



Prognostic Evaluation of the Expression of HER-2/neu in Adenocarcinoma of the Uterine Cervix - An Original Study in a Tertiary Care Centre

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Abstract

Background: Worldwide, cervical carcinoma is second only to breast carcinoma in its incidence and mortality. About 471,000 new cases are diagnosed every year, mainly among the economically disadvantaged in both developing and industrialized nations. Cancer of uterine cervix, the most prevalent cancers among the Indian females, is an important cause of morbidity and mortality among women. Adenocarcinoma currently comprises 10-25% of all cervical carcinomas. The overall survival rate for adenocarcinoma of cervix is worse than from the more common squamous cell carcinoma of the uterine cervix.

Materials and methods: This study was conducted in the Department of Pathology, R.G.Kar Medical College and Hospital. It was cross-sectional observational study, was done in the Department of Pathology, R.G. Kar Medical College and Hospital, Kolkata. A total of 105 cases of cervical carcinoma cases were obtained during the period of January 2017 to June 2018. Out of 105 cases, 20 cases (19%) of Adenocarcinoma of cervix were diagnosed during this period. After confirming the diagnosis of Adenocarcinoma of Cervix histopathologically, sections

were taken for immunohistochemical expression of HER-2/neu. Results were analysed at the end of the study.

Results: HER-2/neu expression areas showed brown coloured membrane staining. Samples with Score 0 and 1+ were considered negative and those with scores 2+ and 3+ were considered positive. Out of 20 cases of adenocarcinoma of cervix, 7 cases (35%) show 1+ positivity, 5 cases (25%) show 2+ positivity, 6 cases (30%) show 3+ positivity. We found in our study that out of 20 cases of cervical adenocarcinoma, 11 cases (55%) were HER-2/neu positive, while rest 9 cases were HER-2/neu negative. In our present study, we noticed that intensity of HER-2/neu expression increased in tumour of higher grade (Mod. Diff. > Well diff.). However, no statistically significant correlation (p=0.198) was established between the grade of Cervical Adenocarcinoma and Expression of HER-2/neu. Increased lymph node metastasis noted in moderately differentiated Adenocarcinoma cases (i.e. poor prognosis).

Conclusion: This study shows the importance of continued basic and traditional research on the HER-2/neu receptors in invasive cervical carcinoma cases. The results of the present study indicate that evaluation of HER-2/neu

expression may provide additional and independent prognostic information to predict the clinical course of cervical adenocarcinoma.

Keywords: Adenocarcinoma, Her-2/neu, cervix.

Introduction

According to the global cancer statistics for 2012, cervical cancer is the fourth most common cancer, affecting women worldwide and it is the most common cause of cancer death in women worldwide^[1]. Carcinoma cervix is the most common malignancy in women in India with an incidence of 9 to 44 per 100000 women^[2]. Cancer of uterine cervix, the most prevalent cancers among the Indian females^[3], is an important cause of morbidity and mortality among women.

Adenocarcinoma currently comprises 10-25% of all cervical carcinomas in developed countries, compared to 5-10% three decades ago^[4]. The mean age at presentation is about 50 yrs^[5]. The overall survival rate for adenocarcinoma of cervix is worse than from the more common squamous cell carcinoma of the uterine cervix^[6]

While curable in early stages, the treatment result of locally advanced cases is still unsatisfactory. Therefore the next step in treatment is testing of molecular targeted therapies to improve outcome of cervical cancer patients^[7].

The c-erbB-2 proto-oncogene, also called HER-2/neu, is a gene localized on Chromosome 17q21 and it encodes a growth factor receptor-like molecule with tyrosine kinase activity. Its structure is similar to that of epidermal growth factor receptor and has 1255 amino acids. The expression of HER-2 is believed to be associated with aggressive biological behavior and metastatic potential^[8].

The HER-2/neu oncogene, in response to ligand specific binding, these receptors initiate tyrosine kinase activity in the intracellular domain. This amplification of tyrosine kinase activity results in the phosphorylation of several tyrosine residues. Phosphorylated tyrosines serve as

binding sites for signal transducers and adapter molecules that initiate signaling pathways; resulting in cell proliferation, adhesion, protection from apoptosis and transformation leading to initiation and development of some malignant tumors^[9].

The oncogenic pathway is thought to be initiated by HER-2/neu mutation, overexpression, structural rearrangements, and/or relief of normal regulatory or inhibitory pathways. The presence of HER-2/neu receptors has also been associated with accelerated tumour progression and resistance to therapy for multiple types of malignancies. The casual relationship of this receptor network to disease progression and resistance to therapy provides a rationale for targeting HER-2/neu^[10].

Aim and objective: To study the expression of HER-2/neu in adenocarcinoma of cervix and its prognostic significance.

Place of study: Department of Pathology, R.G.Kar Medical College and Hospital, Kolkata, West Bengal, India

Duration of study: 18 months (January 2017 to June 2018)

Study design: Observational cross-sectional study

Study materials: Histopathologically confirmed Cervical Adenocarcinoma cases (both cervical biopsy specimen and hysterectomy specimens).

Materials and Methods: This study was conducted in the Department of Pathology, R.G.Kar Medical College and Hospital. It was cross-sectional observational study, was done in the Department of Pathology, R.G.Kar Medical College and Hospital, Kolkata. Specimens of cervical biopsy and hysterectomy specimens were collected in the Department of Pathology.

A total of 105 cases of cervical carcinoma cases were obtained during the period of January 2017 to June 2018. Out of 105 cases, 20 cases (19%) of Adenocarcinoma of cervix were diagnosed during this period.

After getting the samples they were fixed in 10% formal saline overnight. During grossing sections were taken from different part of the fixed samples. Paraffin blocks were prepared using routine histopathological techniques as detailed below. 5micron thin sections were taken on slides & staining were done with routine H&E stain. Both microscopic and macroscopic photograph were taken in all cases.

After confirming the diagnosis of Adenocarcinoma of Cervix histopathologically, sections were taken for immunohistochemical expression of HER-2/neu .All immunohistochemical analysis were carried out by applying the avidin-biotin complex method, on 3µm sections cut from formalin-fixed, paraffin embedded tissues using commercially available mouse-monoclonal antibodies..

Results were analysed at the end of the study.

A golden brown membrane and cytoplasmic staining was taken as a positive reaction. Results were analysed at the end of the study. Intensity of HER-2/neu expression was graded according to the 2014 ASCO/Cap guidelines.

The 2014 ASCO/CAP HER-2 Reporting Guideline

Intensity of HER-2 Expression	Characteristics features
0	No staining is observed or shows membrane staining that is incomplete and is faint/barely perceptible and within <10% of tumour cells
1+	Membrane staining that is incomplete and is faint/barely perceptible and within>10% of tumour cells
2+	Circumferential staining that is incomplete and/or weak/moderate within>10% of tumour cells or Complete intense circumferential

	membrane staining within<10% of tumour cells
3+	Complete intense circumferential membrane staining within >10%of tumour cells

HER-2/neu expression area: Brown coloured membrane staining.

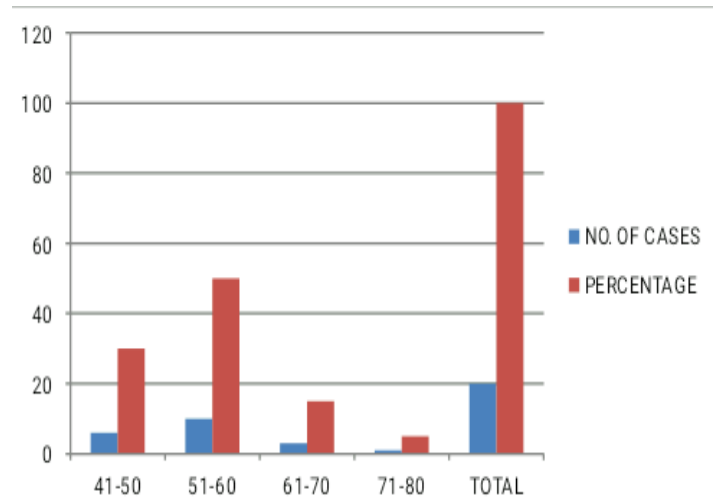
Samples with Score 0 and 1+ were considered negative and those with scores 2+ and 3+ were considered positive.

Results and analysis

Out of 105 cases of cervical carcinoma cases , 20 cases (19%) were cervical adenocarcinoma.

Total 20 cases of cervical adenocarcinoma were analysed in the study.

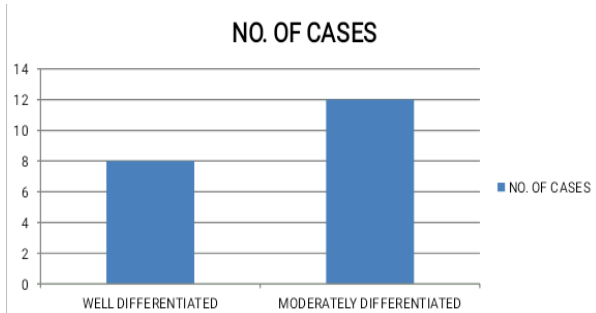
Age range in years	No. of cases	%
41-50	06	30%
51-60	10	50%
61-70	03	15%
71-80	01	05%
Total	20	100%



Grades of Adenocarcinoma cervix	No. of cases	%

Well differentiated	08	40%
Moderately differentiated	12	60%

Table no.2 showing distribution of cases of cervical adenocarcinoma according to its grading

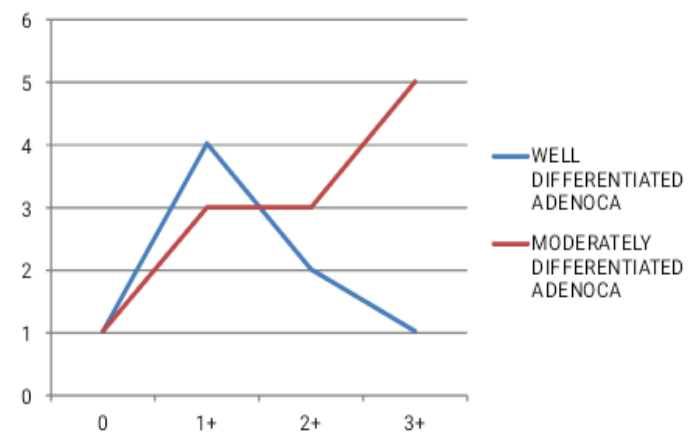
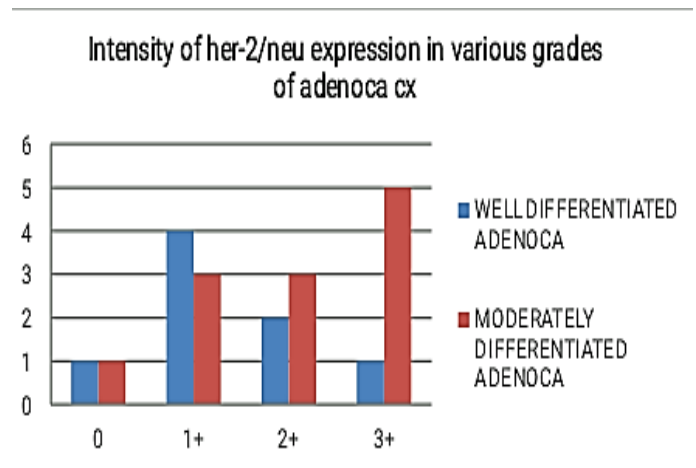


Intensity of HER-2/neu Expression	No. of adenocarcinoma cases (n=20)
0	2 (10%)
1+	7 (35%)
2+	5 (25%)
3+	6 (30%)

Table no. 3(i) showing Intensity of Expression of HER-2/neu in Adenocarcinoma of Cervix

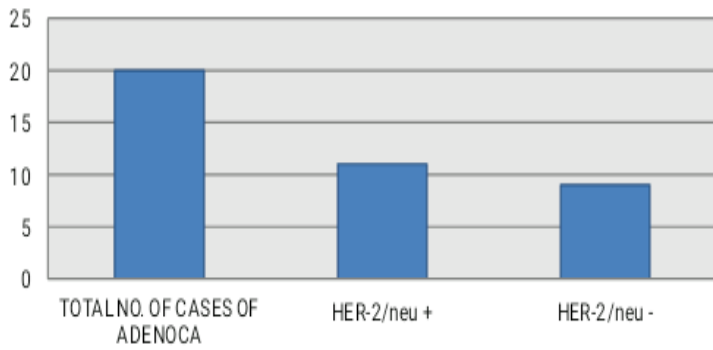
Intensity of HER-2/neu expression	No. of cases of Well differentiated adenocarcinoma of cervix	No. of cases of Moderately differentiated adenocarcinoma of cervix
0	01	01
1+	04	03
2+	02	03
3+	01	05

Table no. 3(ii) showing Intensity of Expression of HER-2/neu in different grades of Adenocarcinoma of Cervix



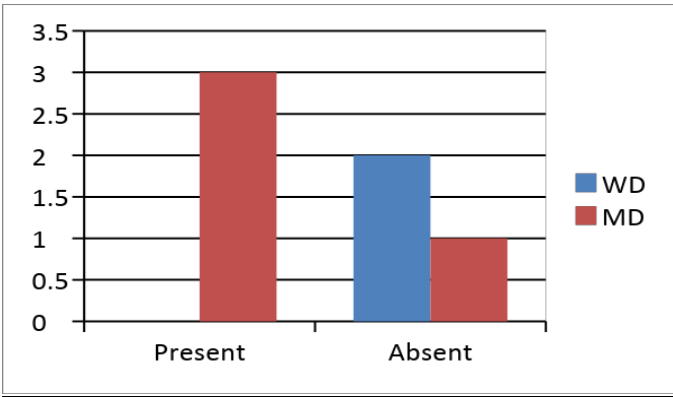
Total no. of adenocarcinoma of cervix cases (n)	HER-2/neu Positive
20	11(55%)

Table no.4 showing distribution of cases of cervical adenocarcinoma according to positive or negative expression of HER-2/neu



Types of cervical adenocarcinoma	Lymph node metastasis present	Lymph node metastasis absent
Well differentiated	0	2
Moderately differentiated	3	1

Table no. 5 showing distribution of cases of cervical adenocarcinoma according to presence or absence of lymph node metastasis



Photomicrographs

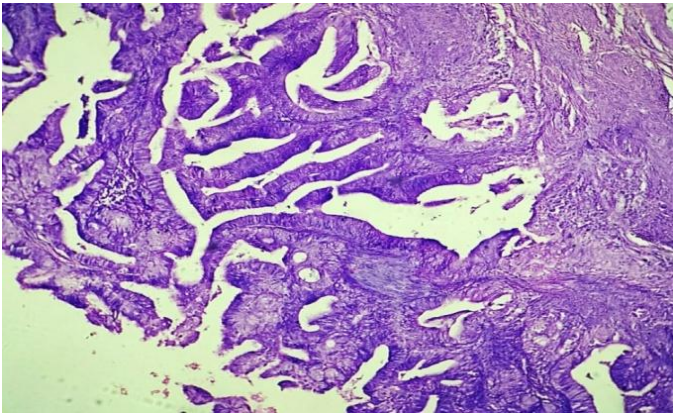


Figure 1: Photomicrograph showing adenocarcinoma of cervix (H & E,100X)

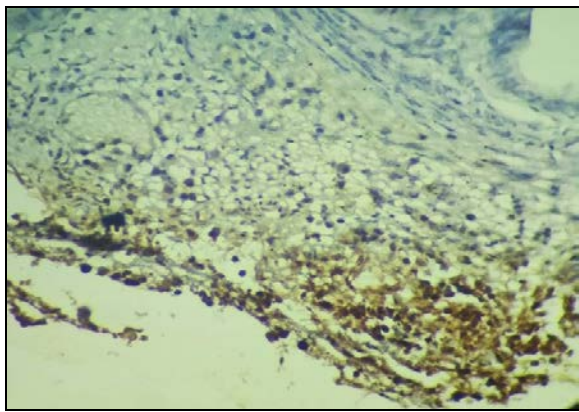


Figure 2: Photomicrograph of HER-2/neu expression in adenocarcinoma of cervix (Intensity of expression 1+)



Figure 3: Photomicrograph of HER-2/neu expression in adenocarcinoma of cervix (Intensity of expression 2+)

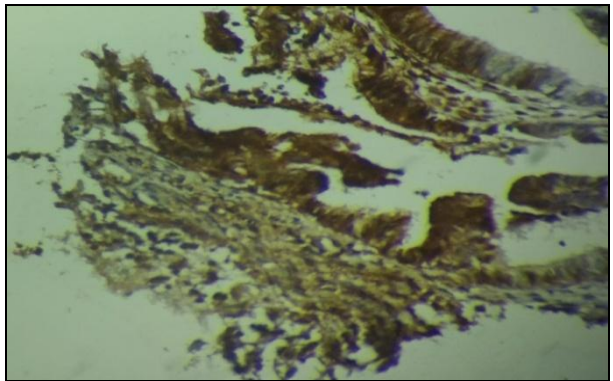


Figure 4: Photomicrograph of HER-2/neu expression in adenocarcinoma of cervix (Intensity of expression 3+)

Discussion

Out of total 105 cases of cervical carcinoma ,20 cases (19%) were adenocarcinoma of cervix (14 cases were cervical biopsy specimen and rest 6 cases were hysterectomy specimens).

Maximum cases were found in the age group of **51-60** yrs.

Median age of presentation is 54.5 yrs.

Out of 20 cases of adenocarcinoma of cervix, 7 cases (35%) show 1+ positivity, 5 cases (25%) show 2+ positivity, 6 cases (30%) show 3+ positivity.

We found in our study that out of 20 cases of cervical adenocarcinoma, 11 cases (55%) were HER-2/neu positive, while rest 9 cases (45%) were HER-2/neu negative.

p-value by Chi-square test is 0.198, which is statistically insignificant

In our present study, we noticed that intensity of HER-2/neu expression increased in tumour of higher grade (Mod. Diff. > Well diff.).

Increased lymph node metastasis noted in moderately differentiated Adenocarcinoma cases (i.e. poor prognosis).

In a study by Mandai et al^[11], HER-2/neu expression was seen in 39 cervical adenocarcinoma. It was reported in that study that significant correlation existed between HER-2/neu expression and lymph node metastasis i.e. the survival and the prognosis of the patients.

In a study by Gupta et al^[12], the positivity rate of HER-2/neu expression was 84.62% of cases. Yong et al^[13] analysed HER-2/neu in 74 cases of cervical adenocarcinoma and reported that significant correlation existed between HER-2/neu expression and lymph node metastasis i.e. the survival and the prognosis of the patients. Yong et al found the expression of HER-2/neu in 34 cases of adenocarcinoma. In this study, positive HER-2/neu staining was associated with increased lymph node metastasis (p=0.041), and lower 5 yrs survival rate.

In a study by Joseph et al^[14] 4 cases of adenocarcinoma were analyzed. Both cases of well differentiated adenocarcinoma showed 2+ positivity while both cases of moderately differentiated adenocarcinoma showed 3+ positivity. However a significant correlation was not

established between HER-2 expression and grade of adenocarcinoma of cervix (p=0.317).

Kihana et al^[15], also did not find any relation between HER-2 expression and grade of cervical adenocarcinoma.

The location of HER-2/neu on the cell surface has contributed to its appeal as an immunotherapy target^[16]. Trastuzumab is a human monoclonal anti-HER-2/neu antibody, has provided a distinct therapeutic advantage in not only breast cancer but also in other cancers. Bellone et al^[17], reported in his study that therapy which targets HER-2/neu may be effective in patients of cervical carcinoma

The high incidence of HER2/neu expression in cervical adenocarcinomas suggests the role of this gene in tumourigenesis. The intensity of HER2/neu expression also increased in cases of Adenocarcinoma. Hence, in future there may be a role of Trastuzumab in treating cases of cervical adenocarcinoma that are positive for HER2/neu. However, studies with greater number of samples are required to establish the prognostic significance of HER2/neu expression in cervical adenocarcinoma.

This study shows the importance of continued basic and traditional research on the HER-2/neu receptors in cases of cervical adenocarcinoma. The results of the present study indicate that evaluation of HER-2/neu expression may provide additional and independent prognostic information to predict the clinical course of cervical adenocarcinoma.

Limitations

1. Since this study was done in a tertiary care centre and the study period is only 18 months; a better conclusive opinion is possible from a study of a larger sample of long duration.

2. Follow up of all the patients could not be accomplished.

3. As most of the specimens(70%) were biopsy specimens, staging of cervical carcinoma could not be accomplished in those cases.

References

1.Sarwade P,,Patil S, Bindu R: Immunohistochemistry Study for HER-2/neu Expression in lesions of uterine cervix, Int J Cur Res Rev; vol 8,issue 13,July 2016.

2.Wadhvani R, Bamnia R, Meena M.Clinicopathological analysis of cancer cervix in tertiary care centre. J Evol Med Den Sci.2013;2 (39): 7381-7385.

3.Shanta V,Krishnamurthi S,Gajalakshmi CK,Swaminathan R,Ravichandran K.Epidemiology of cancer of the cervix: global and national perspective. J Indian Med Assoc 2000;98:49-52.

4.Young RH,Clement PB (2002).Endocervical adenocarcinoma and its variants:their morphology and differential diagnosis.Histopathology 41:185-207.

5.WHO classification of Tumours of Female Reproductive organs,4th edition,2014,page no.184.

6.Hopkins MP, Morley GW.A comparison of adenocarcinoma and squamous cell carcinoma of the cervix.Obstet Gynecol 1991;77: 912-17.

7.Duenas-Gonzalez A,Cetina L, Mariscal I, de la Garza J.Modern management of locally advanced cervical carcinoma.Cancer Treat Rev.2003;29: 389-99.

8.Vaidyanathan K, Kumar P, Reddy CO, Deshmane V, Somasundaram K, Mukherjee G. ErbB-2 expression and its association with other biological parameters of breast cancer among Indian women. *Indian J Canc.* 2010; 47(1):8-15.

9. Yaziji H,Goldstein LC,Barry TS et al.HER-2 testing in breast cancer using parallel tissue-based methods.JAMA 2004;291:1972-1977.

10.Bookman MA,Darcy KM, Clarke-Pearson D,Boothby RA,Horowitz IR.Evaluation of monoclonal humanized anti-HER-2antibody, transtuzumab, in patients with recurrent or refractory ovarian or primary peritoneal

carcinoma with overexpression of HER-2: a phase II trial of the Gynaecologic Oncology.JClinOnc 2003;21: 283-290.

11.Mandai M,Konishi I,Koshiyama M et al.Altered expression of nm23-H1 and c-erb B2 proteins have prognostic significance in adenocarcinoma but not in squamous cell carcinoma of the uterine cervix.Cancer 1995;75:2523-9.

12.Gupta N,Singh S,Marwah N, Kumar S,Chabra S,Sen R.HER-2/neu expression in lesions of uterine cervix: Is it reliable and Consistent? Indian J Pathol Microbiol.2009; 52(4):482-85.

13.Yong H,Shumo C, Shaoyin Y,Daren S.Expression of C-erbB2 and PCNA in cervical adenocarcinoma and its signification.Chin J Canc Res.1998;10(1):68-70.

14. Joseph T,Raghuveer C.V. et al,HER-2/neu expression in cervical intraepithelial neoplasia and cervical carcinoma,International journal of biomedical and advanced research,2015;6(1):47-52 .

15.Kihana T, Tsuda H, Teshima S, Nomoto K, Tsugane S, Sonoda T, et al. *Prognostic significance of the over expression of c-erbB-2 protein in adenocarcinoma of the uterine cervix. Cancer. 1994; 73: 148-153*

16.Diana P.English,Dana M.Roque,Alessandro D.Santin; HER2 Expression Beyond Breast Cancer: Therapeutic Implications For Gynaecologic Malignancies;Mol Diagn. Ther.2013 apr;17(2):85-89.

17.Bellone S,Palmieri M,Gokden M,Joshua J,Roman JJ,Pecorelli S,et al. Selection of HER-2/neu positive tumour cells in early stage of cervical cancer: implications for Herceptin-mediated therapy.Gynaecologic oncology.2003;91(1)231-40.Epub2003