Original Article

# A Comparative Study of HER-2/neu Oncogene in Benign and Malignant Ovarian Tumors

**Sapna Goel[1], Manju Mehra[2], Ajay Yadav[3], Mahak Sharma[4]**

*[1]3rd Year Postgraduate Student, Department of Pathology, SMS Medical College, Jaipur, Rajasthan, India, [2]Professor, Department of Pathology, SMS Medical College, Jaipur, Rajasthan India, [3]Professor & Head of Department, Department of Pathology, SMS Medical College, Jaipur, Rajasthan India, [4]3rd Year Postgraduate Student, Department of Pathology, SMS Medical College, Jaipur, Rajasthan, India*

**Corresponding Author:** *Dr. Sapna Goel, House no. 25/6, Opposite Bal Bhavan, Jind Haryana 126102, Phone no.: +919261696266, E-mail address: dr.sapna02@rediffmail.com*

## Abstract

**Introduction:** Ovarian cancer is the fifth most common malignant cancer and is the most serious disease of female genital tract. The lack of specific symptoms, the relative inaccessibility of the ovaries deep in the pelvis, and the absence of specific marker(s) represent barriers for early detection. HER-2 (human epidermal growth factor receptor-2) proto-oncogene encodes a protein belonging to the EGFR tyrosine kinase receptor family.

**Aims and objectives:** To evaluate the expression of Her-2/neu in ovarian lesions, its relationship to the type of malignancy and correlation with the various clinicopathological parameters, histological grading and staging.

**Materials and methods:** The present study was conducted in the Department of Pathology, SMS Medical College, Jaipur during the year 2012 to 2013 on the 74 consecutive ovarian tumors (33 benign, 4 borderline and 37 malignant) received at histopathology section. The sections were stained by hematoxylin and eosin stain and HER-2/neu immunomarker was applied on each case.

**Observations and Results:** Her-2/neu positivity was seen in 24.3% of ovarian tumors. 48.6% of malignant tumors were Her-2/neu positive and serous adenocarcinoma showing maximum association as compared to other tumors. Her-2/neu expression was significantly associated with tumors in higher grade but had no relation with the age, size and stage of tumor. All the benign and borderline tumors were negative for Her-2/neu.

**Conclusion:** Though stage and grade of a tumor are the most important prognostic indicators, we suggest that Her-2/neu deserves further evaluation as a prognostic marker in epithelial ovarian cancers.

**Keywords:** Her-2/Neu, Markers, Ovarian neoplasms,

## INTRODUCTION

The ovaries are a major endocrine organ, source of female fertility and origin of most complex as well as lethal neoplasms. Ovarian cancer is the fifth most common malignant cancer and is the most serious disease of female genital tract. Approximately 70% of women with ovarian cancer die of this disease. The lack of specific symptoms, the relative inaccessibility of the ovaries deep in the pelvis, and the absence of specific marker(s) represent barriers for early detection. Ovarian cancer includes a broad spectrum of lesions ranging from localized benign tumors to tumors of borderline malignant potential through invasive malignant adenocarcinoma. It is generally impossible to diagnose the nature of the ovarian tumor preoperatively just by clinical examination and even on exploration, though certain investigations like peritoneal fluid cytology, estimation of serum lactic dehydrogenase, fibrin degradation products and immunological tests have been reported to be of some help in predicting the nature of the pathology. The commonest category of the ovarian tumors is epithelial tumors, second commonest being germ cell tumors (GG Swamy and N Satyanarayana 2010).[1]

Among all the ovarian tumors about 80% are benign, out of which 55-65% occur in women less than 40 years of age. Parous women have lower risk as compared to nulliparous women. Etiology is not fully understood although both epidemiological and genetic association has been found. A surgically excised tumor is examined microscopically

and immunohistochemical marker is applied to obtain information which can give clue about prognosis and life expectancy of the patient. HER-2 (human epidermal growth factor receptor-2) proto-oncogene encodes a protein belonging to the EGFR tyrosine kinase receptor family (Coussens et al 1985).[2] Over expression of HER-2 initiates intracellular signaling pathways involved in cell proliferation, differentiation, migration and apoptosis. Amplification or over-expression of this gene has been shown to play an important role in the pathogenesis and progression of certain aggressive types of breast cancer and in recent years it has evolved to become an important biomarker and target of therapy for approx. 30% of breast cancer patients. Over-expression is also known to occur in ovarian (Slamon et al 1989),[3] stomach (Yokota et al 1988)[4] and oral cancer (Xia et al 1997 and Xia et al 1999).[5,6]

The data regarding the expression of HER-2/neu in ovarian tumors is very limited in international as well as Indian literature. Hence in the present study, we evaluated the expression of Her-2/neu in ovarian lesions, its relationship to the type of malignancy and correlation with the various clinicopathological parameters, histological grading and staging.

## MATERIALS AND METHODS

The present study was conducted in the Department of Pathology, SMS Medical College, Jaipur during the year 2012 to 2013 on the 74 consecutive ovarian tumors (33 benign, 4 borderline and 37 malignant) received at histopathology section. The specimens were fixed in 10% formalin for histopathological examination. They were examined grossly according to the standard guidelines, with special emphasis to the size of tumor and presence of capsular breach. Then paraffin embedded tumor section were made in usual manner and thin sections of 5 microns cut by microtome and sections will be stained by haematoxylin and eosin. Mayer's Haematoxylin is used. The Hematoxylin and Eosin stained slides were studied under low power and high power and observations were recorded.

The following parameters were specifically examined:

1. Age of patient: For assessing the relationship between age of patient and Her-2/neu, patients were divided into, with age less than 50 years or more than 50 years.
2. Histologic type: According to WHO classification 2003
3. Histologic grade: Grading was done for epithelial tumors only according to grading proposed by Yoshio and Shimizu et al 1998 [7] on the basis of architectural pattern, nuclear pleomorphism and mitotic activity into grade I, II and III. For assessing association of Her-2/neu with tumor grade tumors were categorized into low grade (I and II) and high grade (III).
4. Tumor stage: Clinical FIGO staging[8] was done on all primary malignant tumors of ovary as per guidelines provided by FIGO society in 2006. Metastatic tumors were excluded. For assessing the association of tumor stage with Her-2/neu expression, tumors were divided into early stage tumors (stage I&II) and tumors with late stage (III and IV).

Representative sections with tumor and the adjacent normal ovarian tissue were processed for HER-2/neu immuno-histochemical staining. A case of Her-2/neu positive Breast carcinoma was kept as positive control.

For HER-2/neu staining, after antigen retrieval, slides were stained with a polyclonal antibody against HER-2/neu (DAKO) oncoprotein by envision system. All the immunostained slides were reviewed and evaluated using following criteria.

### Assessment of the Immunohistochemical Staining for HER-2/neu Overexpression

*Negative expression*

Either no staining or faint to weak membranous positivity in less than 10% of tumor cells was considered Her-2/neu negative

*Positive expression*

Moderate to strong membranous positivity in more than 10% of tumor cells were considered Her-2/neu positive.

## RESULTS

**Table 1: Distribution of ovarian tumors according to Her-2/neu**

| Her-2/neu (n=33) | Benign | Borderline | Malignant |
|---|---|---|---|
| Negative | 33 | 4 | 19 |
| Positive | 0 | 0 | 18 |
| Total | 33 | 4 | 37 |

Above table shows that none of the benign and borderline cases were HER-2/neu positive whereas 48.6% of malignant tumors were Her-2/neu positive while 51.4% were Her-2/neu negative.

**Table 2: Status of Her-2/neu and age**

| Age (years) (n=74) | Her-2/neu negative | Her-2/neu positive | P-value |
|---|---|---|---|
| < 50 | 38 (67.9%) | 12 (66.7%) | 0.927 |
| ≥ 50 | 18 (32.1%) | 6 (33.3%) | Non Significant |
| Total | 56 (75.7% | 18 (24.3%) | |

Among 18 (24.3%) Her-2/neu positive cases, 66.7% were <50 years age and 33.3% were above 50 years while among 75.7% negative cases, 67.9% were present in age less than 50 years and 32.1% in more than 50 years but the difference was not statistically significant. From the above data we can conclude that age had no relation with expression of Her-2/neu.

**Table 3: Status of Her-2/neu and size of tumor**

| Size of tumor | Her-2/neu negative | Her-2/neu positive | P-value |
|---|---|---|---|
| <10 | 22 (39.3%) | 9 (50%) | 0.27 |
| ≥10 | 34 (60.7%) | 9 (50%) | Non Significant |
| Total | 56 (75.7%) | 18 (24.3%) | |

**Table 4: Status of Her-2/neu and classification of ovarian tumors**

| Classification of tumor (n=37) | Her-2/neu status | | No. of cases | P-value |
|---|---|---|---|---|
| | Her-2/neu negative | Her-2/neu positive | | |
| Epithelial Tumors | 10 (39.5%) | 16 (61.5%) | 26 | 0.01 |
| Others (Germ cell and metastatic tumors) | 9 (81.8%) | 2 (18.2%) | 11 | Significant |
| Total | 19 (51.4%) | 18 (48.6%) | 37 | |

Note: Only malignant tumors were included

**Table 5: Status of Her-2/neu and stage of tumor**

| Stage (n=33) | Her-2/neu negative | Her-2/neu positive | P-value |
|---|---|---|---|
| 1 & 2 | 9 (56.3%) | 7 (43.7%) | 0.23 |
| 3 | 6 (35.3%) | 11 (64.7%) | Non significant |
| Total | 15 (45.5%) | 18 (54.5%) | |

**Table 6: Status of Her-2/neu and tumor grade**

| Tumor grade (n=26) | Her-2/neu negative | Her-2/neu positive | P-value |
|---|---|---|---|
| I & II | 7 (63.6%) | 4 (36.4%) | 0.04 |
| III | 3 (20%) | 12 (80%) | Significant |
| Grand Total | 10 | 16 | |

**Table 7: Status of Her-2/neu and Histological type of tumor**

| S. no. | Type of tumor (n=37) | Her-2/neu negative | Her-2/neu positive | P-value |
|---|---|---|---|---|
| 1 | Brenner tumor | 1 (100%) | 0 | 0.007 |
| 2 | Mucinous adenocarcinoma | 1 (33.3%) | 2 (66.7%) | Significant |
| 3 | Poorly differentiated epithelial neoplasm | 2 (66.7%) | 1 (33.3%) | |
| 4 | Serous adenocarcinoma | 5 (27.8)% | 13 (72.2%) | |
| 5 | Endometroid carcinoma | 1 (100%) | 0 | |
| 6 | Dysgerminoma | 1 (100%) | 0 | |
| 7 | Malignant mixed Germ cell tumor | 3 (100%) | 0 | |
| 8 | Yolk Sac tumor | 1 (33.3%) | 2 (66.7%) | |
| 9 | Krukenberg tumor | 4 (100%) | 0 | |
| | Grand Total | 19 | 18 | |

Among 24.3% tumors which were Her-2 positive, 50% had size less than 10 cm and 50% had size more than 10 cm. Among 75.7% cases which were Her-2/neu negative, 39.3% cases had size less than 10 cm and 60.7% had size more than 10 cm but the difference between them was not statistically significant. Hence in our study no association of Her-2/neu was found with the size of tumor.

Out of all epithelial tumors, 61.5% cases were Her-2/neu positive and 39.5% were Her-2/neu negative. In germ cell tumors18.2% cases were Her-2/neu positive and 81.8% were Her-2/neu negative. None of the sex cord and metastatic tumors were Her-2/neu positive. Hence association of epithelial ovarian tumors was more with Her-2/neu than with other ovarian tumors and the difference was statistically significant.

In patients with early stage ovarian cancer, (stage I & II) 43.7% patients were Her-2/neu positive and 56.3% were Her-2/neu negative while in patients with advanced stage ovarian cancer (stage III & IV), 64.7% cases were Her-2/neu positive and 35.3% were Her-2/neu negative but the difference was statistically non significant. This shows that expression of Her-2/neu was not associated with stage of ovarian tumors.

Among low grade tumors, 36.4% were Her-2/neu positive and 63.6% were Her-2/neu negative while in high grade ovarian tumors 80% were Her-2/neu positive and only 20% were Her-2 negative and difference was statistically significant. From this we can conclude that expression of Her-2/neu was more in patients with high grade ovarian tumors.

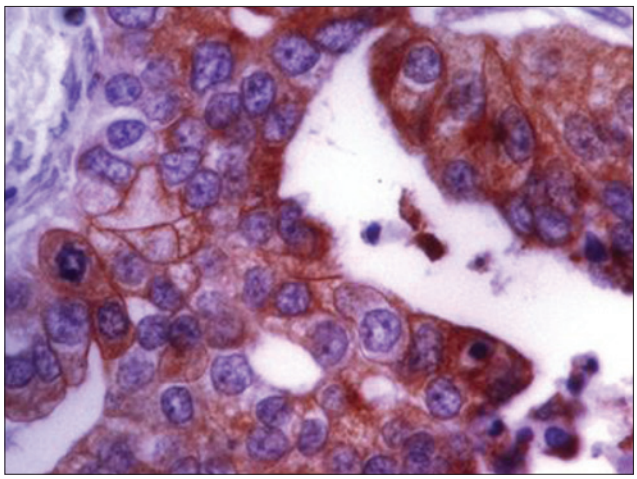

**Figure 1: Microphotograph of Grade III serous adenocarcinoma showing membrane positivity of HER-2/neu**

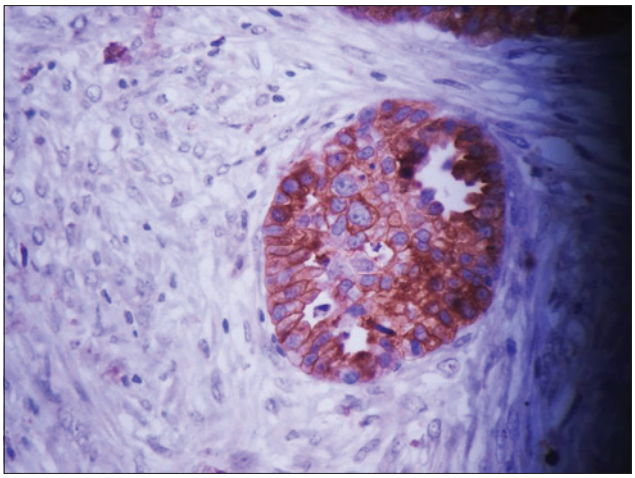

**Figure 2: Microphotograph of Grade III serous adenocarcinoma showing membrane positivity of HER-2/neu**

Out of all studied malignant ovarian tumors, maximum Her-2/neu positivity (72.2%) was present in serous adenocarcinoma and its association with Her-2/neu was statistically significant as compared to others.

## DISCUSSION

The proportion of ovarian cancers overexpressing Her-2/neu is a matter of debate. Various studies have reported that between 5% and 30% of ovarian tumors overexpress Her-2/neu (Hellstrom I *et al* 2001).[9] In this study we evaluated the expression of Her-2/neu in ovarian lesions, its relationship to the type of malignancy and correlation with the clinicopathological factors like age of patient and size of tumor, histological grading and staging, to assess whether Her-2/neu like in breast cancer can be considered as an important prognostic indicator.

In our study 24.3% all ovarian cancers showed Her-2/neu positivity of which none of benign and borderline tumors were positive for Her-2/neu. According to a study done by Kacinski BM et al 1992 on 24 benign, borderline and malignant tumors, only one (4.1%) benign tumor was positive for Her-2/neu.[10] None of borderline tumor was positive. In our study, 48.6% of malignant tumors showed Her-2/neu positivity. The results corresponded to the study of Rubin et al 1993 showing 45.6% positivity in malignant tumors.[11] However Nisha Marwah et al. 2007 showed 38% positivity in malignant ovarian tumors.[12] From these results we can conclude that Her-2/neu is associated more with malignant ovarian tumors than benign or borderline tumors. In our study, no significant association of Her-2/neu was found with the age and size of tumor. Our results were in concordance with several other similar studies Nielsen JS *et al.* 2004 conducted a large study that included 783 ovarian malignant surface epithelial tumors found no correlation of Her-2/neu with prognostic factors like age of patient and size of tumor.[13] Similar results were shown by Sueblinvong T *et al.* 2007 who found no correlation between Her-2/neu and clinicopathologically analyzed factors for 74 cases of surface malignant ovarian tumors.[14] In our study, among all malignant epithelial tumors 61.5% cases were Her-2/neu positive and 39.5% were Her-2/neu negative. In germ cell tumors18.2% cases were Her-2/neu positive and 81.8% are Her-2/neu negative. None of the sex cord and metastatic tumors were Her-2/neu positive. Hence we can conclude that Her-2/neu was statistically significantly associated with epithelial tumors than with other ovarian tumors. Serous adenocarcinoma showed statistically significant association with Her-2/neu among all malignant ovarian tumors with positivity in 72.2% tumors. No significant association was seen with germ cell tumors and sex cord tumors.

Our results were comparable with results of M. C. Marinas *et al.* 2012 who studied 26 benign, borderline and malignant tumors and found that Her-2/neu expression has significant association with serous adenocarcinomas with more intense positivity in high grade serous adenocarcinomas.[15]

In contrary to our study Rubin SC *et al.* 1993 studied 105 patients with advanced epithelial tumors and found no correlation between Her-2/neu and type of tumor. [11] Similar results were shown by Singleton M D *et al.* 2006 on 56 patients with advanced ovarian cancer and found no correlation between the type of tumor and Her-2/neu overexpression. [16] Our results are in concordance with results of several studies done on Non epithelial tumors of ovary. Menczer J et al. 2007 studied 20 patients with non-epithelial ovarian malignancies (12 granulosa cell tumor and 8 germ cell tumor) and found that Her-2/neu was not present in any of these non-epithelial malignancies examined.[17] Histological grading has met with limited clinical acceptance as noted by lack of inclusion of any histological grading system in the classification of ovarian malignancies as adopted by FIGO. Grading of the ovarian tumors is limited to invasive epithelial tumors only.

According to our study, out of total 26 invasive epithelial tumors, we found that 36.4% low grade tumors (Grade I&II) were Her-2/neu positive while 80% of high grade tumors (Grade III and IV) were Her-2/neu positive. Our results were similar to the results of study done by Nisha Marwah et al. 2007 on 75 ovarian tumors (25 benign and 50 malignant).[12] They found that Her-2/neu expression were significantly associated with high grade ovarian tumors. M. C. Marinas *et al.* 2012 studied 26 serous tumors and found that there is statistically significant correlation between high grade (poorly differentiated) serous adenocarcinomas as compared to Her-2/neu expression in low grade (well differentiated) serous carcinomas.[15]

However in contrary, Meden H *et al.* 1992 studied the prognostic significance of Her-2/neu in 243 patients with ovarian cancer and found that Her-2/neu expression had no association with tumor grade and other prognostic indicators.[18]

FIGO stage is the most important prognostic indicator in ovarian tumors. In our study out of 16 (48.5%) patients with early stage ovarian cancer (stage I & II), 43.7% patients were Her-2/neu positive while out of 17 (51.5%) patients with advanced stage ovarian cancer (stage III & IV), 64.7% cases were Her-2/neu positive and 35.3% were Her-2/neu negative but the difference between the two was not statistically significant. From these results we concluded that expression of Her-2/neu can occur in any stage of ovarian cancer.

Hogdall et al. 1998 investigated the overexpression of Her-2/neu from 181 cases of ovarian tumors and studied the overexpression of Her-2/neu in cases from FIGO stage I to IV. [19] However, no statistical correlation was found between the presence of Her-2/neu overexpression and FIGO stage, suggesting that activation of Her-2/neu overexpression can occur both in early and late stages of disease. However in contrary, Seidman JD et al. 1992 conducted a study on 39 serous tumors (20 of low malignant potential) and 19 of serous carcinoma) and found that expression of Her-2/neu may be associated with high stage in serous ovarian neoplasms.[20]

## CONCLUSION

Her-2/neu positivity was seen in 24.3% of ovarian tumors. All the benign and borderline tumors were negative for Her-2/neu. 48.6% of malignant tumors were Her-2/neu positive. Epithelial tumors were significantly associated with Her-2/neu with Serous adenocarcinoma showing maximum association as compared to other tumors. Her-2/neu expression was significantly associated with tumors in higher grade but had no relation with the stage of tumor. No association of Her-2/neu was found with clinical parameters like age of patient and size of tumor.

Though stage and grade of a tumor are the most important prognostic indicators, we suggest that Her-2/neu deserves further evaluation as a prognostic marker in epithelial ovarian cancers.

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