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HER2/neu and Ki-67 as prognostic factors in serous type ovarian carcinoma

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ABSTRACT

Introduction: Serous type ovarian carcinoma occurs in about 59% of all ovary's malignant tumors. The high incidence of advanced ovarian carcinoma at the time of diagnosis causes 5-year survival rate of only about 20%. Thus deep science in predictive biomarkers of prognosis is needed, which later could use in therapeutic considerations. Human epidermal growth factor receptor-2 (HER2/neu) is a proto-oncogene that is involved in cell proliferation, differentiation, and apoptosis. Ki-67 is a marker of proliferative activity that plays an essential role in tumor aggressiveness. This study aimed to prove that HER2/neu and Ki-67 have a role as a prognostic factor in serous type ovarian carcinoma.

Methods: The study design was an analytic cross-sectional study, with total sample were 36 samples from the patient's surgery specimen of serous type ovarian carcinoma, examined at Anatomical Pathology Laboratory Faculty of Medicine, *Universitas Udayana*/Sanglah General Hospital, Denpasar. Histopathological diagnosis for the type of malignancy, grade, and pathological stage according to tumor size was determined on H & E stain. The expression of HER2/neu and Ki-67 was examined with immunohistochemical stain. The correlation between HER2/neu and Ki-67 expression with the degree of differentiation and tumor size was analyzed by the chi-square test, with $p<0.05$ was significant.

Result: The mean of the age was 49.6 ± 11.28, with range was between 26-64 years. The most were in the age group 51-60 years (38.9%). No significant correlation was found between HER2/neu expression with the degree of differentiation ($\alpha = 0.178$) and tumor size ($\alpha = 0.264$). A significant correlation was found between Ki-67 expression with the degree of differentiation ($\alpha = 0.019$) and tumor size ($\alpha = 0.039$).

Conclusion: In this study, Ki-67 can be considered for its role as a prognostic factor, whereas HER2/neu requires further research, with a larger sample and followed by an examination of gene amplification.

Keywords: HER2/neu, Ki-67, prognostic, ovarian carcinoma, serous type
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INTRODUCTION

Epithelial ovarian cancer is the most common pathologic subtype of all ovarian malignancies. There are several histotypes which affected by cell origin, pathogenesis, biomolecular, risk factors and prognosis.[1] Serous type ovarian carcinoma occurs in about 50% of all ovarian malignant tumors. Serous type ovarian carcinoma are the seventh cancer among women in the world and are the most common cause of death among gynecologic malignancies. The high incidence of advanced cancer at the time of diagnosis, causing 5-year survival rate only around 20%, so that more in-depth knowledge of the predictive biomarkers of prognosis is needed, which can later be used in therapeutic considerations.[2,3]

Human epidermal growth factor receptor-2 (HER2/neu) is a proto-oncogene that is involved in cell proliferation, differentiation, migration, and apoptosis. Ki-67 is a marker of proliferative activity that plays an essential role in tumor aggressiveness. Histological type in clinicopathological features affects the variety of tumor biology expression.[4]

The highest positive expression of HER2 occurs in serous type (29%) and mucinous type (38%), then endometrioid type (20%) and clear cell type (23,1%). High expression of Ki-67 occurs in malignant epithelial ovarian tumors compared with benign and borderline tumors.[4,5] This study was to prove the role of HER2/neu and Ki-67 have a role as a prognostic factor in serous type ovarian carcinoma.

RESEARCH DESIGN AND METHODS

Specimen collection

This study design was a cross-sectional analytic observational study that was conducted during the year 2019. The samples of this study were surgical tissue of patients with serous type ovarian carcinoma, whose tissues were examined at Anatomical Pathology Department Faculty of Medicine, *Universitas Udayana*/Sanglah General Hospital, which corresponds to inclusion and exclusion criteria. New patients and never received chemotherapy or radiotherapy were

included in this study. We excluded the specimens containing a lot of necrotic and hemorrhaged tissue; and damaged paraffin blocks. Samples were collected with consecutive sampling, according to the calculation of the sample was 36 samples. This study has permitted by the ethical committee of faculty of medicine, *Universitas Udayana*, with letter number No. 1314/UN14.2.2.VII.14/LP/2019.

Histopathological and Immunohistochemical of HER2/neu and Ki-67 Examination

This study was conducted at the Anatomical Pathology Department Faculty of Medicine, *Universitas Udayana*/Sanglah General Hospital. The specimens were processed and then stained with conventional stain Hematoxylin and Eosin. A histopathologic examination was performed to determine the type of carcinoma. We examined the HER2/neu and Ki-67 expression by immunohistochemically. Their expression got from surgery tissue of histologically diagnosed patient with serous type ovarian carcinoma. The Cell Marque Rabbit Monoclonal Antibody Her2/neu (EP3) and Ki-67 (SPG) Vantagebio were used.

Statistical Analysis

A descriptive characteristic of the data subject was tabulated. The Kolmogorov-Smirnov Test was performed to determine the normality of the data, and the Levene-T Test was performed to find homogeneity and equality. Statistical analysis Pearson chi-square was performed to determine the correlation between HER2/neu and Ki-67 expression with the degree of differentiation and the size of the tumor. Statistical significance for this test was set at 2-sided of 0.05 levels with 95% convidence interval (CIs).

Table 1 Distribution Characteristics of The Sample

Variables		Number	
		n (36)	% (100)
Age	Mean±SD	49.6±11.28	
	21-30	3	8.3
	31-40	4	11.1
	41-50	9	25.0
	51-60	14	38.9
	61-70	6	16.7
Degree of Differentiation	Grade 1	14	38.9
	Grade 2	8	22.2
	Grade 3	14	38.9
Size of Tumor	T1	13	36.1
	T2	10	27.8
	T3	13	36.1

RESULTS

The age range of the study samples was between 26-64 years, with a mean was 49.6±11.28. Most samples were in the 51-60 years group (38.9%). Based on the degree of differentiation, grade 1 and grade 3 have a similar and the most number (38.9% at each). Also, based on tumor size, T1 and T3 have identical and the most number (36.1% at each) (Table 1).

Based on the degree of differentiation, there were negative HER2/neu expression in 32 (88.9%) samples. The most negative HER2/neu expression was found in grade 1 (43.8%) samples, and the most positive was found in grade 2 and grade 3 (50.0% at each). For Ki-67, low expression was 24 (66.7%) samples, and high expression was 12 (33.3%) samples. Grade 1 was found dominant with low Ki-67 expression (54.2%), and grade 3 was found dominant in high-expression (50.0%). HER2/neu expression showed no significant correlation with the degree of differentiation ($\alpha = 0.178$), but contrary with Ki-67 expression ($\alpha = 0.019$) (Table 2).

By size of the tumor, 32 (88.9%) samples were negative HER2/neu expression, and 4 (11.1%) samples were the positive expression. The most negative HER2/neu expression was found in T1 (40.6%), and the most positive was found in T2 and T3 (50.0% at each). Low Ki-67 expression was 24 (66.7%) samples, and high expression was 12 (33.3%) samples. Low Ki-67 expression was dominant in T1 (50.0%), and high Ki-67 expression was dominant in T3 (58.3%). HER2/neu expression showed no significant correlation with size of tumor ($\alpha = 0.264$), but contrary with Ki-67 expression ($\alpha = 0.039$) (Table 3).

Figure 1 shown the sample with H & E staining according to histopathological grade. The results of the negative and positive expression of HER2/neu are shown in Figure 2. The results of low and high expression of Ki-67 expression are shown in Figure 3.

DISCUSSION

Ovarian cancer originating from epithelial occurs in about 90% of cases. Among epithelial ovarian cancers, nonmucinous carcinoma has a high prevalence compared to mucinous carcinoma (3%). Among nonmucinous types, the serous type was found dominant type about 65-70%.[5,15] Epithelial ovarian cancer is also considered an age-related

Table 2 Distribution of HER2/neu and Ki-67 Expression Based on The Degree of Differentiation

Immunohistochemical Expression		Degree of Differentiation (n (%))			Total	*p*
		Grade 1	Grade 2	Grade 3		
HER2/neu	Negative	14 (43.8)	6 (18.8)	12 (37.5)	32 (100)	0.178
	Positive	0 (00.0)	2 (50.0)	2 (50.0)	4 (100)	
Ki-67	Low	13 (54.2)	3 (12.5)	8 (33.3)	24 (100)	0.019
	High	1 (8.3)	5 (41.7)	6 (50.0)	12 (100)	

Table 3 Distribution of HER2/neu and Ki-67 Expression Based on The Size of the Tumor

Immunohistochemical Expression		Size of Tumor (n (%))			Total	*p*
		T1	T2	T3		
HER2/neu	Negative	13 (40.6)	8 (25.0)	11 (34.4)	32 (100)	0.264
	Positive	0 (00.0)	2 (50.0)	2 (50.0)	4 (100)	
Ki-67	Low	12 (50.0)	6 (25.0)	6 (25.0)	24 (100)	0.039
	High	1 (8.3)	4 (33.3)	7 (58.3)	12 (100)	

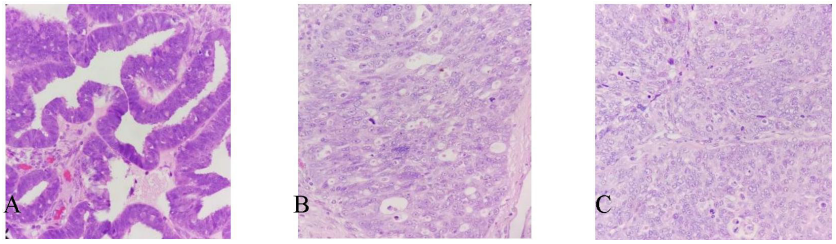

Figure 1 Ovarian Carcinoma serous type (HE, 400×)
A. Grade 1 B. Grade 2 C. Grade 3

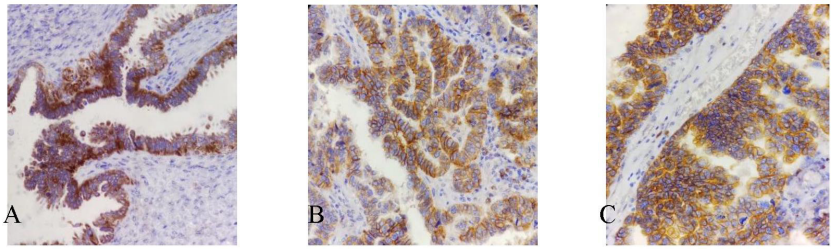

Figure 2 Ovarian Carcinoma serous type (IHC Her2/neu, 400×)
A. Grade 1 (Positive 1) B. Grade 2 (Positive 2)
C. Grade 3 (Positive 3)

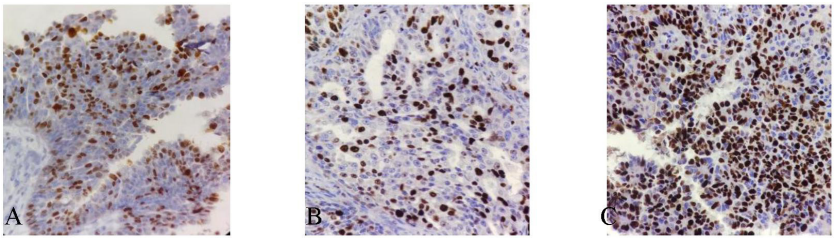

Figure 3 Ovarian Carcinoma serous type (IHC Ki-67, 400×)
A. Grade 1 (Low expression) B. Grade 2 (Low expression)
C. Grade 3 (High expression)

disease and is more often diagnosed with increasing age.[16,17] Epithelial ovarian cancer rarely occurs in premenopausal women, whereas germ cell tumors mainly occur in young women.[17] By using 65 years as the age limit for increasing incidence, in the United States, the incidence <65 years old is 9.34 per 100,000, while at the age >65 years old is 52.7 per 100,000. About 40% patients are ≥ 65 years, some are diagnosed with the late stadium, and constitute two-thirds of cancer mortality.[16] A study by Kim et al. (2019) showed ≥66 years old patients have a worse survival rate than a younger age. About 85% of mortality caused by cancer occur after the age of 55 years.[17,18] The correlation between age ≥ 66 years and worse survival rate is borderline significant.[12] In serous type carcinoma, most occur at the age above 50 years.[5] In this study, the age range of the study samples between 26-64 years, and the most in the age group 51-60 years with a mean was 49.6±11.28.

Of all ovarian epithelial carcinoma, most tumors (75%) show a high degree of differentiation. Among the serous types, there are 76.92% tumors with a high degree of differentiation.[5] In this study, based on the degree of differentiation, most samples were found in grade 2 and 3.

Research by Marinas et al. (2012) obtained tumor size, age, degree of differentiation, and FIGO stage as the main prognostic factors for serous ovarian carcinoma.[3] Among the serous types, 57.5% of cases showed FIGO stage III at the time of diagnosis.[5] In this study, most sample was T1 and T3.

Biologic tumor expression in ovarian cancer also varies, depending on the clinicopathology feature, including histology type.[4] HER2 expression in the normal ovarian epithelium is low, whereas in ovarian cancer is the high expression (8% -66%).[4,12] Nielsen et al. (2004) obtained HER2/neu expression in ovarian carcinoma is 35%. In poorly differentiated carcinomas, the intensity of HER2/neu expression is substantial when compared with

well-differentiated tumors.[3,19] Demir et al. (2014) reported overexpression of HER2 of 18.9-22.4% in ovarian epithelial carcinoma, and more frequently in serous (50%), and mucinous type cancer (12.5%).[2] Tuefferd et al. (2007) obtained positive HER2 in 6.6% of ovarian cancer cases.[12]

HER2 is a tyrosine kinase receptor family member that has role in normal tissue growth and malignancy.[2] HER2/neu gene amplification or overexpression will increase cell growth, DNA damage, and tumor progression. This effect might explain the negative correlation between HER2 expression and patient survival.[4] The prognostic role of HER2/neu in ovarian cancer in previous studies still yields controversial results. Most of the research results show that HER2/neu is a bad predictor for ovarian cancer, while other studies report that HER2/neu does not affect patient survival.[2,4,12] Corkery et al. (2015) showed an association between HER2 and poor survival in serous-type carcinomas,[20] but other studies have shown that HER2 expression was not related to the survival of serous-type ovarian cancer.[21,22] A meta-analysis study conducted by Luo et al. (2019) showed an association between HER2/neu expression and a poor prognosis and could be used as a prognostic biomarker in ovarian cancer.[2,4] Nielsen et al. (2004) obtained a HER2/neu expression related to the degree of tumor differentiation, but not associated with a clinical-stage, age and tumor size.[3,19] There was no correlation between HER2 expression with prognostic factors such as tumor stage, histological type, degree of differentiation, ascites, debulking status, and age.[12]

In this study, the most positive HER2/neu expression were obtained in grades 2 and 3. By tumor size, the most positive HER2/neu expression were obtained at T2 and T3. There was no correlation between HER2/neu expression with the degree of differentiation ($\alpha = 0.178$) and with tumor size ($\alpha = 0.264$). In this study also did not examine gene amplification, and include the results of immunohistochemical 2+ into the negative category.

Ki-67 is a non-histone nuclear protein. Ki-67 expression is a marker of cell proliferation because it is expressed entirely in proliferation tissue and is not expressed in quiescent cells. The Ki-67 gene is located on the long arm of chromosome 10 (10q25). Cell proliferation has an essential role in clinical behavior and the aggressiveness of ovarian carcinoma. Determination of proliferation activity is essential as guidelines for clinical management, as well as diagnostic and prognostic factors.[5]

There was the overexpression of Ki-67 in malignant tumors originating from the ovarian surface epithelium, with a mean of 48.6±26.76 compared to benign and borderline.[5] Research by Marinas et al. (2012) obtained Ki67 proliferation index of 2.1% for benign tumors, 6% at the borderline tumor and 47.7% of malignant ovarian tumors.[3] High Ki-67 expressions correlated with tumor aggression, vascular invasion, tumor metastasis, poorer prognosis and inadequate chemotherapy response.[5]

Among the histological subtypes of ovarian carcinoma, the highest Ki-67 expression was obtained in the serous type, with mean of 65.03±21.67 and the lowest in the transitional type carcinoma. In well-differentiated adenocarcinomas, the Ki-67 proliferation index is below 50%. In contrast, all tumors with low differentiation have a Ki-67 proliferation index above 50%.[3] In serous type, there was statistically significant between high Ki-67 expression with tumors that have a high degree of differentiation (65.34%) compared with tumors with a low degree of differentiation (37.96%). Mean Ki-67 expression in serous types generally higher than the mean non serous types (53.17 ± 13.16). The Ki-67 proliferation index is also associated with advanced stage FIGO (stage III).[5] In this study, the highest high Ki-67 expression was obtained in grade 3, namely 6 (50.0%) samples. With the chi-square statistical test, there was significant correlation between Ki-67 with the degree of differentiation ($\alpha = 0.019$). High Ki-67 expression, mostly obtained at T3, as many as 7 (58.3%) samples. Ki-67 expression was obtained significant correlation with tumor size ($\alpha = 0.039$).

CONCLUSION

In this study, Ki-67 can be considered for its role as a prognostic factor, whereas HER2/neu requires further research, with a larger sample and followed by an examination of gene amplification.

AUTHOR CONTRIBUTION

All authors have contributed to all process in this research, including preparation, data gathering and analysis, drafting and approval for publication of this manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this article.

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