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Original Research Article

Immunohistochemical expression of Her-2/neu in surface epithelial carcinoma of the ovary

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Abstract

Her-2/neu overexpression/amplification has been reported in ovarian cancer and is associated with poor clinical outcome. The aim of our study is to evaluate expression of Her-2/neu in surface epithelial ovarian carcinomas (SEOC) and its relation with different clinicopathological parameters. This retrospective study was carried out on 94 cases of SEOC retrieved from archive of pathology lab at Mansoura Oncology Center during the period from 2010 to 2017.All available H & E stained slides were examined. Immunohistochemical staining was performed and Her-2/neu positivity was assessed. Forty-seven cases were of score (0), twenty-eight cases were of score (1), eleven cases were of score (2) and eight cases were of score (3). Three out of the 8 positive cases were serous cystadenocarcinomas and five were of high tumor grade. We found no statistically significant relation between Her-2/neu expression and any of the clinicopathological features of the tumors. We observed non-statistically significant higher percentage of Her-2/neu negativity among serous carcinoma, endometrioid carcinoma and mucinous carcinoma. On the other hand, 50% of clear cell carcinoma were Her-2/neu positive. In conclusion, Her-2/neu is overexpressed in 8.5% of SEOC. Serous type and high grade carcinomas showed the maximum number of Her-2/neu positive cases. There was no statistically significant association between Her-2/neu overexpression and any of the clinicopathological features.

Keywords: Her-2/neu- surface- epithelial- carcinoma of the ovary

INTRODUCTION

Ovarian cancer is the second most common gynecologic malignancy and the fifth leading cause of cancer death in women in developed countries (Siegel et al., 2014). Surface epithelial ovarian carcinoma (SEOC) represents 73.33% of all ovarian tumors (Mokhtar et al., 2007). The standard of care for SEOC patients is surgery with a maximal cytoreductive procedure followed by systemic chemotherapy. However, despite higher tumor response rates, some patients develop distant metastases or relapse locally, while other patients do not respond even to the initial chemotherapy (English et al., 2013). Therefore, many studies investigate the possible predictive and/or prognostic biomarkers in order to improve survival of SEOC patients**.**

The HER2 (c-erb-B2) gene, located on chromosome 17q11, encodes the HER2 protein which is normally involved in the signal transduction pathways leading to cell growth and differentiation. The HER family are important mediators of normal ovarian follicle development and regulate the growth of ovarian epithelial

cells (Conti et al., 2006). Dysregulation of HER signaling in the ovary due to overexpression of, or mutations in HER family members have been linked to the growth and proliferation of ovarian tumors (Amler et al., 2012).

Despite a typically good response to first-line combination chemotherapy, the prognosis of patients with advanced ovarian cancer remains poor because of acquired chemotherapy resistance. The use of targeted therapies such as trastuzumab might potentially improve patient outcome (Ray-Coquard et al., 2009 b).

Her-2/neu overexpression/amplification has been reported in ovarian cancer and is associated with poor clinical outcome but the exact percentage of Her-2/neu expression in ovarian carcinomas varies widely in the literature between 8% and 66%. So, the aim of our study is to evaluate expression of Her-2/neu in surface epithelial ovarian carcinomas and its relation with different clinicopathological parameters**.**

MATERIAL AND METHODS

This retrospective study was carried out on 94 cases of SEOC retrieved from archive of surgical pathology lab at Mansoura Oncology Center. Available files of all resected ovarian tumors were revised during the period from 2010 to 2017.Inclusion criteria of patients included:1-Cases of SEOC (serous, mucinous, endometroid, clear cell carcinomas and malignant Brenner tumors). 2-Cases with complete clinical and pathological data including☹age of the patient, histological type of the tumor, grading of the tumor, history of neo-adjuvant therapy, primary or recurrent tumor, size of tumor, unilateral or bilateral involvement of the ovaries, lymph nodes involvement, distant metastasis, scetic fluid involvement, absence or presence of necrosis, TNM staging of the tumor).3-Cases with available medical reports and paraffin blocks. Exclusion criteria included:1-Cases with incomplete clinical data, cases with no available paraffin blocks. 2-Cases received neo-adjuvant chemotherapy with no residual tumor tissue (complete response). 3-Cases of metastatic tumors in the ovaries.

All available H & E stained slides were examined by two pathologists (AG and AM) for assessment of histopathological type and grading of the tumor, residual tumor tissue in patients received neo-adjuvant therapy, lymph nodes involvement, scetic fluid involvement and absence or presence of necrosis.

Immunohistochemistry

Immunohistochemical staining was performed on 4 µm thick, formalin fixed, paraffin embedded tissue sections. DAKO kit (DakoREAL™ EnVision™ Detection System, Peroxidase/DAB+, Rabbit/Mouse, Produktionsvej 42, DK-2600, Glostrup, Denemark) was used. The horseradish peroxidase and diaminobenzedene hydrochloride (DAB) are the enzyme and chromogen employed. Briefly, the sections were deparaffinized, followed by incubation in xylene and hydration in a series of decreasing concentration of ethanol. After that, heat-induced epitope retrieval was done using pressure cooker and EDTA buffer (PH 9). The sections were washed in PBS buffer, and immersed in peroxidase-blocking solution of DAKO to inhibit endogenous peroxidase activity. The slides were incubated with primary antibodies, monoclonal mouse antihuman Her2/neu receptor (Clone PgR636, IR06861 (1 ml), concentrated, with dilution of 1:100, Dako, North America, Inc.). Labelled polymer-HRP was then applied to the tissue for 30 min at room temperature. The staining was visualized by adding diaminobenzidine and the sections were then counterstained with hematoxylin, dehydrate, cover-slipped and mounted with DPX mounting media. HER2 positivity was assessed using Ellis and Wolff recommendations (Wolff et al, 2007). We classified score 0 and 1 as negative, score 2+ as equivocal and score 3+ as positive. Cytoplasmic staining was considered to be non-specific. An external control containing her2/neu positive (3+) breast cancer sample was used (Tuefferd et al., 2007). The relation between Her2 expression and different clinicopathological features was assessed.

Data were analyzed using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. Quantitative data were described using median (minimum and maximum) for non-parametric data and mean, standard deviation for parametric data after testing normality using Kolmogrov-Smirnov test. Significance of the obtained results was judged at the 5% level.

RESULTS

This retrospective study was conducted on 94 cases of SEOC, with age ranged from 17-94 years and the mean age was 53.46±11.49 years. Serous adenocarcinoma was the most common histopathological type (55 cases-58.5%), while malignant Brenner tumor was the least common variant (2 cases-2.1%). Twenty-two cases (23.4%) were diagnosed as mucinous adenocarcinoma, eleven cases (11.7%) were endometroid type and four cases (4.2%) were clear cell carcinoma**.** All the clinicopathological features were illustrated in table (1).

Immunohistochemical expression of Her-2/neu

Forty-seven cases (50%) were of score (0), twenty-eight cases (29.8%) were of score (1), eleven cases (11.7%) were of score (2) and eight cases (8.5%) were of

Table 1. Clinicopathological features of the studied epithelial ovarian carcinoma cases.

Clinicopathological Features	N=94	%
Histopathological Type		
Serous carcinoma	55	58.5
Endometrioid carcinoma	11	11.7
Mucinous carcinoma	22	23.4
Clear cell carcinoma	4	4.3
Malignant Brenner tumor	2	2.1
Grade((N=66)#		
Low	10	15.2
Intermediate	7	10.6
High	49	74.2
Stage		
I	24	25.5
II	36	38.3
III	15	16.0
IV	19	20.2
Bilateral affection for both ovaries	51	54.3
Lymph node metastasis	12	12.8
Involvement of ascetic fluid cytology	34	36.2
Recurrence	12	12.8
Distant metastasis	52	55.3
Receiving neo adjuvant chemotherapy	33	35.1
Necrosis	75	79.8

#' The number of cases are 66 as we graded only serous and endometrioid carcinoma cases

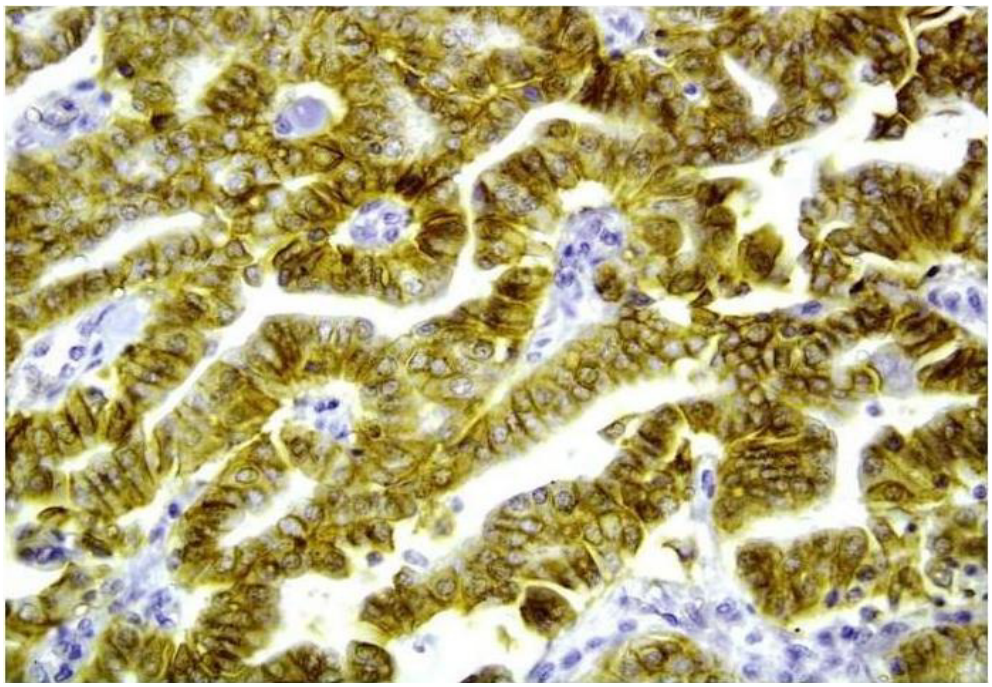

Figure 1. High grade serous adenocarcinoma showed strong complete membranous staining in more than 10% of the tumor cells, score 3+ (HER2/NEU x400).

score (3). Three out of the 8 positive cases (37.5%) were serous adenocarcinomas (Figure 1), two were mucinous carcinoma (Figure 2), two were clear cell carcinomas (Figure 3) and one case was endometroid carcinoma (Figure 4).

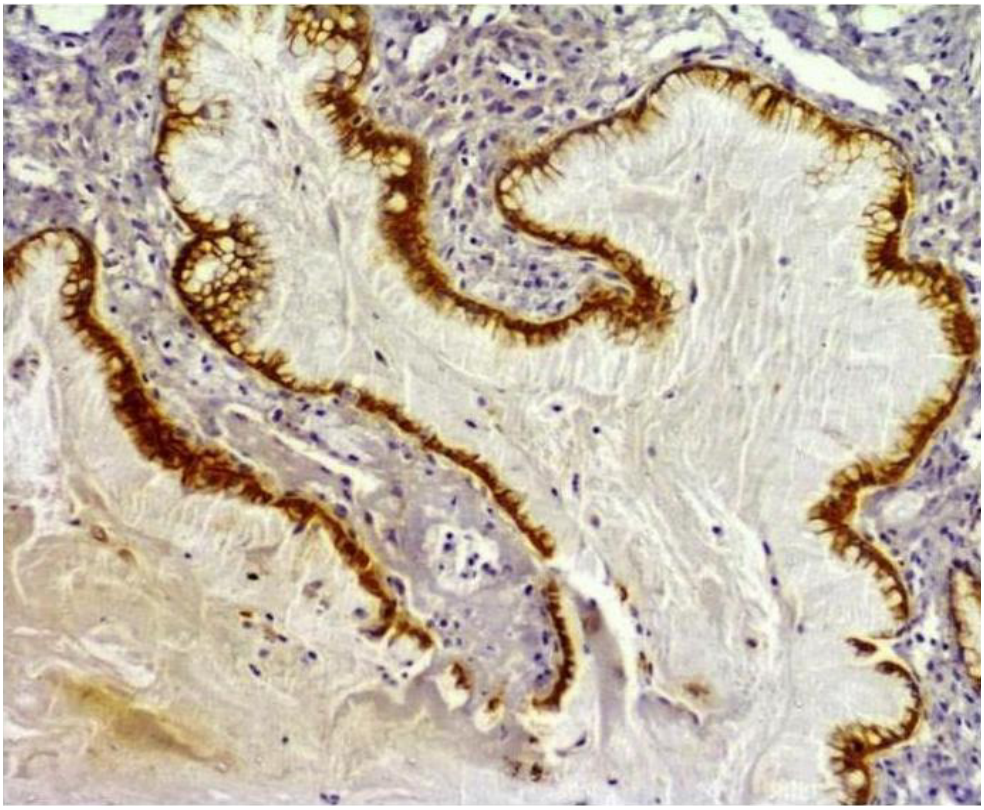

Figure 2. Mucinous adenocarcinoma showed strong complete membranous staining (basolateral) in more than 10% of tumor cells score 3+ (HER2/NEU x400).

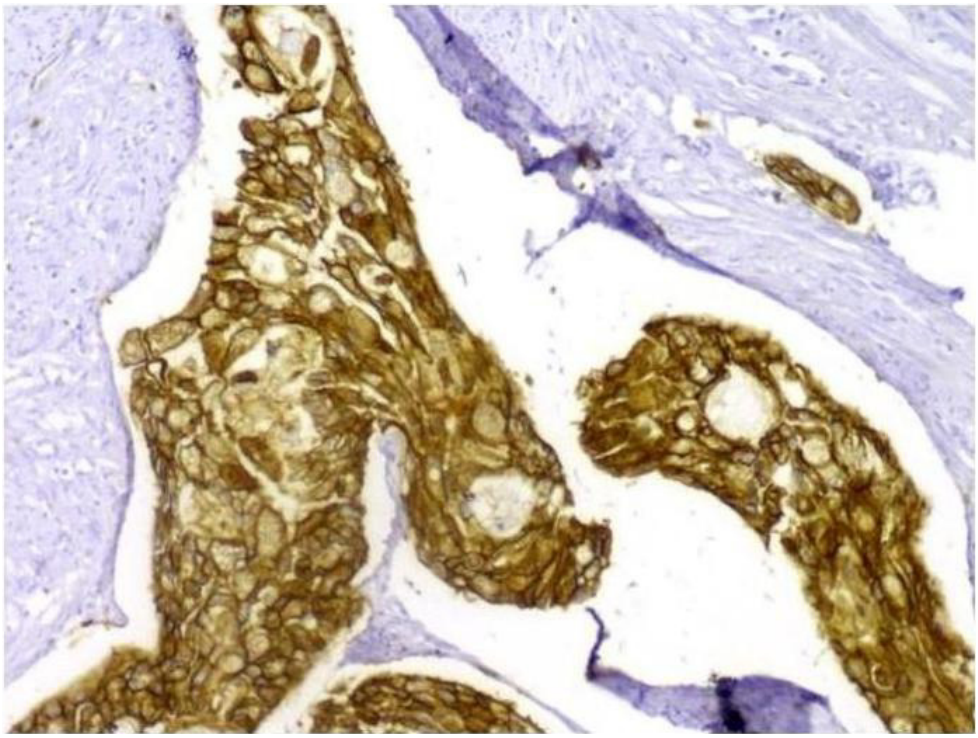

Figure 3. Clear cell carcinoma of the ovary showed strong complete membranous staining in more than 10% of tumor cells, score 3+ (HER2/NEU x400).

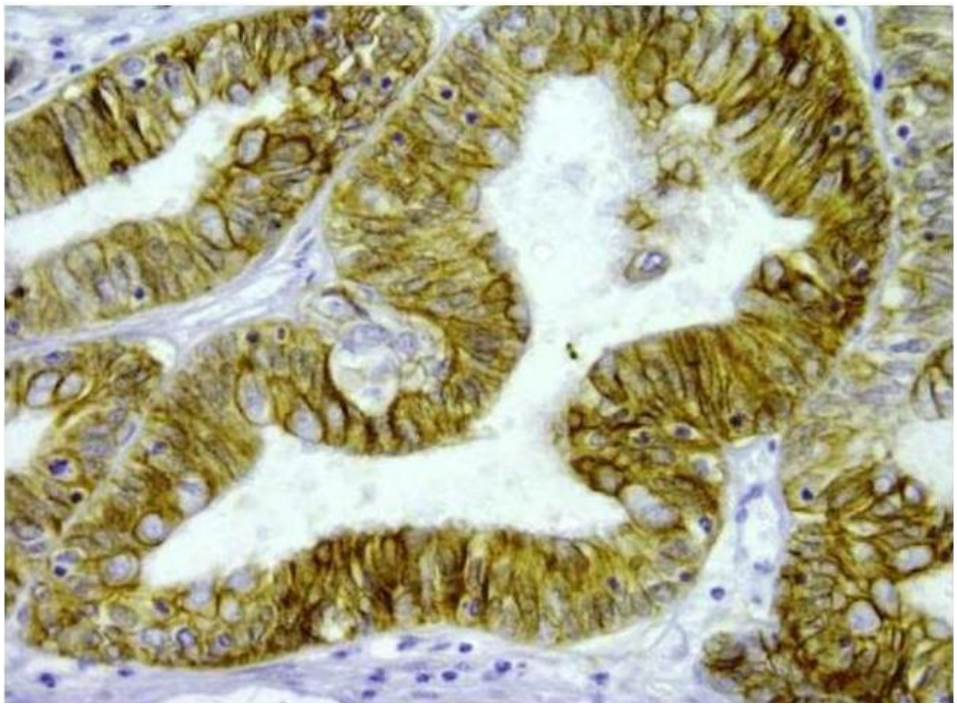

Figure 4. Endometrioid adenocarcinoma neoplastic cells showed strong complete membranous staining in > 10% of tumor cells, score 3+ (HER2/NEU x400).

Table 2. Relation between Her2/neu expression and the clinicopathological features of the studied epithelial ovarian carcinoma cases

Clinicopathologicalfeatures	**Her 2 Neu**			**Test of significance**
	Negative	**Equivocal**	**Positive**	
Age /years Mean± SD	**53.48±12.3**	**52.09±8.5**	**55.13±6.6**	**P=0.85**
	N(%)	N(%)	N(%)	
Histopathological type				
- Serous adenocarcinoma	46(83.6)	6(10.9)	3(5.5)	P = 0.12
- Endometroid adenocarcinoma	9(81.8)	1(9.1)	1(9.1)	
- Mucinous cyst adenocarcinoma	17 (77.2)	3(13.6)	2(9.1)	
- Clear cell carcinoma	2(50.0)	0(0.0)	2(50.0)	
- Transitional cell carcinoma	1(50.0)	1(50.0)	0(0.0)	
Tumor grade (N=66)#				
- Low	10 (18.2)	0 (0.0)	0 (0.0)	P = 0.33
- Intermediate	6 (10.9)	0 (0.0)	1 (25.0)	
- High	39 (70.9)	5 (100.0)	5 (75.0)	
Tumor Stage				
- I	19(25.3)	2(18.2)	3(37.5)	P = 0.7
- II	29(38.7)	3(27.3)	4(50.0)	
- III	12(16.0)	3(27.3)	0(0.0)	
- IV	15(20.0)	3(27.3)	1(12.5)	
Size Mean ±SD	10.16±6.6	10.64±7.8	10.0±6.4	P = 0.97
Tumor recurrence	9(75.0)	2(16.7)	1(8.3)	P=0.85
Bilateral affection for both ovaries	41(80.4)	7(13.7)	3(5.9)	P=0.59
Lymph node metastasis	11(91.7)	1(8.3)	0(0.0)	P=0.55
Involvement of ascetic fluid cytology	26(76.5)	4(11.8)	4(11.8)	P=0.73
Distant Metastasis	41(78.8)	7(13.5)	4(7.7)	P=0.86
Preoperative neo adjuvant chemotherapy	27(81.8)	4(12.1)	2(6.1)	P=0.85
Necrosis				
- Absent	16 (21.3)	2 (18.2)	1 (12.5)	P = 0.9
- Present	59 (78.7)	9 (81.8)	7 (87.5)	

#' The number of cases are 66 as we graded only serous and endometrioid carcinoma cases '

Relation between Her2/neu expression and the clinicopathological features

We found no statistically significant relation between Her-2/neu expression and any of the clinicopathological features of the tumors (Table 2). We observed a noticeable but non-statistically significant higher percentage of Her-2/neu negativity among serous carcinoma (83.6 % of serous carcinoma were Her-2/neu negative versus 5.5% were Her-2/neu positive), endometrioid carcinoma (81.1 % of endometrioid carcinoma were Her-2/neu negative versus 9.1% were Her-2/neu positive), and mucinous carcinoma (77.2 % of mucinous carcinoma were Her-2/neu negative versus 9.1% were Her-2/neu positive). On the other hand, 50% of clear cell carcinoma were Her-2/neu positive (Table 2).

We graded only cases of serous and endometroid types. Five out of the six Her-2/neu positive serous and endometroid cases (83.3%) showed high tumor grade. Similarly, seven out of the eight Her-2/neu positive cases showed presence of necrosis. However, these associations did not reach statistical significant level (P = 0.33, 0.9) respectively (Table 2).

DISCUSSION

In this study, HER2/neu protein expression was analyzed in 94 SEOC and overexpression (score 3+) was detected in 8.5% cases. This figure is slightly lower than detected byJafri et al, 2017 where 10.7% of cases, turned out to be score (3+) positive but higher than detected by Ray-Coquard et al., 2009a where Her-2/neu score 3+ staining was observed in 4.7% of the 320 tumor samples. On the other hand, various other published reports show a higher rate of Her-2/neu protein overexpression in SEOCs. Higher expression has been demonstrated by Goel et al., 2014 which reported that 48.6% of the SEOC cases exhibited Her-2/neu positivity. Also, Lanitis et al., 2012 described positive Her-2/neu expression in 52% of cases included in their study.

The discrepancy in percentage of Her-2/neu overexpression may possibly be justified by a number of reasons that include diverse detection techniques used, e.g. (IHC), (FISH) and (CISH), various technical factors including different immuno-histochemical methods (Hercep test or non-commercial antibodies), difference in staining procedures, subjective analysis, interobserver variability of the slides, differences in sample sizes and in populations included in examination of cases. All these issues complicate the comparison and variability of Her-2/neu expression results among various research studies (Jafri et al., 2017).

In the current study, there were no statistically significant relationships between Her-2/neu expression and any of the studied clinicopathological features. Regarding patient's age, tumor histopathological type, tumor grade, tumor stage, our results were comparable to many studies which revealed no statistically significant relationships between Her-2/neu expression and these four variables (Tuefferd et al., 2007; Berchuck et al., 1991; Demir et al., 2014). However, few studies revealed a significant relationship between Her-2/neu expression and age being more expressed at higher age at diagnosis (Serrano-Olvera et al., 2006 and Reibenwein et al., 2008).

We observed a non-statistically significant higher percentage of Her-2/neu negativity among serous carcinoma as 83.6 % of serous carcinoma were Her-2/neu negative. In contrast, many studies have established the overexpression of Her-2/neu in serous neoplasm (Reibenwein et al., 2008 and Lee et al., 2006).Also, Cloven et al., 2004 reported that the mucinous and clear cell varieties overexpress Her-2/neu more frequently. In this study, 37.5% of positive cases were serous adenocarcinomas which were comparable with results of Marinas et al., 2012 who found that Her-2/neu expression has significant association with serous adenocarcinomas.

For tumor grade, in contrast to this study, many studies showed statistically significant relationship between Her-2/neu expression and grade being more expressed with higher grades (English et al, 2013; Steffensen et al., 2007; Reibenwein et al., 2008; Verri et al., 2005). Unlike this study, Steffensen et al., 2007 and Reibenwein et al., 2008 showed statistically significant relationship between Her-2/neu expression and tumor stage being more expressed with higher stages (III and IV). The discrepancy may be due to difference in staining procedures, subjective analysis, inter-observer variability of the slides, differences in sample sizes and populations included in the studied cases.

Regarding involvement of ascetic fluid cytology by tumor cells, to our knowledge, the only two studies including this variable were similar to ours and established no statistically significant relationship between Her-2/neu expression and malignant ascetic fluid (Tuefferd et al., 2007 and Lassus et al., 2004).

Regarding assessment of Her-2/neu expression in recurrent cases, only 3 cases (25%) out of the 12 recurrent cases included in this study exhibited 2+ or 3+ expression with no statistical significance, a different result was established by the of study of Bookman et al., 2003were out of 837 recurrent cases only 95 tumors (11.4%) exhibited 2+ or 3+ expression.

To the best of our knowledge, the six clinicopathological features included in this study (size of the tumor, bilateral affection of both ovaries, lymph nodes involvement, presence of metastatic deposits in other organs, history of neo-adjuvant chemotherapy, presence or absence of necrosis) haven't been mentioned in the

literature as a point of study in relation to Her-2/neuexpression in malignant SEOC till now.

CONCLUSION

In conclusion, Her-2/neu is overexpressed in 8.5% SEOC. Of the 8 positive cases, serous cystadenocarcinomas and high grade carcinomas showed the maximum number of Her-2/neu positive tumors. There was no statistically significant association between and Her-2/neu protein overexpression and any of the clinicopathological features.

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