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Original Article

Evaluation of prevalence and prognostic significance of human epidermal growth factor receptor 2/neu expression in carcinoma endometrium

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ABSTRACT

Context: Endometrial cancer is a frequently encountered gynecological malignancy. An adverse prognostic immunohistochemical marker had not been identified until recently. Recent studies have used human epidermal growth factor receptor 2 (HER2)/neu expression as a marker for adverse outcomes in carcinoma endometrium, as proven in carcinoma breast.

Aim: The present study was undertaken to correlate HER2/neu expression with prognostic factors in cases of endometrial carcinoma.

Materials and Methods: This was a prospective study on evaluation of HER2/neu expression done on 30 patients with biopsy-proven carcinoma endometrium admitted in the hospital for staging laparotomy. Histopathological examination was performed and HER2/neu expression was assessed by immunohistochemistry and was categorized into (1) Group 1: HER2/neu 0/1+ (negative), (2) Group 2: HER2/neu 2+ (equivocal), and (3) Group 3: HER2/neu 3+ (positive). All the cases were followed up for a period of 1 year.

Statistical Analysis: All data analyses were carried out using statistical software SPSS, IBM version 21.0. $P < 0.05$ was considered as significant.

Results: 0/1+ HER2/neu expression (negative) was detected in 19 patients (63.33%), 2+ HER2/neu expression (equivocal) was detected in 4 patients (13.33%), and 3+ HER2/neu expression (positive) was detected in 7 patients (23.33%). HER2/neu 3+ group was significantly associated with non-endometrioid histology ($P = 0.003$) and high tumor grade ($P = 0.005$). The association of HER2/neu 3+ group with advanced disease and lymph node involvement showed a trend, but it was not statistically significant. No association was found between HER2/neu overexpression and extent of myometrial invasion. There were two recurrences in 1 year follow-up period. HER2/neu expression (2+ and 3+) was seen the recurrence cases.

Conclusion: HER2/neu overexpression was found to be an adverse prognostic marker in carcinoma endometrium, which could form the basis of targeted chemotherapy.

Keywords: Carcinoma endometrium, Human epidermal growth factor receptor 2/neu expression, Grade, Tumor

INTRODUCTION

Endometrial cancer is a frequently encountered gynecological malignancy. In the USA, it is the most common gynecological malignancy and the fourth most common cancer.[1] In India, it ranks third after carcinoma cervix and carcinoma ovary.[2]

There are two pathogenic types of carcinoma endometrium – Type I and Type II: Majority being Type 1 tumors (80–90%). Endometrial tissue histology confirms the diagnosis of carcinoma endometrium.[3,4]

An adverse prognostic immunohistochemical marker for carcinoma endometrium had not been identified until recently. Recent studies suggest the role of human epidermal growth factor receptor 2 (HER2)/neu receptor as an adverse prognostic marker for carcinoma endometrium. HER2/neu is a protein that is encoded by erbB2 gene. This marker is widely used in carcinoma breast histological study. The pathogenetic role and prognostic significance of HER2/neu in endometrial carcinomas have recently become the focus of research.

Following the successful development of targeted therapy against HER2/neu in breast cancer, reports on HER2/neu overexpression have sparked considerable interest in a potential novel HER2/neu based therapy in endometrial carcinoma. At present, there is a need to develop standardized criteria for HER2/neu detection in carcinoma endometrium.

The present study was undertaken to correlate HER2/neu expression as estimated by immunohistochemistry with prognosis in cases of endometrial carcinoma treated during the study period.

MATERIALS AND METHODS

This was a prospective study on evaluation of HER2/neu expression in carcinoma endometrium, conducted in the Department of Obstetrics and Gynaecology, Pathology, and Radiotherapy of our institute. Ethical clearance was taken from the Institute Ethics Committee.

Thirty new patients with carcinoma endometrium who were admitted to the hospital for staging laparotomy and were willing to participate in the study were included in the study. Patients with other gynecological malignancies and carcinoma breast, not willing to participate, and who received prior treatment were excluded from the study.

Informed consent was taken from all 30 patients. After taking a detailed history of patients, general, systemic, per speculum, per rectal, per vaginal, and per abdominal examination was performed. Investigations performed on patients included complete blood count, renal and liver function tests, and blood sugar: Fasting and postprandial, serum electrolytes, electrocardiogram, chest X-ray, ultrasound abdomen/pelvis, contrast-enhanced computed tomography (CECT)/magnetic resonance imaging abdomen/pelvis, and serum cancer antigen (CA)-125 levels. All patients underwent staging laparotomy. Formalin-fixed paraffin-embedded blocks of hysterectomy specimens were retrieved, and 4 μ thick sections obtained from these blocks were stained with routine Hematoxylin and Eosin stains. Histology, grade, myometrial invasion, lymph node involvement, and stage of endometrial carcinoma were assessed. HER2/neu expression was assessed in all hysterectomy specimens by immunohistochemistry. HER2/neu expression scoring was done using guidelines by the American Society of Clinical Oncology and the College of American Pathologists (2018) for HER2/neu expression in carcinoma breast [Table 1].[5]

All patients were followed up at 1 month, 3 month, 6 months, and 1 year after surgery. At each visit detailed history and clinical examination were done. Pap smear was taken at 3 months, 6 months, and 1 year. If any suspicious findings were noted on any visit, in a patient CECT abdomen/pelvis was done. CECT abdomen/pelvis was routinely performed in all the patients, 6 months after surgery. Details regarding adjuvant treatment (radiotherapy/chemotherapy) were noted (if received).

All data analyses were carried out using statistical software SPSS, IBM version 21.0. For outcome variables in case of frequency data, Chi-square test or Fischer-exact test was used to compare the categories. Continuous variables were tested for normality assumption using appropriate statistics. Means of groups were compared using one way ANOVA. In case of non-normal distribution, median values were computed by Kruskal–Wallis nonparametric test. For all statistical tests, a probability of $P < 0.05$ was considered as statistically significant.

RESULTS

Age of patients ranged from 33 years to 76 years. Mean age was 58.47 years. Mean body mass index (BMI) was 27.7 kg/m^2. Parity of majority of patients (33.33%) was para 2. Mean age of menarche was 12.47 years. Majority

Table 1: ASCO/CAP (2007) HER2/neu scoring guidelines.

Scoring	HER2/neu expression characteristics
0	Reaction absent or present in <10% of tumor cells
1+	Weak interrupted membrane reaction in >10% of the tumor cells
2+	Weak moderate, continuous membrane reaction, in >10% of the tumor cells intense, continuous membrane reaction in ≤30% of the tumor cells
3+	Intense, continuous membrane reaction in >30% of the tumor cells

ASCO/CAP: American Society of Clinical Oncology and the College of American Pathologists, HER2: Human epidermal growth factor receptor 2

of the patients (83.3%) were postmenopausal; mean age at menopause was 51.46 years. Most common presenting complaint was postmenopausal bleeding (73.33%) followed by menorrhagia (20%) and vaginal discharge (6.67%). In the majority of cases (66.7%), duration of symptom was <6 months. Hypertension was the most common comorbidity present in 50% of the patients followed by obesity (23.33%) and diabetes (20%). Majority of patients (76.67%) had CA-125 <35 U/ml. Significant correlation was seen between the CA-125 and surgicopathological stage of disease ($P < 0.001$). In 60% of the cases, myometrial invasion was <½ of the myometrium. Endometrioid adenocarcinoma was the most common histopathological finding present in 66.67% of the patients [Table 2]. Pelvic lymph node involvement was noted in 16.67%. No patients showed paraaortic lymph node involvement. Table 2 also depicts the histological grading and FIGO staging in the study patients.

Of total patients, 53.33% required no adjuvant treatment. Radiotherapy was received by 16.67% of patients and 30% of patients received both chemotherapy and radiotherapy.

Recurrence occurred in two patients – one patient had endometrioid, Grade 1, Stage IA (HER2/neu 2+) endometrial carcinoma. She developed vault recurrence. The other patients had papillary serous carcinoma, Grade 3, Stage IIIC1 (HER2/neu 3+). She developed recurrence in the pelvic and para-aortic lymph nodes. Of the 30 patients, one patient died due to gangliothalamic infarct due to low platelet count during adjuvant chemotherapy.

On immunohistochemistry, positive HER2/neu overexpression (3+) was detected in 23.33% of patients [Table 2]. Figure 1 shows the microscopic view of histology slides showing HER2/neu staining patterns obtained by immunohistochemistry. Based on HER2/neu expression, the study patients were grouped into three categories according to ASCO/CAP (2018) guidelines: [5] (1) Group 1: HER2/neu 0/1+ (negative), (2) Group 2: HER2/neu 2+ (equivocal), and (3) Group 3: HER2/neu 3+ (positive).

These three groups were compared on various criteria. Age and BMI were comparable amongst the three groups ($P = 0.104$ and $P = 0.148$, respectively). Advanced disease was more common in HER2/neu 3+ cases compared to HER2/neu 0/1+ and 2+, but the difference was not statistically significant ($P = 0.251$). Myometrial invasion >½ was comparable between HER2/neu 0/1+ group and 3+ group ($P = 0.263$). Lymph node metastasis was more common in HER2/neu 3+ group, but the difference was not statistically significant ($P = 0.327$).

The three groups were compared in terms of histopathology – endometrioid and non-endometrioid (papillary serous and clear cell). In HER2/neu 3+ group, 85.71% of patients had non-endometrioid histopathology (one had clear cell histopathology and five were papillary serous on histopathology). In comparison, in Group 1 – 21.05% of patients and in Group 2 – none of the patients had non-endometrioid histopathology and this difference was statistically significant ($P = 0.003$). Grade 3 tumors were significantly more common in HER2/neu 3+ group ($P = 0.005$) [Table 3].

Table 2: Pathological characteristics of carcinoma endometrium patients.

Pathological feature	*n* (%)
Myometrial invasion	
No myometrial invasion	2 (6.67)
<½ myometrial invasion	18 (60.00)
>½ myometrial invasion	10 (33.33)
Histopathology	
Endometrioid adenocarcinoma	20 (66.67)
Papillary serous carcinoma	8 (26.67)
Clear cell carcinoma	2 (6.67)
Histological grade	
1	11 (36.67)
2	8 (26.67)
3	11 (36.67)
FIGO stage	
IA	18 (60.00)
IB	4 (13.33)
II	1 (3.33)
IIIA	2 (6.67)
IIIC1	5 (16.67)
HER2/neu expression	
0/1+	19 (63.33)
2+	4 (13.33)
3+	7 (23.33)

HER2: Human epidermal growth factor receptor 2

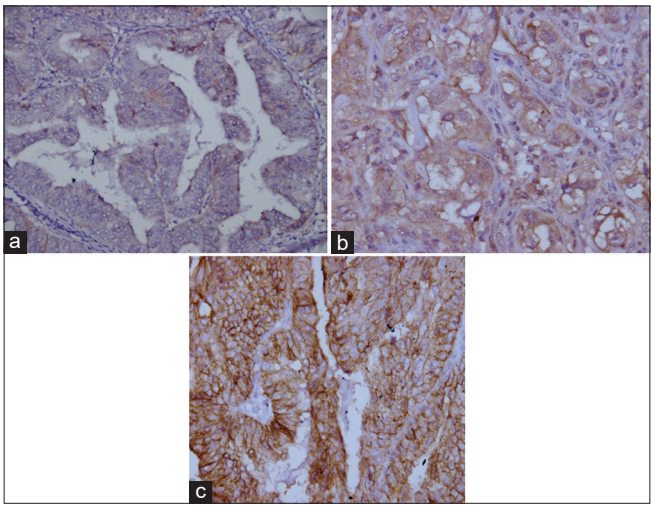

Figure 1: (a) 0 human epidermal growth factor receptor 2 (HER2)/neu expression (negative). (b) 2+ HER2/neu expression (equivocal). (c) 3+ HER2/neu expression (positive).

Table 3: Comparison of various parameters in relation to HER2/neu expression.

Parameter	HER2/neu 0/1+ (%)	HER2/neu 2+ (%)	HER2/neu 3+ (%)	*P* value
Stage				
Stage I and II	15 (78.95)	4 (100)	4 (57.14)	0.251
Stage III	4 (21.05)	0 (0)	3 (42.86)	0.251
Myometrial invasion				
No or <1/2	11 (57.89)	4 (100)	4 (57.14)	0.263
>1/2	8 (42.11)	0 (0)	3 (42.86)	0.263
Lymph node involvement				
Absent	17 (89.47)	4 (100)	3 (57.14)	0.092
Present	2 (10.53)	0 (0)	4 (42.86)	0.092
Histopathology				
Non-endometrioid	4 (21.05)	0 (0)	6 (85.71)	0.003
Endometrioid	15 (78.95)	4 (100)	1 (14.29)	0.003
Histological grade				
Grade ½	14 (73.68)	4 (100)	1 (14.29)	0.005
Grade 3	5 (26.32)	0 (0)	6 (85.71)	0.005

HER2: Human epidermal growth factor receptor 2

DISCUSSION

In our study, we evaluated 30 cases of endometrial carcinoma. All these cases underwent surgical staging. Following this, HER2/neu expression was studied by immunohistochemistry on tissue obtained from the hysterectomy specimen. HER2/neu expression of the cases was then correlated with the prognostic factors of endometrial carcinoma.

The most important parameters that provide an opinion regarding the potential clinical and biological behavior of endometrial cancer are age, hormonal status, and other molecular markers including HER2/neu, P53, PTEN, and microsatellite instability.[6]

In present study, the mean age of the 30 endometrial carcinoma cases was 58.47 years. In studies by Morrison *et al.*, Srijaipracharoen *et al.*, Togami *et al.*, and Lapińska-Szumczyk *et al.*, the mean age of endometrial carcinoma patients was 64 years, 62 years, 56.3 years, and 63.6 years, respectively.[7-10]

In the present study, the mean BMI of patients of endometrial carcinoma was 27.7 kg/m^2. In a study by Everett *et al.*, the mean BMI was 34 kg/m^2.[11] Modesiit *et al.* and Dossus *et al.* reported that the mean BMI in their studies was 29.8 kg/m^2 and 26.5 kg/m^2, respectively.[12,13]

In the present study, the mean age at menarche was 12.47 years. Of all patients, 83.3% of patients were postmenopausal. The mean age of menopause was 51.46 years. In study by Lapińska-Szumczyk *et al.*, number of postmenopausal, premenopausal, and perimenopausal women was 86.33%, 7.0%, and 6.5%, respectively.[10] Dossus *et al.* reported that the mean age at menarche was 13 years. The number of premenopausal patients was 26.2% and 73.8% of patients were postmenopausal. The mean age at menopause was 50.9 years.[13]

In the present study, 50% of patients were hypertensive, 23.33% of patients were obese, and 20% of patients were diabetic. In a study by Anderson *et al.*, 10% of patients were diabetic and 45.4% of patients were hypertensive.[14] Lucenteforte *et al.* found that compared to nonobese nondiabetic women, the relative risk for developing endometrial cancer in nonobese diabetic women was 1.4 and increased to 5.1 for obese diabetic women.[15] Comorbidities are known predisposing risk factors in tumor pathogenesis.[6]

A significant correlation was seen between pre-operative CA-125 and surgicopathological stage of disease ($P < 0.001$). In a study by Dotters, CA 125 levels of >35 U/mL were strongly predictive of extrauterine disease.[16] In a similar study by Sebastianelli *et al.*, a statistically higher number of patients from the Stages III or IV group had a serum CA-125 level ≥35 U/L (58%) compared to the Stages I or II group (16%) ($P < 0.001$).[17]

In addition to many clinical and pathological features, some of the biological molecules are suggested as prognostic biomarkers in endometrial carcinoma. Of these, the estrogen and progesterone receptors are significant in predicting the hormone therapy response and prognosis. The HER2/neu overexpression and amplification have been well known in the pathogenesis and targeted therapy of breast cancer and recently in gastric and gastroesophageal junction cancers. Overexpression in endometrial carcinoma has been mentioned in endometrial serous cancer and is related to worse outcome. There is an increase in interest for development of effective new therapies against biologically aggressive tumors.[6]

In the present study, 0/1+ HER2/neu expression was detected in 63.33% of patients, 2+ HER2/neu expression was detected in 13.33% of patients, and 3+HER2/neu expression was

detected in 23.33% of patients on immunohistochemistry. In a study by Kato *et al.*, on 36 cases of carcinoma endometrium 0/1+ HER2/neu expression was detected in 24 patients (66.67%), 2+ HER2/neu expression was detected in 10 patients (27.78%), and 3+HER2/neu expression was detected in 2 patients (5.55%).[18] Mohapatra and Shivalingaiah found only one of 35 cases to have HER2/neu overexpression (3+).[6]

In the present study, the advanced disease was more common in HER2/neu 3+ cases compared to HER2/neu 0/1+ and 2+, but the difference was not statistically significant ($P = 0.251$). Kato *et al.* also found no significant difference in disease stage amongst the groups.[18] Conversely, in the study by Morrison *et al.*, Togami *et al.,* and Halle *et al.*, the difference was statistically significant.[7,9,19]

The difference between the three groups was not statistically significant in terms of myometrial invasion ($P = 0.263$). Togami *et al.*, Kato *et al.,* and Halle *et al.* also found no significant difference.[9,18,19] In contrast to the findings of these studies Morrison *et al.* and Srijaipracharoen *et al.* reported a statistically significant difference.[7,8]

The spread of disease to lymph nodes was more common in HER2/neu 3+ group, but the difference was not statistically significant ($P = 0.092$). Togami *et al.* and Kato *et al.* also reported the difference to be not statistically significant.[7,9] Contrary to this, Morrison *et al.* found that the difference was statistically significant.[18]

Non-endometrioid histology was more common in HER2/neu 3+ group in comparison to HER2/neu 0/1+ and 2+ group. This difference is statistically significant ($P = 0.003$). According to Morrison *et al.* and Halle *et al.* also non-endometrioid histology was significantly more in the HER2/neu 3+ group.[7,19] Srijaipracharoen *et al.* and Peiró *et al.* did not find a significant correlation between the histopathology type and HER2/neu expression.[8,20]

Grade 3 tumor was more common in HER2/neu 3+ cases as compared to HER2/neu 0/1+ and 2+ cases. This difference was statistically significant ($P = 0.005$). Morrison *et al.* and Halle *et al.* found significantly more cases of Grade 3 tumor in HER2/neu 3+ cases.[7,19] Srijaipracharoen *et al.* and Peiró *et al.* found no significant difference in tumor grading among the HER2/neu positive and negative cases.[8,20]

In the present study, patients with endometrial carcinoma were followed over a period of 1 year. Recurrence occurred in two patients (6.67%). HER2/neu expression (2/3+) was seen in both cases. In a study by Kato *et al.*, five patients with recurrent endometrial carcinoma were studied and HER2/neu expression (2/3+) was seen in 4 cases (80%).[18] In a similar study by Togami *et al.*, recurrence occurred in 12.6% cases over a period of 5 years. Of total patients, 90% of the cases showed HER2/neu expression (2/3+).[9]

Our study had some limitations. The first one is that as our study was conducted in a setting that caters to patients belonging mainly to the lower or middle socioeconomic strata; hence, the data mainly reflect the situation in this cohort. The second limitation is that due to this study being conducted at a single center, its results cannot be generalized to study the prevalence of endometrium carcinoma in the general population. The sample size also limited this study.

CONCLUSION

In the present study of 30 cases of carcinoma endometrium, HER2/neu expression was assessed by immunohistochemistry and its prognostic significance was evaluated. HER2/neu overexpression (3+) was significantly associated with non-endometrioid histology and high tumor grade. No association was seen between HER2/neu overexpression (3+) and myometrial invasion, stage or lymph node involvement. HER2/neu expression (2/3+) was seen in recurrences. However, in view of the small number of cases and a short follow-up period, no definite conclusion can be drawn. Studies with a larger number of patients and a longer follow-up period may provide further insight into the role of HER2/neu expression in carcinoma endometrium so that patients may benefit from target-based chemotherapy for aggressive endometrial cancers.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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