

EVALUATION OF HER2/NEU EXPRESSION IN CARCINOMA CERVIX

Suppulakshmi N¹, Bhuvanewari MG¹¹Assistant Professor, Department of Pathology, Coimbatore Medical College and Hospital, Tamilnadu, India

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Corresponding Author:

Dr. Bhuvanewari MG,

Email: drmgbsiddu@gmail.com

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**Abstract**

Background: Given the literature and controversy surrounding various cervical lesions, this prospective study aimed to investigate the expression of Human Epidermal Growth Factor Receptor-2 (HER-2/neu) in cervical lesions and its relationship to the histopathological grade and type of tumor. Immunohistochemistry (IHC) was used to assess HER-2/neu expression because it is the most reliable way of detecting HER-2/neu overexpression. **Materials and Methods:** The prospective hospital-based study was conducted for two years at the Department of Pathology at Tirunelveli Medical College after ethical consideration from the Institutional Ethics Committee. Patients with confirmed biopsies of carcinoma cervix and positive results from hysterectomy specimens were included in the study. The immunohistochemistry evaluation was conducted using the standard IHC method. A golden-brown membrane and cytoplasmic staining were used for positive deduction, and the intensity of expression was graded according to ASCO guidelines. **Result:** One hundred patients were included in the study, with 75 presenting with squamous cell carcinoma and 21 with adenocarcinoma. Most patients were in the age group of 51-60 years of age (30.0%), and a total 17% prevalence of HER2/Neu expression was reported, out of which 94.11% were in squamous cell carcinoma patients. A significant correlation (p-value <0.05) was reported between HER2/Neu expression, lymph node metastasis, and clinical staging. **Conclusion:** HER2/Neu expression was prevalent in 17% of patients with cervical cancer; however, results report an increase in HER2/expression with high clinical staging.

INTRODUCTION

Cervical and ovarian cancers are the most frequent gynecological cancers in women worldwide, including India. Cervical cancer is declining, yet it is still the second most frequent malignancy among women, trailing only after breast cancer. In India, 122,844 women are diagnosed with cervical cancer each year, with 67,477 dying due to the disease.^[1] The high prevalence of cervical cancer in underdeveloped countries such as India is primarily due to a lack of awareness initiatives and proper screening programs, which means that most women arrive in the late stages of the disease.^[2] The discovery of new prognostic or predictive indicators is critical in developing effective anticancer medicines. One of the most extensively researched growth receptor families is the HER family. HER-2/neu is a membrane-bound receptor with a tyrosine-kinase activity that plays an important role in malignant transformation and carcinogenesis by interacting with other HER family members to enhance intracellular signalling, aside from its

function in the progression of various cancers. It has also been thoroughly evaluated as a therapeutic target.^[3] The role of HER-2/neu expression in gynecological cancers is still being studied to reach a consensus.^[4]

The carcinoma cervix has well-characterized histopathologically precursor lesions (CIN), which proceed slowly to the well-differentiated tumor. Cervical epithelial cells take 10 to 15 years to progress from CIN to cancer. Many critical tumor progression markers are expressed throughout this transformation period. As a result of the protracted time of transformation, the carcinoma cervix provides a wonderful chance to research the expression of biomarkers of tumor growth.^[5]

Due to the benefits listed above, the current study was conducted to assess the expression pattern of HER2 neu in different histological types of cervical cancer lesions better to understand the role of HER2 neu in cervical carcinogenesis.

MATERIALS AND METHODS

The prospective study was conducted at the Department of Pathology, Tirunelveli Medical College, for two years (2012 to 2014). After ethical approval from the ethics committee and acceptance of patient consent, the current study was conducted in patients with carcinoma cervix.

The study was initiated after approval from the Institutional Ethical Committee of Tirunelveli Medical College, Tirunelveli. In addition, we collaborated with the Department of Gynecology, Tirunelveli Medical College, from 2012 to 2014 to test several cervical biopsies and hysterectomy specimens.

Inclusion Criteria

The patient's cervical biopsies diagnosed with cervical cancer and hysterectomy specimens diagnosed with cervical cancer were included.

Exclusion Criteria

Patients diagnosed with other inflammatory conditions, benign lesions, cervical biopsies diagnosed with carcinoma in situ and mesenchymal lesions, and hysterectomy specimens reported other findings than cervical carcinoma were excluded.

Patient approval was taken before the initiation of the study with data collection of demographic details, clinical staging, lymph node status, parametrial extension, and pathology records from the patient's case sheet. Cervical biopsy and hysterectomy specimens were fixed with 10% formalin and processed as per the routine. Patient biopsy specimens were presented in toto and hysterectomy specimens were carefully sectioned to include endocervix and ectocervix. Sections of 4-5 μ thickness were cut and stained with hematoxylin and eosin. The slides were observed under a light microscope, and the sections were subjected to immunohistochemical staining.

The evaluation was conducted with 3 μ m sections using microtome from the paraffin selected blocks, and sections were coated to adhesive slides using poly-L-lysine and incubated at 60 °C for 1 hour. The slides were subjected to two changes of xylene, and

deparaffinization was conducted. The overall process of immunohistochemistry is given in the supplementary data.

HER2/Neu positivity was graded per ASCO guidelines, and positive test reaction was recorded on a crisp brown golden membranous and cytoplasmic staining. The intensity was also graded under the ASCO scoring system as strong, complete membrane staining in more than 10% of malignant cells (3+); weak to moderate complete staining in more than 10% of malignant cells (2+); no fewer than 10% cells staining (0 to 1+) respectively.

Statistical analysis was performed using the Statistical Package for Social Science Software Version 11.5, HER2/Neu expression and the correlation of variables were calculated using Pearson's Chi-square. A P value of <0.05 was considered statistically significant.

RESULTS

A total of 100 patients with cervical cancer were included in the study, out of which 75 cases were of squamous cell carcinoma (75%), followed by 21 cases of adenocarcinoma (21%), and 3 cases of small cell carcinoma (3%). One patient was reported with adenosquamous carcinoma [Table 1].

Based on the age distribution, most patients were 51-60 (30%) and 61-70 (30%), respectively. Further, 22% of the patients were in the age group of 41-50 years of age, followed by 11% of cases in the age group of 30-40 years of age and 7% of patients in the age group of 71-80 years.

The FIGO classification categorized 64 patients in Stage I (64.0%), followed by 22 patients in stage II (22.0%), and 14 patients diagnosed with stage III staging (14.0%) [Table 1].

A total of 17 patients tested positive for HER2/Neu cases, where five patients (22.7%) were reported in the age group of 41-50 years of age and 61-70 years of age with five patients (16.6%). The positive results of HER2/Neu were also seen in 51-60 years of age (20.0%) and one patient aged 71-80, respectively [Table 2].

Table 1: Demographic data of the study

		Total cases (n=100)
Types of carcinomas	Squamous cell carcinoma	75
	Adenocarcinoma	21
	Small cell carcinoma	3
	Adenosquamous carcinoma	1
Age group (Years)	30-40	11
	41-50	22
	51-60	30
	61-70	30
	71-80	7
FIGO stage	Stage I	64
	Stage II	22
	Stage III	14

Table 2: Distribution of positive HER2/Neu results.

		No of HER2 positive cases (n=17)
Age group (Years)	30-40	0
	41-50	5 (22.7%)

	51-60	6 (20%)
	61-70	5 (16.6%)
	71-80	1 (14.2%)
Types of carcinomas	Squamous cell carcinoma	16 (94.11%)
	Adenocarcinoma	0
	Small cell carcinoma	1 (0.05%)
	Adenosquamous carcinoma	0

The squamous cell carcinoma type of cancer reported the highest expression of HER2/Neu, with 16 patient (94.11%) and one patient in the category of small cell carcinoma (0.05%) [Table 2].

Correlation of HER2/Neu expression with clinical staging of carcinoma cervix

The study reports a significant difference concerning clinical staging, as stage III cases reported an increased HER2/Neu expression with 50.0% of positivity and a significant correlation between HER2/Neu expression and clinical staging (p-value 0.0011) [Table 3].

Table 3: Correlation HER2/Neu expression with clinical staging of carcinoma cervix

Clinical staging	Total cases	HER2 positive cases (n=17)	HER2 negative	P-value
Stage I	64	6 (9.37%)	58	0.0011
Stage II	22	4 (18.18%)	18	
Stage III	14	7 (50%)	7	
Stage IV	0	0	0	

Increased cancer staging was reported with an increased expression of HER2/Neu.

Lymph Node Metastasis

Among 100 cases of cervical carcinoma, nine patients were reported with lymph node metastasis with squamous cell carcinoma. Among 9 cases, one patient was well-differentiated carcinoma, 7 cases were presented with moderately differentiated carcinoma and poorly differentiated carcinoma was seen in one patient [Table 4].

Table 4: Distribution of lymph node metastasis in squamous cell carcinoma

Lymph node metastasis in grades squamous cell carcinoma	No of cases (n=9)
Well-differentiated carcinoma	1 (11.11%)
Moderately differentiated carcinoma	7 (77.7%)
Poorly differentiated carcinoma	1 (11.11%)

Table 5: Correlation of HER2/Neu expression with lymph node involvement

Lymph node metastasis	Total cases	HER2 positive	HER2 negative	P-value
Present	9	5 (55.55%)	4	0.001
Absent	91	12 (13.1%)	79	

Table 6: Correlation of squamous cell carcinoma with HER2/Neu expression in cervical cancer

Lymph node metastasis	Total cases	HER2 positive	HER2 negative	P-value
Present	9	5 (55.55%)	4	0.007
Absent	66	11	55	

Correlation between the HER2/Neu expression and lymph node metastasis reported a significant difference (p-value=0.001) with positive expression in 5 patients out of 9 cases with lymph node metastasis [Table 5].

In addition, cases with positive metastasis lymph node involvement were diagnosed with squamous cell carcinoma, out of which five patients were of moderately differentiated carcinoma and HER2/Neu expression (55.55%). A significant difference was reported between the HER2/Neu expression and squamous cell carcinoma (p-value = 0.007) [Table 6].

[Figure 1] demonstrates the IHC staining of HER2/Neu with a score of +3, representing neoplastic squamous cells with membranous staining of HER2/Neu in 400x.

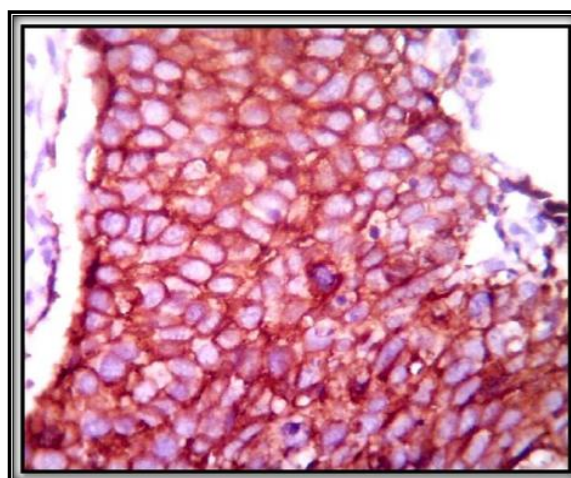


Figure 1: IHC staining of HER2/Neu (+3 score) Neoplastic squamous cells representing strong membranous staining of HER2/Neu in 400x

[Figure 2] demonstrates the membranous squamous cells staining with IHC staining of HER2/Neu (score +3), 400x.

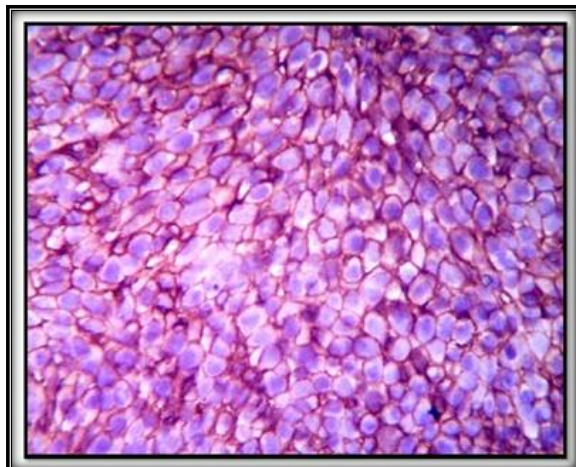


Figure 2: IHC staining of HER2/Neu score (+3) at 400 x

[Figure 3] demonstrates the IHC staining of HER2/Neu score +2; 400x neoplastic squamous cells with less intense membranous staining.

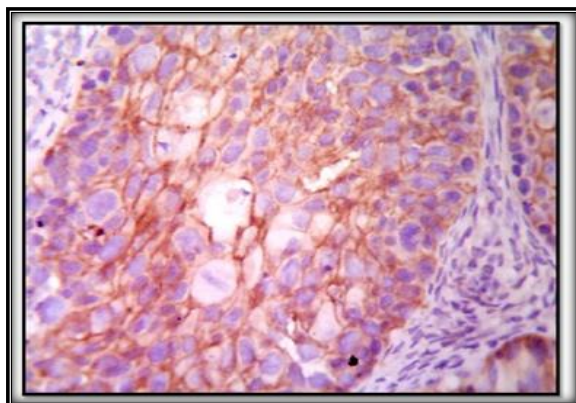


Figure 3: IHC staining of HER2/Neu (score +2); 400x

Neoplastic squamous cells representing a less intense membranous staining score

[Figure 4] represents the Histological evaluation with well-differentiated squamous cell carcinoma.

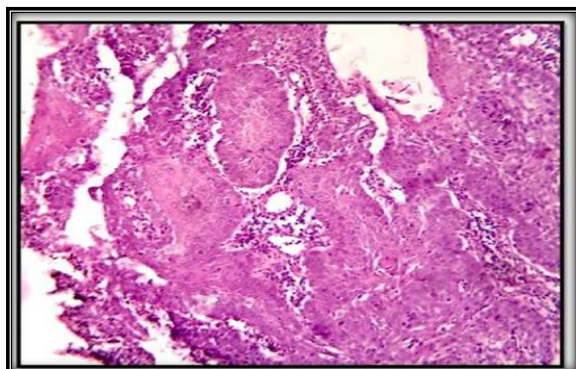


Figure 4: Histopathological evaluation with well-differentiated squamous cell carcinoma representing malignant squamous cells infiltrating the stroma as finger-like projections (at 100x)

DISCUSSION

Carcinoma of the uterine cervix continues to be a leading cause of death among women in the developing world, including India. According to recent data from the ICMR's Atlas of Cancer in India project, cervical cancer is the second most common cancer in women after breast cancer in most urban population-based registries in India, ranging from 10.9 to 65.4 in various registries, with an average incidence of around 25 per 100,000 women.^[6] HER family members are activated when a ligand and a dimer of the same monomer or another member of the HER family are linked. Tyrosine autophosphorylation of cytoplasmic signal proteins after activation sends signals to the nucleus, regulating elements of cell growth, division, differentiation, and migration.^[7]

Overexpression of HER2 receptors results in the transmission of excessive signals to the nucleus for cell growth. This may cause the converted cell to grow more aggressively. There have been findings in the literature that HER-2/neu overexpression corresponds with the decreased benefit of adjuvant therapy, such as in cases of breast cancer treated with tamoxifen. Several studies have indicated that HER-2/neu regression inhibits the malignant behaviours of cancer cells overexpressing this oncoprotein. Making it a good target for developing anti-cancer medicines that target HER-2/neu overexpression.^[8]

Given the literature and controversy surrounding the expression of HER-2/neu in various cervix disorders, this prospective study was designed to investigate the expression of HER-2/neu oncoprotein in cervical lesions and the correlation of these receptors with the histopathological grade and type of tumor at the time of diagnosis. We excluded chronic cervicitis and metaplasia from the current study. In our study, SCC cases (75%) outnumbered adenocarcinoma cases (21%) which was in concordance with the World Health Organization (WHO) too, which states that squamous cell carcinomas account for ~70%–80% of cervical cancers while adenocarcinomas for 10%–15%.^[9]

The current study reports a prevalence of HER2/Neu expression in 17% of the patients, with an increased expression of HER2/Neu in stage III carcinoma in about 50.0%. The current study's positivity rate is comparable to that reported by Sharma N et al. and Sarvade P et al., who reported 36.6% and 44%, respectively.^[10,11] In contrast to our study, Conesa et al. reported a lower incidence for the expression of HER2/Neu; however, this can be due to stringent criteria for positive correlation in patients with scores more than 3.^[12]

The present study reported a significant correlation between HER2/Neu expression with the stage of the tumor, which was also reported by Gupta et al. with a p-value <0.05.^[13] In addition, Joseph et al. reported a higher prevalence of HER2/Neu expression in 70% of premalignant cases.^[14] The current study reports

the expression of HER2/Neu majorly in squamous cell carcinoma, than in adenocarcinoma.

The incidence of HER2/Neu expression with cervical carcinoma was reported in 17% of the patients with positive results. The current study's findings are thus inconsistent with previous studies, except for some resemblance to the study of Gupta et al. The present study has HER-2 neu positivity in squamous cell carcinoma and small cell carcinoma, and negative in Adenocarcinoma which is in contrast with Study of Bajpai et al,^[15] in which Adenocarcinoma shows maximum positivity than Squamous cell carcinoma.

Supplementary Data

Process for immunochemistry

- 3µm thickness sections were cut using microtome from the selected paraffin blocks.
- The sections are taken in poly L-lysine-coated adhesive slides.
- The slides are incubated at 60 c for 1 hour
- The slides are subjected to 2 changes of xylene, 5 minutes each, for deparaffinization.
- They are then transferred to absolute alcohol for 5 minutes, followed by 80% and 70% alcohol for 5 minutes to rehydrate the tissue sections.
- Tissue sections are then placed in running tap water for 5 minutes and washed in distilled water
- Antigen retrieval was performed using a pressure cooker in citrate buffer
- Then the sections are cooled to room temperature, and the slides are washed with distilled water
- Endogenous peroxidase activity is removed by incubating the tissue sections with enough 3% peroxide block drops in a humidity chamber for 5 minutes.
- The sections are then washed in TRIS wash buffer. Primary antibody (Her2 neu) is added over the tissue sections and incubated for 30 minutes.
- The tissue sections are then washed in TRIS wash buffer.
- Followed by that primary amplifier is added for 15 minutes to enhance the process of the primary antibody, which is then washed in TRIS wash buffer
- A secondary antibody is added and incubated for 20 minutes and then washed with TRIS wash buffer
- AB chromogen (1ml DAB buffer +1 drop DAB chromogen) is added over the tissue, incubated for 4 minutes, and washed with two changes of distilled water.
- Counterstaining was done with haematoxylin for 30 seconds and washed in tap water.
- Two changes of 100 % alcohol do dehydration.
- Mounting is done by DPX mountant and observed under a microscope.

Buffer Preparations

1. Citrate buffer

Citric acid - 1.92 gms Distilled water - 1000ml pH is adjusted to 6.2 with 1N NaoH

2. Tris wash buffer

Tris - 0.605 gm

Sodium chloride - 8 gm

1 N Hcl - 4ml

Distilled water - 1000 ml

CONCLUSION

The study reports a 17% prevalence of HER2/Neu expression in cervical carcinoma, majorly in the clinical staging of stage III carcinoma. In addition, significant differences were reported between the HER2/Neu expression, clinical staging, and lymph node metastasis. However, current study findings were in parallel to few studies; hence, there is a need to conduct a larger sample size study to assess the correlation and prognostic significance in cervical carcinoma patients.

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