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EGFR and HER2/NEU immunoexpression in ovarian neoplasms in Bundelkhand Region

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***Abstract*---**Introduction: Ovarian Cancers account for the greatest number of deaths from malignancies of female genital tract and it is the fifth leading cause of cancer fatalities in women. Surface epithelial tumours are the most common, followed by germ cell tumours. Objective: To analyse the expression of EGFR and HER 2neu using Immunohistochemisty in different Ovarian tumours with special reference to surface Epithelial tumours. Material and Methods: 52 cases of different ovarian tumours were studied. Cases included total abdominal hysterectomy with bilateral saphingoophorectomy, oophorectomy, and cystectomy specimens. Expression of EGFR (ErbB1) and HER2-neu (ErbB2) was determined by immunohistochemical reactions performed with the Super Sensitive™ IHC Detection system by the Biogenix™. Tests were performed according to the instructions of each kit. Results: The mean age of presentation for epithelial tumours was found to be 42.48 years. For malignant epithelial tumours mean age of presentation was found to be 51.1 years. EGFR positivity was found in 28.57 % of surface epithelial ovarians tumours, and HER2/neu positivity was seen in 20% of surface epithelial ovarian tumours. As far as malignant serous papillary adenocarcinoma is concerned, we found 33.3% positivity for HER2/neu and 50% positivity for EGFR. Conclusion: Positivity of EGFR and HER 2/neu are significantly more in surface epithelial ovarian tumours as compared to non epithelial tumours. Higher intensity of HER2/neu staining was associated more with high grade serous carcinoma as compared to low Grade serous carcinoma.

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Introduction

Ovaries are the female gonads, which plays a major role in the female aspect of reproduction. Ovarian Cancers account for the greatest number of deaths from malignancies of female genital tract and it is the fifth leading cause of cancer fatalities in women.[1.] they represent about 30% of all cancers of female genital organs. There are more than 30 different types of ovarian cancer, which are classified by the type of cells from which they start.

In developed countries it is about as common as cancers of the corpus uteri (35%) and invasive cancer of the cervix (27%).[2] In India, ovarian cancer is the third leading site of cancer among women, trailing behind cervix and breast cancer.[3] Tumours of the ovaries arise ultimately from one of the three ovarian components - Surface Epithelium derived from coelomic epithelium, germ cells (which migrate to the ovaries from the yolk sac and are pleuripotent) and stroma of the ovary.

Out of the these epithelial tumours are the most common, comprising of about 60% of all ovarian tumours[5.]and serous carcinoma being the commonest histological subtype. Ovarian cancer has the worst prognosis among all gynecological malignancies. The overall 5-year survival is approximately 45%, primarily due to the late stage at diagnosis of the disease.[3.]EGFR and its family members play a variety of roles in oncogenesis and tumour progression in different cancer.

Overexpression of the EGFR protein has been detected in 9% to 62 % of Human Ovarian cancers. Increased EGFR expression has been associated with high tumour grades, high cell proliferation index and poor patient outcome.[4.] EGFR and HER2/neu belong to the same family of Growth factors. Ovarian cancer and breast cancer share the overexpression of HER2/neu.

The situation of HER2/neu in ovarian cancer is less clear with contradicting results of HER2/neu expression in the prognosis of the disease. In our study we intend to analyse the expression of EGFR and HER 2neu using Immunohistochemisty in different ovarian tumours with special reference to surface epithelial tumours.

Material and Methods

The study is conducted in the Department of Pathology, Maharani Laxmibai Medical College Jhansi during the year 2015 and 2016. Cases included total abdominal hysterectomy with bilateral saphingoophorectomy, oophorectomy, and cystectomy specimens. Total 52 cases of different ovarian tumours were studied.

Immunohistochemistry

The sections were cut and picked on poly-L-Lysine coated slides. Expression of EGFR (ErbB1) and HER2-neu (ErbB2) was determined by immunohistochemical reactions performed with the Super Sensitive™ IHC Detection system by the Biogenix™. Tests were performed according to the instructions of each kit. This Detection System uses the streptavidin-Biotin Technology wherein the biotinylated secondary antibody which is bound to primary antibody reacts with enzyme labelled streptavidin and is then visualised by a chromogen. The Super sensitive ™* Polymer HRP Detection System uses a non Biotin polymeric technology wherein the secondary antibody conjugated to polymer HRP reagent is bound to the primary antibody and is then visualised by the chromogen. These detection systemsmay be used in immunohistochemical applications manually, or using BioGenex automated Staining Systems.

The EGFR staining was scored based on membrane staining intensity – 0 for no staining, 1 for faint cytoplasmic staining in > 10% tumour cells, 2 for moderate membranous staining and 3 for strong membranous staining. *HER2* positivity was assessed using Ellis and Wolff recommendations. A score of 1+ was defined as barely perceptible membrane staining in more than 10% of cells, a score of 2+ was defined as weak-to-moderate complete membrane with staining present in more than 10% of tumor cells, and a score of 3+ was defined as strong complete membrane staining in more than 10% of tumor cells. We classified 2+ as equivocal and 3+ as positive. Cytoplasmic staining was considered to be non-specific.

Statistical analysis

At the End of the study, all the data were subjected to statistical analysis, using statistical package for social sciences (SPSS) software, version 20.

Results

Sections from 52 formalin fixed and paraffin embedded surgical specimen of benign, borderline as well as malignant ovarian neoplasms were screened to grade as well as classify the tumours. These cases were then assessed for the immunoexpression of EGFR and HER2/neu using Super Sensitive™* IHC Detection System by BioGenex.

Table 1
Distribution of cases according to Histological type

	Benign	Borderline	Malignant
Epithelial tumours	22	4	9
Germ cell tumours	11	0	2
Sex cord stromal tumours	0	0	2
Metastatic tumours	0	0	2

Table 2
EGFR and HER 2/neu Immunoreactivity in Surface Epithelial tumours (n=35)

	Benign	Borderline	Malignant
EGFR			
Positive	5	1	4
Negative	17	3	5
HER 2 /neu			
Positive	4	1	3
Negative	18	3	6

Table 3
EGFR and HER2/neu positivity in surface epithelial tumours

	Positivity (%)	Negativity (%)
EGFR Immunoexpression	28.57	71.43
HER2/neu Immunoexpression	20.0	80.0%

Table 4
EGFR expression and HER 2/neu immunoexpression in Different Histological types of Ovarian Neoplasms. (n=35)

	Epithelial Tumours	Germ cell tumours	Sex Cord stomal tumours.
EGFR Expression			
Positivity (%)	28.57	0.7	0
Negativity (%)	71.43	99.3	100
HER 2/neu			
Positivity (%)	20%	0.7%	0%
Negativity (%)	80%	99.3%	100%

Table 5
EGFR and HER2/neu Expression in ovarian Surface epithelial tumours

	Benign	Borderline	Malignant
EGFR positivity	5	1	4
HER2/neu Positivity	4	1	3

Table 6
EGFR immunostaining intensity in Surface Epithelial tumours

EGFR staining	Benign	Borderline	Malignant
0	16	2	4
1+	2	1	1
2+	5	1	2
3+	0	0	2

Table 7
HER2/neu immunostaining intensity in Surface Epithelial tumours

	Benign	Borderline	Malignant
0	17	2	5
1+	1	1	1
2+	4	1	1
3+	0	0	2

Discussion

Compared to the 20–30% rate of *HER2*/neupositivity observed in breast cancers.[5] the rate in ovarian cancer is lower and intratumoral heterogeneity is frequently detected. The good concordance between *HER2/neu*status in primary tumour and corresponding distant locations suggests that *HER2/neu* clonal selection occurs before tumor dissemination. It is worth noting that some tumours showed the same heterogeneous pattern in both primary and distant locations with adjacent positive and negative areas, detected by both IHC and FISH methods. In ovarian cancer, the prognostic influence of HER2/neu is still a matter of debate and the therapeutic capacity of the available drugs to target the HER2/neu pathway is insufficiently explored.

In our study, out of all epithelial ovarian tumours (N=35), 20% were found to be HER2/neu positive and 80 % were negative for HER2/neu. Amongst all germ cell tumours only one case was Positive for HER2/neu. Both sex cord stromal tumours as well as metastatic tumours were negative for both HER2/neu as well as EGFR. Hence we found that HER2/neu positively is seen more with Epithelial ovarian neoplasms as compared to the non-epithelial counterparts.

Our results were comparable to that of study conducted by S Goel et al., however their overall HER 2/neu positivity for epithelial ovarian tumours was 61.5% cases.[6] The reason being, their study included 74 consecutive cases of ovarian neoplasms, out of which 37 cases were malignant,33 benign and 4 borderline. The number of malignant cases clearly outnumbered our proportion of malignant cases. Hence, the positivity was far greater than our study. Out of the three HER 2/neu positive cases of serous papillary adenocarcinoma, two were of high grade serous carcinoma, and one low grade serous carcinoma. Hence the high grade tumours were associated more with HER2/neu positivity. The only malignant Brenner tumour was both HER 2/neu as well as EGFR negative.

Over all positivity for HER 2/neu among malignant epithelial tumours was found to be 33.3% which is comparable to the study by MA Ajani et al who found HER2 /neu positivity in 37% cases. They also mentioned the rate of HER2/neu positivity in Epithelial ovarian cancers reported in the literature which ranges from 7-50%. [7]

Manisha Sarkar et al also concluded in their study that serous type is the most common type of epithelial ovarian tumours. Grade 3 HER2/neu positivity was associated more with advanced disease.[8]
Verri E et al. in their study found HER 2/neu over expression in 53 out of 194 investigated cases and concluded that HER 2/neu over expression is associated with increased risk of progression and Death.[9] In contrary to our study Rubin S C

et al who studied 105 patients with advanced epithelial tumours found no correlation between HER 2/neu and type of tumours.[10] Likewise Singleton MD et al also found no correlation between the type of tumours and HER 2/neu expresson.[11]

Increased EGFR expression has been associated with high tumour grade and high proliferation index, however prognostic impact of EGFR expression in ovarian cancers still remains controversial.[12] In our study, we found EGFR expression in 5 benign, 1 borderline and 4 malignant epithelial ovarian tumours. Over all EGFR positivity in epithelial ovarian tumours was found to be 28.57 %. This was greater as compared to HER 2/neu positivity in the same cases. Psyrri et al found that there was a correlation between EGFR over expression and ovarian cancer patient survival.[13] However Denis et al found EGFR expression was more frequent in advanced tumours but not related to poorer outcome of patients.[14]

Ke Wang et al. in their study found that high levels of EGFR expression in tumour stroma were associated with aggressive clinical conditions in epithelial ovarian cancer and high expression of EGFR in tumour stroma was an independent prognostic factor for epithelial ovarian cancer patients. a significant positive correlation was seen between the expression levels of EGFR in tumour stroma and the expression levels of Ki67 in tumour cells. Over expression of EGFR in tumour stroma was associated with aggressive clinical condition in ovarian cancers.[12]

As per study by Rossela De et al. malignant Brenner tumours give strong positivity for EGFR on contrary our only case of malignant Brenner tumour was negative for both EGFR and HER2/neu.[15] The germ cell tumours came out to be both EGFR and HER2/neu negative. As summarised by Ignacio Duran et al EGFR and HER2/neu are expressed in a small proportion of Germ cell tumours. The expression of these markers have no prognostic or predictive significance. [16]

Skirnisdóttir et al. studied that Co-expression of HER-2/neu and EGFR was most frequently seen in serous tumors and positive staining for HER-2/neu alone was associated with mucinous tumors. Both endometrioid and clear cell tumours belonged to the largest subgroup with concomitant negativity for both HER-2/neu and EGFR. In a multivariate Cox analysis, the tumour grade and EGFR status of the tumours were independent and significant prognostic factors. A therapeutic strategy for epithelial ovarian cancer might be to decrease EGFR expression by gene therapy in combination with adjuvant radiotherapy or chemotherapy[17]

Hogdall EV et al concluded that HER-2 overexpression has prognostic value both in univariate and multivariate survival analyses. Therefore, the clinical relevance of their observation should be established conclusively by therapy that targets HER-2 in a prospective Phase II clinical trial.[18]

Literature data on EGFR and HER2/neu immunoexpression are controversial as regards association with clinical and histopathological prognostic factors. Nielsen JS et al. found HER2/neu and EGFR over expression in 35% and 62 % of cases respectively. They indicated the association of HER2/neu expression with tumour grade and no other correlation with clinical stage or prognostic factors (age, size

FIGO stage). As far as our study is concern due to lack of adequate data, we were not able to classify our cases according to FIGO classification. Their team proposed that the panel consisting of EGFR, HER2/neu and P53 tumours may provide prognostic information for borderline tumours.[19]

Lassus H et al. indicate EGFR protein overexpression in 17% of serous ovarian adenocarcinomas and association with tumour grade, residual tumour size and patient age.[20] In a study conducted in 2008 on a cohort of 50 serous ovarian carcinomas, found that 64% of lesions were positive to EGFR, in correlation with tumour grade and survival.[21]

Meta-analysis conducted by P De Graeff et al. showed a significant relationship between over expression of EGFR and poor patient outcome, although significant heterogeneity was present. The meta-analysis also showed overexpression of HER 2/neu was associated with poor overall survival[22].

Conclusion

Positivity of EGFR and HER 2/neu are significantly more in surface epithelial ovarian tumours as compared to non epithelial tumours. Higher intensity of HER2/neu staining was associate more with high grade serous carcinoma as compared to low Grade serous carcinoma. EGFR and HER2/neuimmunoexpression is not significant in Germ cell tumours and Sex cord Stromal tumours. Malignant surface epithelial tumours are associated with higher intensity of staining with both EGFR and HER2/neu as compared to benign counterparts.

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