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HER2 Overexpression in Borderline and Malignant Ovarian Tumors: A Cross-sectional Study in an Iranian Population and Literature Review

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Abstract

Background: Different studies have investigated the overexpression of human epidermal growth factor receptor 2 in ovarian cancers, in addition to the association between the level of its overexpression and tumor characteristics (tumor grade, subtype, stage, and prognosis). However, the prognostic significance of human epidermal growth factor receptor 2/neu dysregulation in epithelial ovarian tumors is controversial. The current study aims to assess human epidermal growth factor receptor 2 overexpression in different types and stages of epithelial borderline and malignant ovarian tumors in a population of Iranian patients.

Methods: We conducted this cross-sectional study on 100 patients diagnosed with epithelial borderline and malignant ovarian tumors who referred to the Cancer Institute of Imam Khomeini Hospital at Tehran between 2012 and 2014. After selection of the appropriate tissue block, we prepared slides for immunohistochemical staining with the human epidermal growth factor receptor 2 marker. Human epidermal growth factor receptor 2 positivity was evaluated and scored according to Ellis and Wolff recommendations. Cases with equivocal immunohistochemical results (score 2) also underwent chromogenic *in situ* hybridization.

Results: The most prevalent tumor in our study was serous carcinoma (54%). Human epidermal growth factor receptor 2 scores were: 0 in 69%, 1+ in 26%, 2+ in 4%, and 3+ in 1% of tumors. Chromogenic *in situ* hybridization examination of cases with human epidermal growth factor receptor 2 score of 2 showed negative results for human epidermal growth factor receptor 2 gene amplification. We observed no association between human epidermal growth factor receptor 2 and the level of tumor differentiation, histologic subtype, clinical stage, tumor size, and patient's age.

Conclusion: Controversial results and wide range of prevalence in human epidermal growth factor receptor 2 overexpression in different studies could be due to several causes. Technical considerations, tumor heterogeneity, and lack of standard guidelines for interpretation could influence the results. We did not find any relationship between human epidermal growth factor receptor 2 overexpression and prognostic indices of grade, clinical stage or histologic subtype as many other reports. Future studies should be conducted on larger numbers of patients with different disease stages and adequate numbers of different histologic subtypes.

Keywords: HER2, Overexpression, Ovary, Borderline, Malignant, Tumor, Iran

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Introduction

Ovarian cancer is the fifth most common cancer in women and the leading cause of cancer deaths among genetic cancers worldwide.[1] Ovarian epithelial cancers are the most common ovarian malignant tumors that usually remain asymptomatic until metastasis; therefore, they are diagnosed at advanced stages of the disease in more than two-thirds of patients.[2,3] Ovarian cancer is one of the major issues in the field of surgery, which requires serious and often complex treatment, and wastes the psychological and physical energy of the patient.[4] Epithelial tumors of the ovary constitute approximately 75% of ovarian neoplasms and 90% of all ovarian cancers are surface epithelial carcinomas.[5] Approximate distribution of surface ovarian epithelium tumors shows that about 85% of these tumors are serous and mucinous, and one third are carcinomas.[6] According to the WHO Committee for the classification and histological typing of ovarian tumors, there is a borderline group between benign and malignant groups based on histology and behavior manifestations.[7] The tumors of this group have cell proliferation more than benign types and some degree of nuclear atypia without apparent invasion or destruction in the stroma.[8]

The human epithelial growth factor receptors (HERs) are the receptors of trans-membrane tyrosine kinase enzymes that play a key intermediary role in cell growth and development as well as cell survival.[9] The activity of HER tyrosine kinase stimulates intracellular signal pathways such as MAPK and PI3K/Akt.[10] Overexpression of HER2 is one of the most common and frequent pathways of oncogenesis in different cancers. Human epithelial growth factor receptor families are important mediators in the development of ovarian follicles and play an essential role in regulating the growth of ovarian epithelial cells.[11] Disturbance in the regulation of HER signals in the ovary has a strong relationship with the growth and development of ovarian tumors due to increased expression or mutation in HER.[12] The role of HER2 overexpression in ovarian cancers has been studied previously. According to studies, 5%-35% of ovarian tumors are associated with increased HER2 expression.[13,14] However, there are inadequate and contradictory results in the effects of HER2 on disease prognosis and its potential treatment role. Our study aims to assess HER2 overexpression in different stages of epithelial borderline and malignant ovarian tumors in an Iranian population.

Materials and Methods

We conducted this cross-sectional study on 100 patients diagnosed with epithelial borderline and malignant ovarian tumors who referred to the Cancer Institute at Imam Khomeini Hospital between 2012 and 2014. Baseline characteristics were collected by a review of the patient's medical records. The appropriate block for the preparation of slide for immunohistochemical staining with the HER2 marker was selected based on the highest amount of tumor tissue and minimum necrosis. We used the c-erbB-2(CB11) kit (Biocare Company) which contains monoclonal antibodies. After preparing the appropriate thickness (2 to 3 μm) of the paraffin blocks, paraffin degradation was performed by placing the tissue sections in a hot water bath. Antigen retrieval by placing the slides in a microwave oven for 45 minutes and inhibition of endogenous peroxidase by ethanol solution and oxygenated water were performed, respectively. For blocking the non-specific proteins reaction, incubation for 10-15 minutes at room temperature with Biocare's background sniper accomplished. Then the tissues were incubated with primary antibody, probe, polymer, and chromogen, respectively. Finally, counter-staining with hematoxylin was performed. We assessed for HER2 positivity according to Ellis and Wolff's method as follows: 0 (no staining observed or membrane staining that is incomplete and is faint/barely perceptible and within ≤10% of the invasive tumor cells); 1+ (incomplete membrane staining that is faint/barely perceptible and within >10% of invasive tumor cells); 2+ (membrane staining that is incomplete and/or weak/moderate and within >10% of the invasive

tumor cells or complete and circumferential membrane staining that is intense and within ≤10% of the invasive tumor cells); and 3+(circumferential membrane staining that is complete and intense within >10% of tumor cells) as shown in figure 1.

Chromogenic *in situ* hybridization (CISH) was performed on 1-µm-thick formalin-fixed paraffin-embedded sections of tissue blocks that had unequivocal results (score 2) according to the immunohistochemistry (IHC) examination. The sections were deparaffinized by incubation in an oven at 60 C overnight and dewaxed by xylene and ethanol. Subsequently, temperature pretreatment and enzyme digestion were performed. The sections underwent denaturation and hybridization with HER2/CEN-17 CISH probes. After post-hybridization, detection and visualization by red and green chromogens and contrast staining with hematoxylin solution were performed.

Based on the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) HER2 test guidelines for breast cancer, 3+ IHC results were considered positive. According to CISH, a dual-probe Her2/CEP-17 ratio ≥2.0 regardless of the average HER2 copy number or dual-probe Her2/CEP-17 ratio <2.0 with an average HER2 copy number ≥6.0 signals/cell were considered positive results.[15]

Table 1. Patients' mean age and maximum tumor size in different groups of ovarian borderline and carcinoma with scores of 0-3 human epidermal growth factor receptor 2 (HER2) expression on immunohistochemistry (IHC) study.

HER2 score (N)	Age (years) (*P*=0.452)	Size (cm) (*P*=0.281)
0 (69)	48.12±13.8	10.37±5.6
1 (26)	44.92±13.0	8.23±6.1
2 (4)	38±13.5	6.5±3.8
3 (1)	46	10

Next, we assessed the results and their relationships with age, stage of disease, degree of tumor differentiation, and histologic tumor type.

Statistical analysis

Results were presented as mean±standard deviation (SD) for quantitative variables and summarized by absolute frequencies and percentages for categorical variables. Normality of data was analyzed using the Kolmogorov-Smirnov test. Categorical variables were compared using the chi-square test or Fisher's exact test when we observed more than 20% of cells with expected counts of less than 5. Quantitative variables were also compared with the ANOVA test or Kruskal-Wallis H test. We used the statistical software SPSS version 16.0 for Windows (SPSS, Inc., Chicago, IL, USA). *P*-values of 0.05 or less were considered statistically significant.

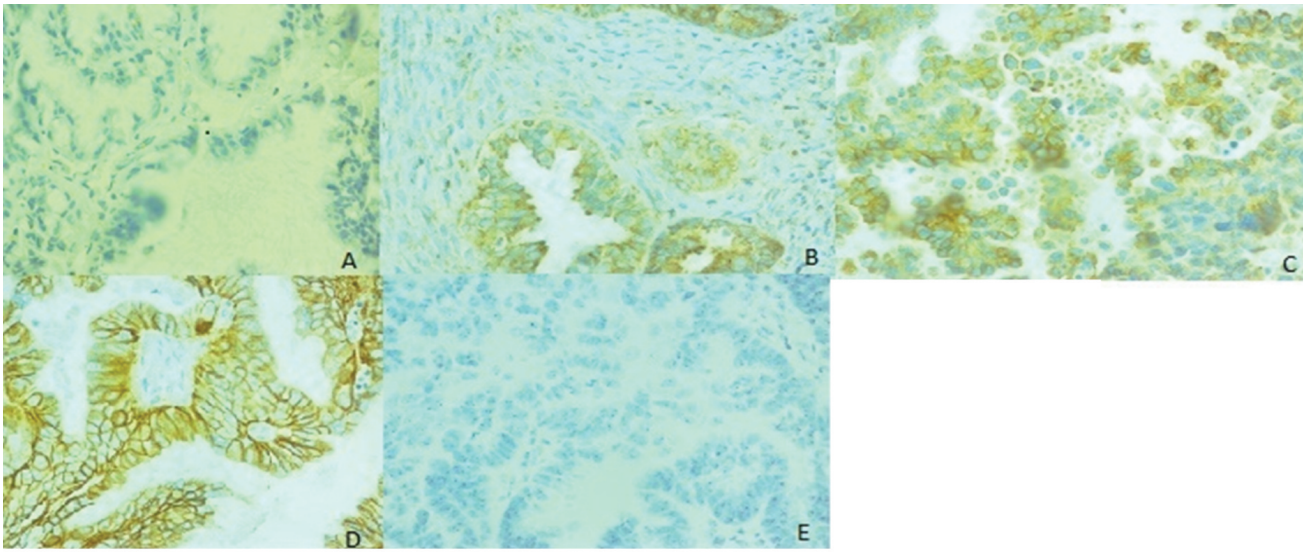

Figure 1. Immunohistochemistry (IHC) study shows A: negative, B: score 1, C: score 2, D: score 3 of HER2 overexpression, and E: Chromogenic *in situ* hybridization assay (CISH) shows negative result.

Table 2. Frequency of human epidermal growth factor receptor 2 (HER2) expression based on immunohistochemistry (IHC) scores in different clinical stages of the patients at the time of presentation.

Clinical stage		HER2 results				Total
		0	1+	2+	3+	
IA	Count	21	8	0	0	29
	% within HER2 score	30.4%	30.8%	0%	0%	29.0%
IB	Count	4	0	0	0	4
	% within HER2 score	5.8%	0%	0%	0%	4.0%
IC	Count	2	2	1	0	5
	% within HER2 score	2.9%	7.7%	25.0%	0%	5.0%
IIA	Count	5	2	1	0	8
	% within HER2 score	7.2%	7.7%	25.0%	0%	8.0%
IIB	Count	2	0	0	0	2
	% within HER2 score	2.9%	0%	0%	0%	2.0%
IIIA	Count	7	3	0	0	10
	% within HER2 score	10.1%	11.5%	0%	0%	10.0%
IIIB	Count	10	4	0	0	14
	% within HER2 score	14.5%	15.4%	0%	0%	14.0%
IIIC	Count	17	7	2	1	27
	% within HER2 score	24.6%	26.9%	50.0%	100.0%	27.0%
IV	Count	1	0	0	0	1
	% within HER2 score	1.4%	0%	0%	0%	1.0%
Total	Count	69	26	4	1	100
	% within HER2 score	100.0%	100.0%	100.0%	100.0%	100.0%

Results

This study included a total of 100 patients with borderline and malignant epithelial ovarian tumors. Patients had an average age of 46.94±13.63 years. The mean tumor size was 9.71±5.77 cm (range: 1 to 25 cm). Based on histology, 54% of patients had serous carcinoma, followed by serous borderline tumor (20%),

Table 3. Frequency of human epidermal growth factor receptor 2 (HER2) score in different histologic tumor subtypes based on WHO classification.

Histologic subtype		HER2 results				Total
		0	1+	2+	3+	
CC	Count	2	0	0	0	2
	% within HER2 score	2.9%	0%	0%	0%	2.0%
EA	Count	6	3	0	0	9
	% within HER2 score	8.7%	11.5%	0%	0%	9.0%
MB	Count	7	0	0	0	7
	% within HER2 score	10.1%	0%	0%	0%	7.0%
MC	Count	3	1	0	0	4
	% within HER2 score	4.3%	3.8%	0%	0%	4.0%
SB	Count	14	5	1	0	20
	% within HER2 score	20.3%	19.2%	25.0%	0%	20.0%
SC	Count	33	17	3	1	54
	% within HER2 score	47.8%	65.4%	75.0%	100.0%	54.0%
SMB	Count	2	0	0	0	2
	% within HER2 score	2.9%	0%	0%	0%	2.0%
SMC	Count	2	0	0	0	2
	% within HER2 score	2.9%	0%	0%	0%	2.0%
Total	Count	69	26	4	1	100
	% within HER2 score	100.0%	100.0%	100.0%	100.0%	100.0%

CC: Clear cell carcinoma; EA: Endometrioid adenocarcinoma; MB: Mucinous borderline tumor; MC: Mucinous carcinoma; SB: Serous borderline tumor; SC: Serous carcinoma; SMB: Seromucinous borderline tumor; SMC: Seromucinous carcinoma

Table 4. Frequency of human epidermal growth factor receptor 2 (HER2) score in different histologic grades of ovarian carcinomas.

		HER2 results				Total
		0	1+	2+	3+	
Histologic grade	Count	28	6	1	0	35
	% within HER2 score	40.6%	23.1%	25.0%	0.0%	35.0%
SC, High grade	Count	27	12	2	0	41
	% within HER2 score	39.1%	46.2%	50.0%	0.0%	41.0%
SC, Low grade	Count	8	5	1	1	15
	% within HER2 score	11.6%	19.2%	25.0%	100.0%	15.0%
EC, FIGO II	Count	3	0	0	0	3
	% within HER2 score	4.3%	0.0%	0.0%	0.0%	3.0%
EC, FIGO I	Count	3	3	0	0	6
	% within HER2 score	4.3%	11.5%	0.0%	0.0%	6.0%
Total	Count	69	26	4	1	100
	% within HER2 score	100.0%	100.0%	100.0%	100.0%	100.0%

EC: Endometrioid carcinoma; SC: Serous carcinoma; FIGO: International Federation of Gynecology and Obstetrics.

endometrioid carcinoma (9%), mucinous borderline tumor (7%), mucinous carcinoma (4.0%), clear cell carcinoma (2.0%), seromucinous borderline tumor (2.0%), and seromucinous carcinoma (2.0%). With respect to tumor differentiation, we observed high grade serous carcinoma in 41%, low grade serous carcinoma in 15%, FIGO I endometrioid carcinoma in 6%, and FIGO II endometrioid carcinoma in 3%. Human epidermal growth factor receptor 2 results were: 0 in 69%, 1+ in 25%, 2+ in 4%, and 3+ in 1%. None of the equivocal cases overexpressed HER2 according to the CISH study.

There were no significant differences in patients' mean age (P=0.425) and maximum tumor size (P=0.281) with different scores of HER2 expression (Table 1). Table 2 summarizes the frequency of different clinical stages at the time of presentation and HER2 scores in different groups. There was no significant association between HER2 expression and clinical stage (P=0.929). We did not find any relationship between HER2 expression and histologic type of the tumors (P=0.991; Table 3). Patients with different histologic grades of ovarian carcinoma did not show a significant difference in HER2 expression (P=0.567; Table 4).

Discussion

Most ovarian cancers have a good response to first-line chemotherapeutic agents. However, high mortality rate for advanced staged tumors due to acquired resistance to the usual drugs highlights the use for targeted therapies. Overexpression of HER2, as a member of the epidermal growth receptors, has been investigated in different studies. Table 6 summarizes 27 previously published studies of HER2/neu expression and/or amplification in ovarian epithelial neoplasms. The method of scoring and accepted threshold for positive results differs in most of these studies (Table 6). Although most studies have considered an IHC HER2 score of ≥2+ as positive, a wide range of prevalence in HER2 protein overexpression in 4% up to 69% of ovarian tumors has been previously reported. Association of the HER2/neu gene or protein abnormalities with tumor subtype, stage, grade, size, and patient's age is also inconsistent in different studies. A few studies have investigated HER2 gene amplification and reported a prevalence that ranged from 2% to 12.5%.

In the present study, we used the ASCO/CAP guideline recommendations for breast cancer biomarker scoring to score and interpret the results. In terms of HER2 expression, according to IHC, 69% (69/100) of the tumors were negative, whereas 26% (26/100) had a score of 1+, 4.0% (4/100) were 2+, and 1% (1/100) had a score of 3+. Chromogenic *in situ* hybridization was performed on the 4% (4/100) of cases which had equivocal results (score 2+) according to IHC; all

Table 5. Review of previously published studies that evaluated human epidermal growth factor receptor 2 (HER2) overexpression in ovarian cancers, prevalence of positive results, method, scoring method, and correlation between tumor size, stage, grade, subtype, and patient's age.

Author	Country	Method	n/N (%) n:Number of positive cases N: Total number of patients	HER2 overexpression scoring method	Correlation between HER2 expression and tumor subtype	Correlation between HER2 expression and tumor stage	Correlation between HER2 expression and tumor grade	Correlation between HER2 expression and tumor size	Correlation between HER2 expression and age
Rubin et al.[16]	United States	IHC	25/105 (24%): Strong	Negative, weak (diffuse cytoplasmic); Positive: moderate (1+, 2+), strong (3+)	No	No	No	-	-
Rubin et al.[17]	United States	IHC	28/40 (70%): Moderate, 8/40 (20%): Strong	Negative, weak (diffuse cytoplasmic); Positive: moderate (1+, 2+), strong (3+)	Yes (clear cell carcinoma)	No	No	-	-
Singleton et al.[18]	United States	IHC	10/56 (18%)	Positive: 1+ (unequivocal), 2+ (moderate), 3+ (moderate to strong), 4+ (uniform strong intensity)	No	No	No	-	-
Meden et al.[19]	Germany	IHC	48/266 (18%)	Positive: >5% of cells with membranous staining	-	-	-	-	-
Felip et al.[20]	Spain	IHC	23/106 (21.7%)	1+ (light staining), 2+ (moderate staining), 3+ (intense staining)	No	Yes	No	-	No
Fajac et al.[21]	France	IHC FISH	23/52 (44%) 9/65 (14%)	1+, 2+, 3+ ≥2.5	No	No	No	-	-
Simpson et al.[22]	United States	IHC	69/200 (35%)	1+ (weak); 2+ (moderate); 3+ (strong)	-	-	-	-	-
van Haaften-Day et al.[23]	Australia	IHC	11/22 (50%)	1+ (<25%); 2+ (25%-50%); 3+ (50%-75%); 4+ (>75%)	-	-	-	-	-
Goff et al.[24]	United States	IHC	7/64 (11%)	Membranous staining, 1+, 2+, 3+	-	No	No	-	-
Eltabbakh et al.[7]	United States	IHC	9/42 (21%)	Semi-quantitated: number of positively stained tumor cells	-	Yes	-	-	-
Auranen et al.[25]	Finland	IHC	385/559 (68.9%)	Membranous staining, 1+, 2+, 3+	-	-	-	-	-
Høgdall et al.[26]	Denmark	IHC	95/181 (52.5%)	1+, 2+, 3+	-	Yes	-	-	Yes
Høgdall et al.[26]	Denmark	IHC	95/181 (52.5%)	1+, 2+, 3+	-	Yes	-	-	Yes
Bookman et al.[27]	United States	IHC	95/837 (11%)	2+, 3+	-	-	-	-	-

Author	Country	Method	n/N (%) n:Number of positive cases N: Total number of patients	HER2 overexpression scoring method	Correlation between HER2 expression and tumor subtype	Correlation between HER2 expression and tumor stage	Correlation between HER2 expression and tumor grade	Correlation between HER2 expression and tumor size	Correlation between HER2 expression and age
Mano et al.[28]	Belgium	IHC FISH	3/72 (4.2%) 8/64 (12.5%)	3+ HER2: CEP>2	-	-	-	-	-
Camilleri-Broët et al.[29]	France	IHC	15/95 (16%)	Moderate intense staining of >10% tumor cells	-	No-	-	-	-
Nielsen et al.[30]	Denmark	IHC	272/783 (35%)	2+, 3+	-	-	-	-	-
Lassus et al.[31]	Finland	IHC CISH	66/390 (17%) 26/381 (7%)	Low/weak, moderate, strong >5 copies per cell	-	No	Yes	No	Yes
Lee et al.[32]	Canada	IHC	5/102 (5%)	≥1+	-	-	-	-	-
O'Neill et al.[33]	Ireland	IHC	17/47 (36%)	1+ (<10%), 2+ (10%-25%), 3+ (26%-50%), 4+ (51%-75%), 5+ (>75%)	Yes	-	Yes	-	-
Verri et al.[34]	Italy	IHC	27/194 (14%)	2+, 3+	No	No	No	-	-
Mayr et al.[35]	Germany	IHC FISH	1+ (11.3%); 2+ (41.1%); 3+ (2.8%) Low amplification (2.7%); high amplification (3.7%, 2+ and 3+ in IHC)	1+, 2+, 3+ Low amplification: 4-10 gene signals in >10% of nuclei	No	No	No	-	-
Tuefferd et al.[36]	France	IHC FISH	41/320 [12.7% (2+: 8%; 3+: >4.7%)] 38/62 (61%)	2+, 3+ HER2/ CEP >2.2	No	No	No	-	No
Steffensen et al.[37]	Denmark	IHC FISH	18/160 (11%) [6.9%: 2+; 4.4%: 3+] 10/145 (7%)	1+, 2+, 3+ HER2/ CEP >2	-	-	-	-	-
Vermeij et al.[38]	Belgium	IHC FISH Molecular	6/31 (19%); 2+: 9%; 3+:10% 3/6 (10%) 0	2+, 3+ HER2/ CEP >2 Tyrosine kinase mutation	-	-	-	-	-
Hoopmann et al.[39]	Germany	IHC	2/44 (7.7%)	3+	No	No	No	-	No
Kadkhodayian et al.[40]	Iran	IHC	22% (2+); 6% (3+)	2+, 3+	Yes	No	-	-	-
Current study	Iran	IHC CISH	5/100: 4% (2+); 1% (3+) 0	2+: Equivocal, 3+: Positive HER2/CEP17 ratio ≥2.0	No	No	No	No	No

showed negative results for gene amplification. Another study in Mashhad, Iran reported that 28% (14/50) of ovarian epithelial tumors showed IHC expression (2+ and 3+) of HER2 which had only a statistically significant correlation with tumor histologic type.[40]

While overexpression of HER2 appeared to be more common in mucinous carcinoma,[41] there was no statistically significant difference between HER2 expression in various histologic subtypes of ovarian carcinoma in the current study. Serous borderline and carcinomas comprised the vast majority of our cases that had more prevalence of HER2 overexpression. None of the 3 mucinous carcinomas and 7 borderline mucinous tumors overexpressed HER2.

We did not find any significant correlation between patients' age, tumor size, clinical stage, and histologic grade as shown in some study results summarized in table 6.

Controversial results and a wide range of prevalence in HER2 overexpression in different studies could be due to several causes. In addition to the influence of the IHC staining method, sensitivity and specificity of the kits, differences in antibody clonality, changes in antigen expression due to inappropriate fixation and interobserver variability in scoring the results, lack of standard guidelines for interpretation of the results in ovarian tumors, and tumor heterogeneity are the other important factors which can impact the results. McCaughan et al. have reported 20% intratumoral heterogeneity in expression of HER2 in epithelial ovarian carcinomas in their study. This intratumoral heterogeneity not only alters the IHC results, it may have an influence on efficacy of HER2 targeted therapies.[42]

The clinical significance of HER2 overexpression in ovarian tumors is also controversial. While some studies have found HER2 overexpression to be an independent risk factor of decreased survival, others noted that patients with negative HER2 had a better response to chemotherapy and improved survival.[43] A multicenter study of 320 patients in France reported no significant relationship between HER2 expression and other prognostic factors, progression-free, and overall survival.[37]

In conclusion, 5% of the tumors in our study expressed HER2 which was positive in only 1% according to ASCO/CAP guidelines. We did not find any relationship between histologic grade, subtype, and clinical stage. Large sample studies with adequate numbers of different histologic types and clinical stages of ovarian cancer, the use of standard guidelines for IHC or molecular studies, and interpretation of results is needed for future researches.

Conflict of Interest

None declared.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer.* 2015;136(5):E359-86. doi: 10.1002/ijc.29210.
2. Allemani C, Weir HK, Carreira H, Harewood R, Spika D, Wang XS, et al. Global surveillance of cancer survival 1995-2009: analysis of individual data for 25,676,887 patients from 279 population-based registries in 67 countries (CONCORD-2). *Lancet.* 2015;385(9972):977-1010.
3. Sankaranarayanan R, Ferlay J. Worldwide burden of gynaecological cancer: the size of the problem. *Best Pract Res Clin Obstet Gynaecol*. 2006;20(2):207-25.
4. Ebell MH, Culp MB, Radke TJ. A systematic review of symptoms for the diagnosis of ovarian cancer. *Am J Prev Med.* 2016;50(3):384-94.
5. Kurman RJ, Shih IeM. The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory. *Am J Surg Pathol.* 2010;34(3):433-43.
6. Kindelberger DW, Lee Y, Miron A, Hirsch MS, Feltmate C, Medeiros F, et al. Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: Evidence for a causal relationship. *Am J Surg Pathol*. 2007;31(2):161-9.
7. Eltabbakh GH, Belinson JL, Kennedy AW, Biscotti CV, Casey G, Tubbs RR. p53 and HER-2/neu overexpression in ovarian borderline tumors. *Gynecol Oncol.* 1997;65(2):218-24.
8. Bjørge T, Lie AK, Hovig E, Gislefoss RE, Hansen S, Jellum E, et al. BRCA1 mutations in ovarian cancer and borderline tumours in Norway: a nested case-control study. *Br J Cancer.* 2004;91(10):1829-34.
8. Cho KR, Shih IeM. Ovarian cancer. *Annu Rev Pathol*. 2009;4:287-313. doi: 10.1146/annurev.*pathol*.

4.110807.092246.
9. Pritchard KI, Shepherd LE, O'Malley FP, Andrulis IL, Tu D, Bramwell VH, et al. HER2 and responsiveness of breast cancer to adjuvant chemotherapy. *N Engl J Med*. 2006;354(20):2103-11.
10. Ellis IO, Bartlett J, Dowsett M, Humphreys S, Jasani B, Miller K, et al. Best Practice No 176: Updated recommendations for HER2 testing in the *UK. J Clin Pathol.* 2004;57(3):233-7.
11. Lanitis E, Dangaj D, Hagemann IS, Song DG, Best A, Sandaltzopoulos R, et al. Primary human ovarian epithelial cancer cells broadly express HER2 at immunologically-detectable levels. *PLoS One*. 2012;7(11):e49829. doi: 10.1371/journal.pone.0049829.
12. Wang D, Zhu H, Ye Q, Wang C, Xu Y. Prognostic value of KIF2A and HER2-Neu overexpression in patients with epithelial ovarian cancer. *Medicine (Baltimore).* 2016;95(8):e2803.
13. de Toledo MC, Sarian LO, Sallum LF, Andrade LL, Vassallo J, de Paiva Silva GR, et al. Analysis of the contribution of immunologically-detectable HER2, steroid receptors and of the "triple-negative" tumor status to disease-free and overall survival of women with epithelial ovarian cancer. *Acta Histochem.* 2014;116(3):440-7.
14. Hsieh CY, Chen CA, Chou CH, Lai KP, Jeng YM, Kuo ML, et al. Overexpression of Her-2/NEU in epithelial ovarian carcinoma induces vascular endothelial growth factor C by activating NF-kappa B: implications for malignant ascites formation and tumor lymphangiogenesis. *J Biomed Sci.* 2004;11(2):249-59.
15. Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *J Clin Oncol.* 2013;31(31):3997-4013. doi: 10.1200/JCO.2013.50.9984.
16. Rubin SC, Finstad CL, Wong GY, Almadrones L, Plante M, Lloyd KO. Prognostic significance of HER-2/neu expression in advanced epithelial ovarian cancer: a multivariate analysis. *Am J Obstet Gynecol.* 1993;168(1 Pt 1):162-9.
17. Rubin SC, Finstad CL, Federici MG, Scheiner L, Lloyd KO, Hoskins WJ. Prevalence and significance of HER-2/neu expression in early epithelial ovarian cancer. *Cancer.* 1994;73(5):1456-9.
18. Singleton TP, Perrone T, Oakley G, Niehans GA, Carson L, Cha SS, et al. Activation of c-erbB-2 and prognosis in ovarian carcinoma. Comparison with histologic type, grade, and stage. *Cancer*. 1994;73(5):1460-6.
19. Meden H, Marx D, Rath W, Kron M, Fattahi-Meibodi A, Hinney B, et al. Overexpression of the oncogene c-erb B2 in primary ovarian cancer: evaluation of the prognostic value in a Cox proportional hazards multiple regression. *Int J Gynecol Pathol.* 1994;13(1):45-53.
20. Felip E, Del Campo JM, Rubio D, Vidal MT, Colomer R, Bermejo B. Overexpression of c-erbB-2 in epithelial ovarian cancer. Prognostic value and relationship with response to chemotherapy. *Cancer.* 1995;75(8):2147-52.
21. Fajac A, Benard J, Lhomme C, Rey A, Duvillard P, Rochard F, et al. c-erbB2 gene amplification and protein expression in ovarian epithelial tumors: evaluation of their respective prognostic significance by multivariate analysis. *Int J Cancer.* 1995;64(2):146-51.
22. Simpson BJ, Phillips HA, Lessells AM, Langdon SP, Miller WR. c-erbB growth-factor-receptor proteins in ovarian tumours. *Int J Cancer.* 1995;64(3):202-6.
23. van Haaften-Day C, Russell P, Boyer CM, Kerns BJ, Wiener JR, Jensen DN, et al. Expression of cell regulatory proteins in ovarian borderline tumors. *Cancer.* 1996;77(10):2092-8.
24. Goff BA, Shy K, Greer BE, Muntz HG, Skelly M, Gown AM. Overexpression and relationships of HER-2/neu, epidermal growth factor receptor, p53, Ki-67, and tumor necrosis factor alpha in epithelial ovarian cancer. *Eur J Gynaecol Oncol.* 1996;17(6):487-92.
25. Auranen A, Grénman S, Kleml PJ. Immunohistochemically detected p53 and HER-2/neu expression and nuclear DNA content in familial epithelial ovarian carcinomas. *Cancer.* 1997;79(11):2147-53.
26. Høgdall EV, Christensen L, Kjaer SK, Blaakaer J, Bock JE, Glud E, et al. Distribution of HER-2 overexpression in ovarian carcinoma tissue and its prognostic value in patients with ovarian carcinoma: from the Danish MALOVA Ovarian Cancer Study. *Cancer.* 2003;98(1):66-73.
27. Bookman MA, Darcy KM, Clarke-Pearson D, Boothby RA, Horowitz IR. Evaluation of monoclonal humanized anti-HER2 antibody, trastuzumab, in patients with recurrent or refractory ovarian or primary peritoneal carcinoma with overexpression of HER2: a phase II trial of the Gynecologic Oncology Group. *J Clin Oncol.* 2003;21(2):283-90.
28. Mano MS, Awada A, Di Leo A, Durbecq V, Paesmans M, Cardoso F, et al. Rates of topoisomerase II-alpha and HER-2 gene amplification and expression in epithelial ovarian carcinoma. *Gynecol Oncol.* 2004;92(3):887-95.
29. Camilleri-Broët S, Hardy-Bessard AC, Le Tourneau A, Paraiso D, Levrel O, Leduc B, et al. HER-2 overexpression is an independent marker of poor prognosis of advanced primary ovarian carcinoma: a multicenter study of the GINECO group. *Ann Oncol.* 2004;15(1):104-12.
30. Nielsen JS, Jakobsen E, Hølund B, Bertelsen K, Jakobsen A. Prognostic significance of p53, Her-2,

and EGFR overexpression in borderline and epithelial ovarian cancer. *Int J Gynecol Cancer.* 2004;14(6):1086-96.
31. Lassus H, Leminen A, Vayrynen A, Cheng G, Gustafsson JA, Isola J, et al. ERBB2 amplification is superior to protein expression status in predicting patient outcome in serous ovarian carcinoma. *Gynecol Oncol.* 2004;92(1):31-9.
32. Lee CH, Huntsman DG, Cheang MC, Parker RL, Brown L, Hoskins P, et al. Assessment of Her-1, Her-2, And Her-3 expression and Her-2 amplification in advanced stage ovarian carcinoma. *Int J Gynecol Pathol.* 2005;24(2):147-52.
33. O'Neill CJ, Deavers MT, Malpica A, Foster H, McCluggage WG. An immunohistochemical comparison between low-grade and high-grade ovarian serous carcinomas: significantly higher expression of p53, MIB1, BCL2, HER-2/neu, and C-KIT in high-grade neoplasms. *Am J Surg Pathol.* 2005;29(8):1034-41.
34. Verri E, Guglielmini P, Puntoni M, Perdelli L, Papadia A, Lorenzi P, et al. HER2/neu oncoprotein overexpression in epithelial ovarian cancer: evaluation of its prevalence and prognostic significance. Clinical study. *Oncology.* 2005;68(2-3):154-61.
35. Mayr D, Kanitz V, Amann G, Engel J, Burges A, Löhrs U, et al. HER-2/neu gene amplification in ovarian tumours: a comprehensive immunohistochemical and FISH analysis on tissue microarrays. *Histopathology.* 2006;48(2):149-56.
36. Tuefferd M, Couturier J, Penault-Llorca F, Vincent-Salomon A, Broët P, Guastalla JP, et al. HER2 status in ovarian carcinomas: a multicenter GINECO study of 320 patients. *PLoS One.* 2007;2(11):e1138.
37. Steffensen KD, Waldstrøm M, Brandslund I, Jakobsen A. Prognostic impact of prechemotherapy serum levels of HER2, CA125, and HE4 in ovarian cancer patients. *Int J Gynecol Cancer.* 2011;21(6):1040-7. doi: 10.1097/IGC.0b013e31821e052e.
38. Vermeij J, Teugels E, Bourgain C, Xiangming J, in 't Veld P, Ghislain V, et al. Genomic activation of the EGFR and HER2-neu genes in a significant proportion of invasive epithelial ovarian cancers. *BMC Cancer.* 2008;8:3. doi: 10.1186/1471-2407-8-3.
39. Hoopmann M, Sachse K, Valter MM, Becker M, Neumann R, Ortmann M, et al. Serological and immunohistochemical HER-2/neu statuses do not correlate and lack prognostic value for ovarian cancer patients. *Eur J Cancer Care (Engl).* 2010;19(6):809-15. doi: 10.1111/j.1365-2354.2009.01112.x.
40. Kadkhodayan S, Ghaffarzadegan K, Homaee F, Esmaeily H, Torabi SH. Expression of HER-2/neu and P16 in epithelial ovarian tumors and its correlation with clinicopathologic variables. [Article in Persian] *Iran J Obstetrics Gynecol Infertil.* 2015;17(133):1-7.
41. Omar N, Yan B, Salto-Tellez M. HER2: An emerging biomarker in non-breast and non-gastric cancers. *Pathogenesis.* 2015; 2(3):1-9
42. McCaughan H, Um I, Langdon SP, Harrison DJ, Faratian D. HER2 expression in ovarian carcinoma: caution and complexity in biomarker analysis. *J Clin Pathol*. 2012;65(7):670-1; author reply 671-2. doi: 10.1136/jclinpath-2011-200616.
43. English DP, Roque DM, Santin AD. HER2 expression beyond breast cancer: therapeutic implications for gynecologic malignancies. *Mol Diagn Ther.* 2013;17(2):85-99. doi: 10.1007/s40291-013-0024-9.