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ORIGINAL RESEARCH

Expression of HER-2/neu biomarker in premalignant and malignant epithelial lesions of uterine cervix

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Abstract

Background: Cervical malignancy is a serious global public health issue that affects women and major cause of death in low and middle-income nations. Even when using cisplatin-based combination therapy, individuals with severe or recurring illness have a poor prognosis, Therefore, there is a growing need to identify new therapeutic targets that can supplement or replace the present treatment for these individuals.

Objectives: To study the expression of HER-2/neu biomarker in premalignant and malignant epithelial lesions of uterine cervix and its correlation with general clinical parameters and histological type, grade, and tumor stage.

Material and methods: A cross-sectional study was conducted on 72 histologically diagnosed cases of premalignant and malignant epithelial lesions of uterine cervix in 1 year. Detailed gross and microscopic examination followed by HER-2/neu immunostaining was performed in all cases.

Results: Out of 72 cases of cervical lesions, premalignant were 9 in number whereas 63 cases were malignant. HER-2/neu expression was noted in 21 cases (29.2 %) out of 72 cases. Higher HER-2/neu expression intensity (2+ and 3+) was seen in malignant lesions (17.5 %, n=11) than in premalignant lesions (11.1 %, n=1). In comparison to well-differentiated SCC (n=2), the percentage of lesions with higher expression (2+ and 3+ scores) was considerably higher in poorly and moderately differentiated groups (n=8). HER-2/neu expression intensity increases with the advancement of clinical stage of malignant cervical lesions.

Conclusion: Over-expression of HER-2/neu biomarker is associated with poor prognosis and aggressive behavior of premalignant and malignant cervical lesions.

Keywords: Cervix, Premalignant, malignant, immunostaining, HER-2/neu

Introduction

Cervical malignancy is a serious global public health issue that affects women. It ranks second among those under 50 years old and the fourth most common cause of death from cancer in women overall.[1] 16 % of all cervical cancer cases and 15.2 % of all cervical cancer deaths worldwide occur in India, respectively. Every year, 122,844 women in India are diagnosed with cervical malignancy and 67,477 of them lose their life from the disease.[2] The high prevalence of cervical cancer in poor countries like India is mostly attributed to a lack of awareness campaigns and official screening programs as a result of which the majority of women present with cervical cancer in advanced stages.

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Cervical malignancy is the first cancer in humans for which an infectious agent has been identified as a causative agent. The primary etiological agent of cervical carcinogenesis is the human papillomavirus. It is well-recognized that human papillomavirus subtypes 16, 18, 31, 33, and 51 contribute to the development of cervical cancer. HPV infects the transitional or transformational zone of cervix. The viral genome is incorporated into the DNA of the immature squamous cells in the squamocolumnar junction and initiates the pathogenesis of infection.[2]

Most lesions can be detected early on by screening programs before they progress to malignancy. Numerous tissue indicators have been investigated in cervical cancer, opening the door to targeted therapy and treatment for later stages of the condition. One of the growth receptor families that has been explored the most is the HER family. The c-erbB-2 proto-oncogene, also known by the name HER-2/neu, (HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR-2) is a gene on chromosome 17q21 that encodes for growth factor receptor-like molecule with the activity of tyrosine kinase.[3] There is a structural similarity of HER-2/neu with epidermal growth factor receptor and has 1255 amino acids. HER-2/neu presence can be detected by Immunohistochemistry or by fluorescence in situ hybridization (FISH) technique. Its expression is thought to be linked to poor prognosis, aggressive biological behavior and propensity for metastasis in many human malignancies, like breast, stomach, esophagus, colon, uterine endometrial carcinomas, ovary, bladder, lung, head and neck.[4,5]

Molecular targeted medicines are currently seen as the next natural step in enhancing cervical cancer patient's prognosis. The relevance of HER-2/neu in cervix lesions underscores the need for innovative therapeutic approaches. HER-2/neu expression in cervix lesions can help in the innovative therapeutic approaches and this, in the case of cervical cancer, can serve as a guidance for targeted therapy. HER-2/neu expression in carcinoma cervix and their relationship with disease stage, grade, and response to treatment is not well established and very few research has been conducted in this aspect. Thus, the study is undertaken to determine HER-2/neu expression in premalignant & malignant cervical lesions.

Material and method

A cross-sectional study was conducted for a period of one year. 72 histologically diagnosed cases of premalignant and malignant epithelial lesions of uterine cervix were included and subjected to gross and microscopic examination per standard protocol.[6]

Histological diagnosis was documented according to the latest WHO classification of tumors of uterine cervix[7] and was staged according to FIGO staging.[8] Thereafter tissue blocks were taken up for IHC by using rabbit monoclonal anti-human HER-2/neu Receptor IgG antibody and interpretations were done according to ASCO/CAP HER-2/neu reporting guidelines.[9]

Table 1: ASCO/CAP HER-2/neu reporting guideline

Intensity of HER-2 expression	Staining pattern
0	No staining or incomplete membrane staining and with <10% of tumor cells
1+	Incomplete membrane staining with >10% of tumor cells
2+	Incomplete circumferential staining and/or weak/moderate with > 10% of tumor cells or Complete intense circumferential membrane staining with < 10% of tumor cells.
3+	Intense complete circumferential membrane staining with > 10% of tumor cells.

Statistical analysis was done using MS EXCEL &SPSS version 23.0. A *p-value* of <0.05 was considered statistically significant

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Results

A total of 72 cases of cervical lesions were included in this study with patient age ranging from 26 to 78 years and a mean age of 48.9 years. The maximum number of patients belonged to 51-60 years (31.9 %) followed by 41-50 years (29.2%). Among premalignant lesions, the maximum study population belonged to 41-50 years whereas amongst malignant lesions, the maximum study population belonged to 51-60 years. In this study clinical symptoms related to cervical lesions were evaluated and found that the most common symptom was bleeding per vaginum (88.9 %) followed by white discharge and abdominal pain. Out of 72 cases, 9 cases (12.5 %) were premalignant lesions and the rest 63 cases (87.5 %) were malignant lesions. In premalignant lesions, CIN I accounted for 22.2 % of cases (n=2) whereas CIN II & CIN III accounted for 22.2 % (n=2) and 55.5 % (n=5) cases respectively. Out of 63 cases of malignant lesions included in this study, the most common malignancy was squamous cell carcinoma with 57 cases (79.1%) followed by adenocarcinoma with 6 cases (8.3%). (figure 1) Out of 57 cases of squamous cell carcinoma, the majority of cases were moderately differentiated carcinoma (61.4 %, n= 35), followed by poorly differentiated carcinoma (21%, n= 12). Well-differentiated carcinoma was the least in number (10 cases).

In the present study, HER-2/neu immunohistochemical staining was applied to 72 cervical lesions. Out of 72 cervical lesions, 21 cases (29.2 %) showed HER-2/neu expression. (Table 2) In 9 premalignant lesions, HER-2/ neu expression was observed in 3 patients (33.3 %) while out of 63 malignant lesions, HER-2/neu expression was noted in 18 cases (28.5 %). (Table 3,4) 16 cases (28 %) of squamous cell carcinoma showed HER-2/neu expression and in 6 cases of adenocarcinoma, 2 cases (33.3 %) showed HER-2/neu expression. (Table 4) It was observed that in comparison to the well-differentiated SCC, the number of lesions with higher scores (2+ and 3+) was considerably higher in the poorly and moderately differentiated SCC. However, the p-value of this correlation was more than 0.05.(Table 5)

In this study, a higher proportion of cervical carcinoma cases showed 2+ and 3+ expression in higher stage (stage III and IV) in comparison to lower stage (Stage I and II). (Table6)

Correlation of HER-2/neu expression and lymph node involvement and parametrium infiltration in cases of cervical malignancy was done. Higher HER-2/neu expression was noted where lymph nodes and parametrium were involved. ($p > 0.05$)

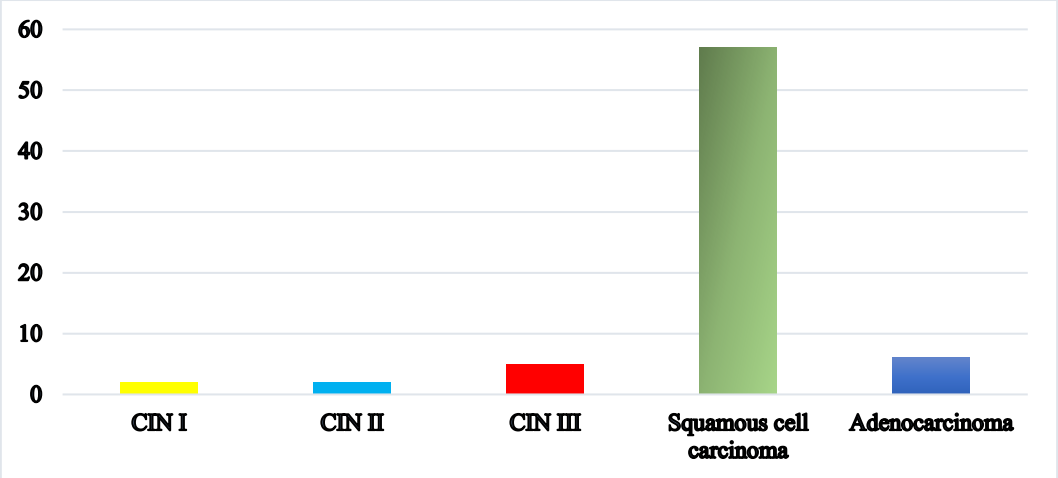

Figure 1: Shows the distribution of cases based on histopathological diagnosis.

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Table 2: Distribution of cases based on HER-2/neu expression

HER-2/neu exp	Frequency	Percent
0	51	70.8
1+	9	12.5
2+	8	11.1
3+	4	5.6
Total	72	100.0

Table 3: Distribution of cases based on HER-2/neu expression in premalignant lesions

Premalignant Cervical lesions	HER-2/neu Expression			
	0	**1+**	**2+**	**3+**
CIN I	2	0	0	0
CIN II	1	1	0	0
CIN III	3	1	1	0
TOTAL	6	2	1	0

Table 4: Distribution of cases based on HER-2/neu expression in malignant lesions

Malignant Cervical lesions	HER-2/neu Expression				p-value
	0	**1+**	**2+**	**3+**	
Squamous cell carcinoma	41	6	7	3	0.291
Adenocarcinoma	4	1	0	1	

Table 5: Distribution of cases based on HER-2/neu expression and histopathological grades of squamous cell carcinoma of cervix.

Histopathological Grade	HER-2/neu exp				p-value
	0	**1+**	**2+**	**3+**	
Well-differentiated	5	3	2	0	
Moderately differentiated	29	2	3	1	0.536
Poorly differentiated	7	1	2	2	

Table 6: Distribution of cases based on HER-2/neu expression and stage of cervical malignancy

Stage	HER-2/neu exp				p-value
	0	**1+**	**2+**	**3+**	
I	9	2	0	0	
II	22	5	3	0	0.038
III	12	0	3	2	
IV	2	0	1	2	

Table 7: Comparison of HER-2/neu expression in cervical lesions between various studies

Study	HER-2/neu expression
Bajpai *et al.*[11]	**35.7 %**
Sarwade *et al.*[5]	**44 %**
Choudhary *et al.*[10]	**56 %**
Gupta *et al.*[12]	**63 %**
Present study	**29.2 %**

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Table 8: Comparison of HER-2/neu expression and grades of SCC of cervix in various studies

Study	p-value
Joseph *et al.*[9]	0.16
Gupta *et al.*[12]	<0.05
Sarwade *et al.*[5]	0.935
Present study	0.536

Table 9: Comparison of HER-2/neu expression and stage of carcinoma cervix in various studies

Study	p-value
Joseph *et al.*[9]	0.71
Gupta *et al.*[12]	<0.05
Sarwade *et al.*[5]	0.073
Present study	0.038

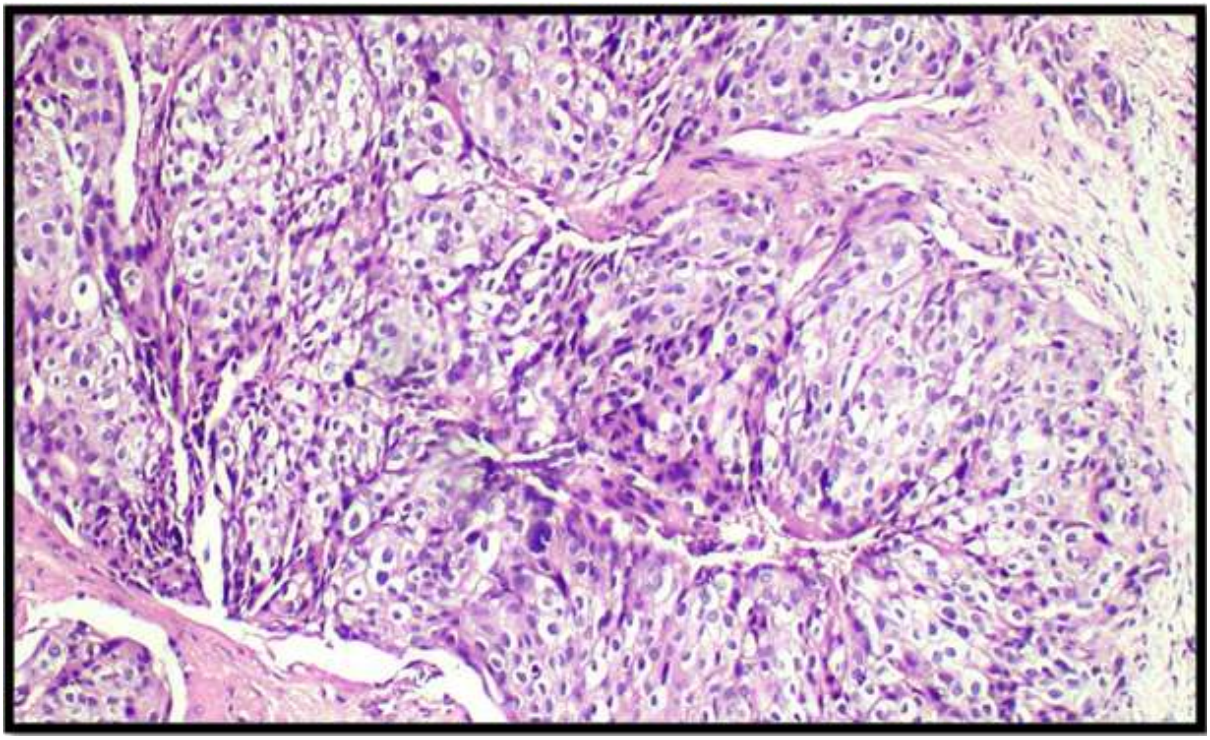

Fig 2:H&E-stained section of Squamous cell carcinoma showing nests of atypical squamous cells, in the stroma(400X)

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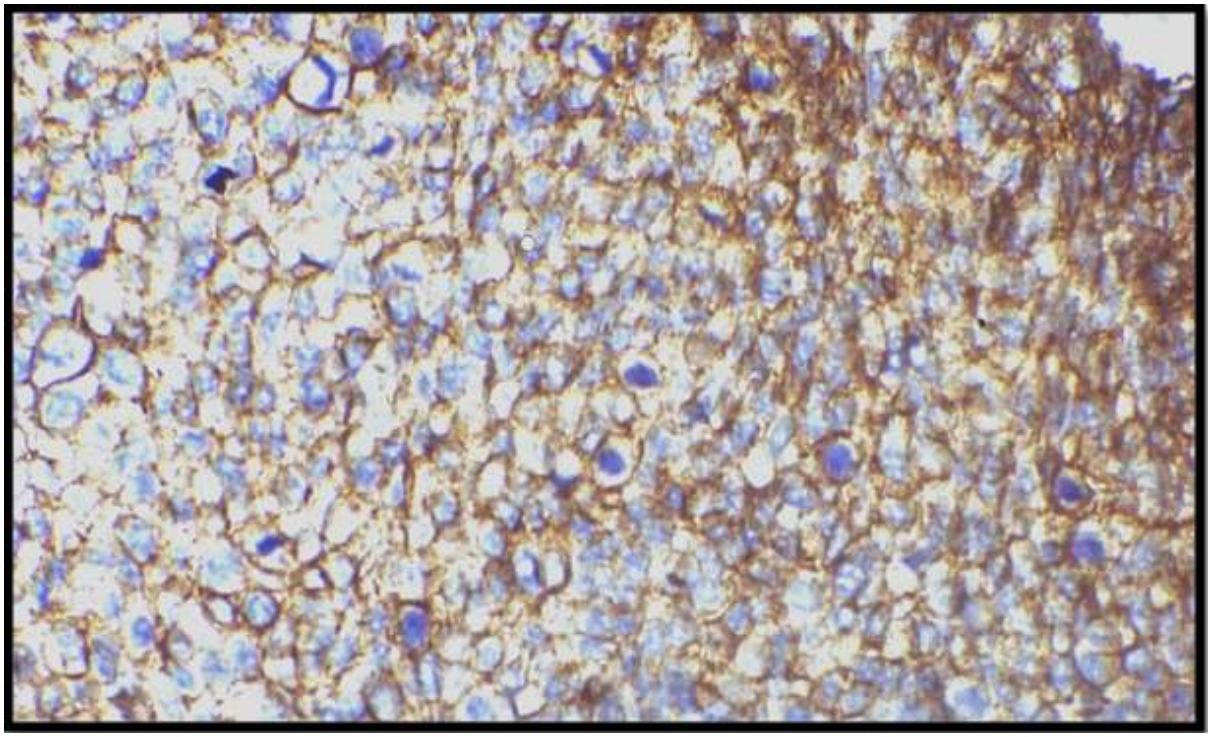

Fig 3: IHC stained section of squamous cell carcinoma showing 3+ or complete intense circumferential staining in >10% of tumor cells(400X)

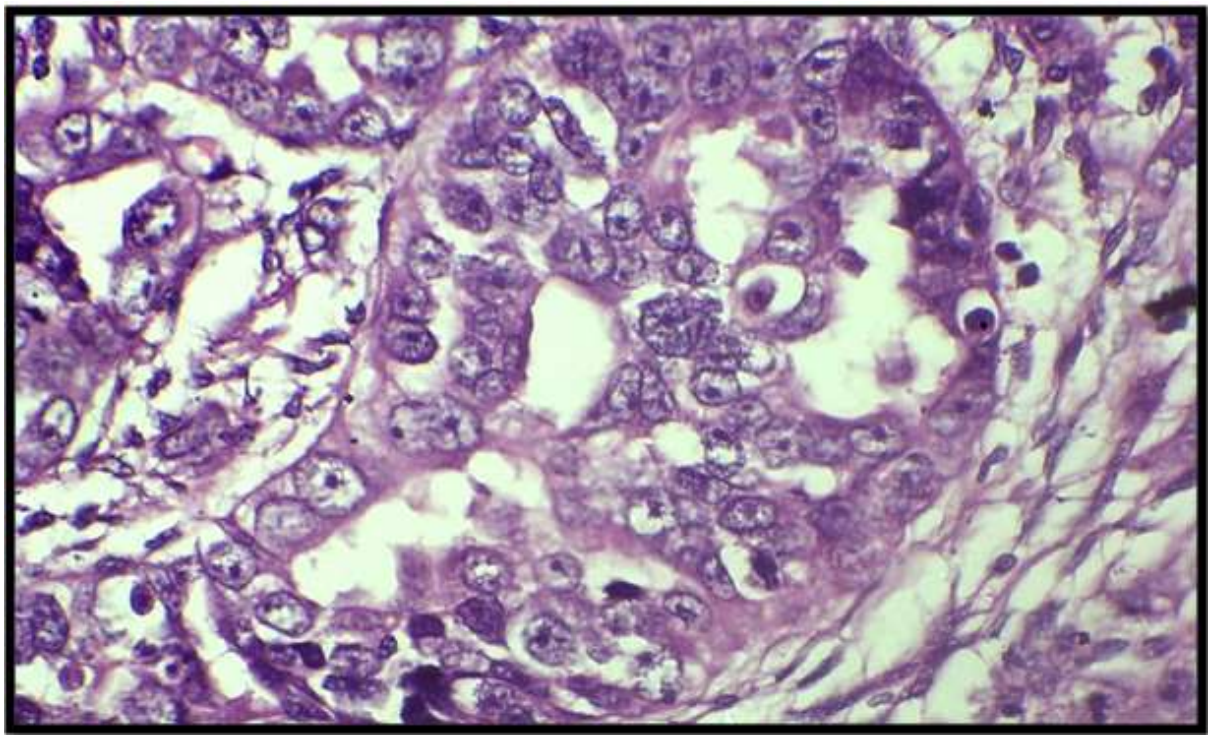

Fig 4:H &E-stained section of Adenocarcinoma showing tumor cells forming glands (100X)

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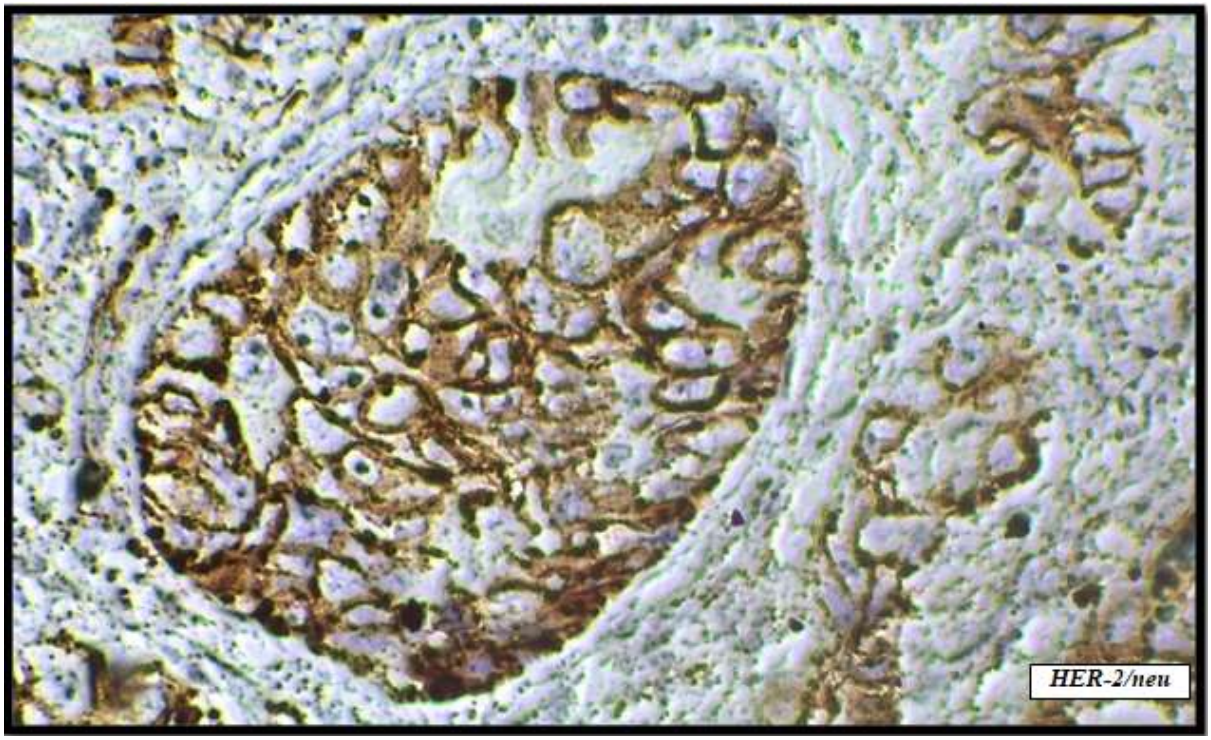

Fig 5: IHC stained section of adenocarcinoma showing 3+ or complete intense circumferential staining in >10% of tumor cells(400X)

Discussion

Cervical carcinoma is a major burden all over the world mainly affecting developing countries like India and remains a major cause of death due to cancer in India. Various types of metaplastic changes of cervical epithelium occur leading to premalignant and malignant changes. Invasive carcinoma develops from premalignant cervical lesions over time. Therefore, most lesions can be detected early on by screening programs, before they progress to malignancy. In this study on 72 patients, HER-2/neu expression was correlated in premalignant and malignant cervical lesions with various clinical parameters and histological types and grades.

In the present study, 72 cases of cervical lesions were included. Out of 72 cases, 9 cases (12.5 %) were premalignant lesions and the rest 63 cases (87.5 %) were malignant lesions.

Out of 72 cervical lesions, 21 cases (29.2 %) showed HER-2/neu expression. The result of this study was well correlated with the study conducted by Bajpai *et al.*[11] and Sarwade *et al.*[5] whereas discordant with study of Choudhary *et al.*[10] and Gupta *et al.*[12](Table 7) After an extensive literature search, it was observed that HER-2/neu expression in uterine cervix lesions had inconsistent findings. Most studies have shown a range of 14–100% HER-2/neu positive staining in cervical lesions.[13,14]

In 9 premalignant lesions, HER-2/neu expression was observed in 3 patients (33.3 %). In 3 positive premalignant lesions, one case of CIN II showed 1+ expression and 2 cases of CIN III showed 1+ and 2+ expression respectively. 3+ expression was not shown in any premalignant cases. It suggested that as the grade of disease increased, the intensity of HER-2/neu expressions also increased. This study showed concordance with the results of Li *et al.*[15] where they found 37.5 % of premalignant patients showed HER-2/neu positive staining. However, Gupta *et al.*[12], Protrka *et al.*[16]and Joseph *et al.*[9] observed 60 %, 66 % & 70 % HER-2/neu expression respectively. However, Bajpai *et al.*[11] found only 4 patients showing HER-2/neu positivity out of 27 premalignant cases (14.8 %).

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In this study, the correlation of HER-2/neu expression of premalignant & malignant lesions and found that higher score (2+ and 3+) was seen more in malignant lesions (17.5 %, n= 11) than premalignant lesions (11.1 %, n=1). Similar results were also observed by Bajpai *et al*.[11] and Joseph *et al.*[9] This suggested that there was a gradual progression in HER-2/neu expression score as the lesion moved to a higher spectrum.

In this study, out of 63 malignant lesions, HER-2/neu expression was observed in 18 cases (28.5 %). According to various studies expression of HER-2/neu in cervical carcinoma varied from 12.1% to 96.8%. Differences in the number of cases investigated, different age groups of patients included in the study, and variations in the tumor stage may be the reason for this variability.

In this study, correlation of the grade of squamous cell carcinoma with HER-2/neu expression was done and found that well-differentiated squamous cell carcinoma showed 1+ and 2+ scores. As the degree of differentiation increased from well to moderately and poorly differentiated carcinoma proportion of 2+ and 3+ cases also increased. (Table 8)

In this study, the correlation of HER-2/neu expression with the stage of cervical malignancy was done, and found that a proportion of cases of cervical carcinoma that showed higher expression (2+ and 3+ scores) were in the higher stage (stages III and IV) in comparison to the lower stage (Stages I and II). Gupta *et al*.[12] observed similar findings in their study. (Table 9)

In this study, the correlation between HER-2/neu expression and lymph node status was done in 63 cases of cervical malignancy. In 63 cervical cancer cases, lymph nodes were positive in 17 cases which were detected radiologically. Out of 17 cases, 7 cases showed HER-2/neu expression. 1 case showed 1+ expression while 2+ and 3+ expression was seen in 6 cases (3 each). This suggested that the presence of lymph nodes in cervical carcinoma correlates with higher expression of HER-2/neu biomarker. A similar finding was mentioned by Joseph *et al.*[9] and Gupta *et al*.[12] Mandai *et al*[17]and Yong *et al*[18] analyzed HER-2 expression in 39 and 74 cases of cervical adenocarcinoma respectively and stated that a significant correlation existed between lymph node metastasis & staining of HER-2/neu biomarker.

In this study, the correlation between HER-2/neu expression and parametrium infiltration was studied in 63 cases of cervical carcinoma. Out of 63 cases of cervical malignancy, parametrium infiltration was seen in 33 patients which were detected radiologically. Out of 33 patients, higher HER-2/neu intensity scores (2+ and 3+) were seen in cases where parametrium was involved.

Conclusion

In premalignant lesions, HER-2/neu expression rises as the lesion advances, representing its involvement in higher-grade lesions. HER-2/neu expression intensity increases with the advancement of grade and stage of cervical malignant lesions. HER-2/neu expression was correlated with the histological types of cervical malignancies, indicating a potential function for HER-2/neu in carcinogenesis.

In this study, HER-2/neu expression in cervical lesions was observed to be low, and statistical significance could not be established in few of them may be due to smaller study sample and limited study period. Since each study reveals a distinct pattern, it is necessary to continue tracking these trends with bigger sample numbers.

This study has suggested that HER-2/neu expression is a poor prognostic factor. So early immunohistochemistry detection of HER-2/neu can guide the course of disease in the initial stage. Molecular targeted therapy against HER-/neu may help in decreasing morbidity and mortality in cervical cancer patients.

Conflict of interest:

None

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