pathological and clinical features, but a significant correlation between

Table 7. The relation between membrane HER2 expression and disease-free survival and overall survival probabilities

Follow-up time	Overall survival probability		Disease-free survival probability		p-value
(months)	HER-2 positive	HER-2 negative	HER-2 positive	HER-2 negative	
24	57%	88%	40%	45%	0.07
60	25%	29%	13%	22%	0.64

overexpression of HER2 with an increased risk of death and progression was observed especially in women with stage I and II ovarian cancer [36]. In our study, there was no significant relationship between cytoplasmic HER2 expression and stage of disease, overall and disease-free survival. The rate of membrane expression of HER2 was 29.8% (score 2+ and 3+ considered as positive). HER2 membrane staining intensity (score 2+ and 3+) in borderline tumors, endometrioid carcinomas and serous adenocarcinomas was 75%, 50% and 31.3%, respectively, while it was negative (score 0 and 1+) in all clear cell carcinomas and mucinous adenocarcinomas. The most HER2 membrane staining distribution (positive staining in > 50% of cells) was observed in serous borderline tumor, endometrioid carcinoma and serous adenocarcinoma, respectively. Overall survival probability and disease-free survival probability (at 24 and 60 months) in membrane HER2-positive cases were lower than negative cases. The study of Coronado *et al***.** indicated that HER2 was expressed in 24.2% of ovarian tumors and its expression was correlated with clear cell and undifferentiated types [17]. In the study by Wu *et al*, HER2 was expressed in 6.7% of ovarian tumors and its expression was restricted to ovarian carcinoma and was not encountered in borderline tumors [42]. Eltabbakh *et al.* have detected HER2 overexpression in 20-25% of borderline tumors (especially in seromucinous) [43].

In our study, the most HER2 membrane expression intensity was observed in stage III. Riener *et al.* reported that overexpression of HER2 is significantly associated with the stage of tumor [44]. Coronado *et al.* reported overexpression of HER2 was related to advanced-stage [17]. Eltabbakh *et al.* demonstrated that overexpression of HER2 was significantly higher in stage III than stage I [43].

Conclusion

In conclusion, according to the results, p16 expression may be used as a prognostic factor of overall survival and stage of disease, while HER2 expression may not be used as a prognostic factor of overall survival.

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