

The Expression of HER2/neu in Cervical Carcinoma

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Introduction: Cervical carcinoma is the fourth most deadly cancer in women. It affects 16 per 100,000 women per year. Approximately 70% of cervical carcinoma occur in developing countries. HER-2/neu gene expression occurs in cervical cancers and also seen in other tumors like breast, stomach, ovary, uterine serous endometrial carcinomas, colon, bladder, lung, head and neck and esophagus. Our aim was to evaluate the expression of HER2/neu in cervical carcinoma.

Material & Methods: This cross-sectional study was taken at the Department of Pathology, SSMC and Mitford Hospital Dhaka from the period of 1 January 2016 to 31 December 2017. Total 70 cervical biopsies and hysterectomies specimen with histologically confirmed carcinoma of cervix. Sample technique was purposive & convenient sampling. All the patient with carcinoma of cervix diagnosed by H&E stain were enrolled in this study. A descriptive analysis was performed for all data. Ethical clearance and permission was taken from the institutional ethical committee of SSMC.

Results: It was observed that more than one third (41.4%) of patients belonged to age 51-60 years. The mean age was 53.96±9.14 years. (Age range 35 to 75 years). Regarding distribution of the patients according to age of menarche, it was observed that more than half (57.1%) of patients belonged to age of menarche 11-12 years. The mean age of menarche was found 12.23±1.49 years which ranged from 9 to 16 years. Almost three fourth (70.0%) of patients were over 16 years when they got married. The mean age at marriage was 16.27±2.69 years which ranged from 13 to 21 years. 12 cases of SCC grade I, 75% cases shows 1+ membrane staining. 37 patients belonged to SCC grade II, among them 51.4% cases show 2+ membrane staining, 21.6% cases show 1+ membrane staining and 13.5% cases show 3+ membrane staining. Rest of the cases of SCC grade 2 expressed no definite membrane staining. Among 5 cases of SCC grade III, all of them shows 3+ membrane staining. Among 12 ADC cases, 58.3% cases show 1+ membrane staining and rest the cases do not show definite membrane staining. In 4 adenosquamous cases, 75% cases show 1+ membrane staining and rest of the cases do not show any membrane positivity.

Conclusion: HER2/neu amplification in cervical carcinoma suggests the role of this gene in tumorigenesis and histological behavior of carcinoma cervix. As HER2/neu expression is present in a significant proportion of cases with carcinoma cervix.

Keywords: Cervical carcinoma, Expression, Cancer, Membrane, Kinase.

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1. Introduction

Cervical carcinoma is the fourth most deadly cancer in women¹. It affects 16 per 100,000 women per year². Approximately 70% of cervical carcinoma occur in developing countries¹. In industrialized countries with well-established cytology screening programs, the incidence of cervical cancer ranges from 4 to 10 per 100,000 women. It is common in lower socio economic groups, in women with early sexual activity and/or multiple sexual partners and in smokers³. HER-2/neu also known as c-erbB-2, CD 340 and p 185, stands for "Human Epidermal Growth Factor Receptor 2". It is a cell membrane surface bound receptor tyrosine kinase and is involved in signal transduction pathway leading to cell growth and differentiation⁴. It is encoded by proto-oncogenes HER-2/neu located on long arm of human chromosome 17(17q21q22). It plays an important role in coordinating the complex Erb B signaling network that is responsible for regulating cell growth and differentiation⁵. The amplification of this gene is associated with resistance to treatment and poor survival, suggesting that cells over expressing HER-2/neu may manifest a more aggressive biological behavior and may have a selective growth advantage over HER-2/neu-negative tumor cells⁶. HER-2/neu has been shown to be

over expressed in breast and female genital tract malignancies⁶. Overexpression of this gene leads to constitutive activation of tyrosine kinase residues. HER-2/neu immunostaining was performed by streptavidin-biotin peroxidase method. Higher expression of HER-2/neu was noted in malignant lesions as compared to benign lesions. The over-expression of HER-2 oncoprotein is associated with poor prognosis, metastatic potential and aggressive biological behavior. HER-2/neu gene expression occurs in cervical cancers and also seen in other tumors like breast, stomach, ovary, uterine serous endometrial carcinomas, colon, bladder, lung, head and neck and esophagus. HER2 is over expressed in a number of solid tumors⁷. Its over expression and prognostic significance in breast cancer led to the development and approval of the use of trastuzumab (Trastuzumab, Genentech, and South San Francisco, CA), a recombinant monoclonal antibody to HER2, for the treatment of patients with metastatic breast carcinomas over expressing HER2⁷. Its over expression has been detected in CIN and cervical cancer and is believed to be associated with poor prognosis, aggressive biological behavior and enhanced metastatic potential⁸. There are variable reports on expression of HER-2/neu in cervical carcinoma either due to heterogeneity of the lesion or due to poor antigen retrieval⁹. Very few studies so far been carried out on expression of HER2/neu in cervical cancer and pre-cancerous lesions in our country.

2. Methods

This cross-sectional study was taken at the Department of Pathology, SSMC and Mitford Hospital Dhaka from the period of 1 January 2016 to 31 December 2017. Total 70 cervical biopsies and hysterectomies specimen with histologically confirmed carcinoma of cervix were selected according to following inclusion and exclusion criteria. Sample technique was purposive & convenient sampling. All the patient with carcinoma of cervix diagnosed by H&E stain were enrolled in this study. Routine tissue processing was done employing paraffin embedded method. After processing the tissues were sectioned at 4 μ m thickness stained with H&E. Total 8 (eight) cases of chronic cervicitis diagnosed by H&E were taken as control for each batch of cases. The tumors were classified initially on H&E stained sections according to WHO classification. The latter is subdivided into well, moderate and poorly differentiated variety. Tumors with acinar differentiation or widespread mucin secretion in at least 75% of the tumor volume was labelled as adenocarcinoma. A lesions exhibiting both squamous and acinar differentiation with the minor component constituting at least one third of the tumor was considered as adenosquamous carcinoma. After histopathological examination relevant points were taken from the report and included in the predesigned proforma. Immunostaining for HER-2 was done at Delta Medical College and Hospital. For immunohistochemistry staining 4-micrometer thick tissue sections were taken on poly-L lysine coated slide from the paraffin blocks of tumor. The tissue was then processed for immunostaining for HER-2/neu. Only the membrane staining intensity and pattern was evaluated on a scale of 0 to 3. Scores of 0 and 1+ were considered negative. Immunoreactivity of 2+ and 3+ was scored as positive. Statistical analyses were carried out by using the Statistical Package for Social Sciences version 20.0 for Windows (SPSS Inc Chicago Illinois USA). A descriptive analysis was performed for all data. Ethical clearance and permission was taken from the institutional ethical committee of SSMC.

Inclusion Criteria

- Adult female patients with cervical cancer diagnosed histopathologically attending Sir Salimullah Medical College & Mitford Hospital and other renowned hospital during the study period were included in the study.

Exclusion Criteria

- Patients with a coexistent pathology of any other organ like uterus and ovary etc. in addition to cervix was excluded.
- Tissue blocks with extensive necrosis or hemorrhage was also excluded from the study.
- Patients who refused to be included in the study.
- Patients with metastatic carcinoma of cervix.
- Patients who underwent radiotherapy and or chemotherapy.

3. Results

It was observed that more than one third (41.4%) of patients belonged to age 51-60 years. The mean age was 53.96 \pm 9.14 years. (Age range 35 to 75 years)(Figure 1). Regarding distribution of the patients according to age of menarche, it was observed that more than half (57.1%) of patients belonged to age of menarche 11-12 years. The mean age of menarche was found 12.23 \pm 1.49 years which ranged from 9 to 16 years (Table 1). Almost three fourth (70.0%) of patients were over 16 years when they got married. The mean age at marriage was 16.27 \pm 2.69 years which ranged from 13 to 21 years (Table 2). Regarding menstrual status of the patients, more than three fourth (78.6%) of patients were premenopausal and 15 (21.4%) postmenopausal (Figure 2). Parity of patients, it was observed that more than one third (34.3%) of patients were para four followed by

28.6% were para five. Other findings are depicted in the (Figure 3). It was observed that only 7(10.0%) patients disclosed positive history of exposure (Table 3). Majority of (82.9%) patients underwent hysterectomy and 12(17.1%) underwent biopsy (Table 4). Out of total 70 cases of carcinoma cervix, 54 cases were diagnosed as SCC, 12 cases belong to ADC and 4 cases belonged to adenosquamous by WHO classification. Out of 54 cases of SCC, 12 cases belonged to grade I. Among them 75% cases expressed 1+ staining and rest of the cases showed no membrane staining. 37 cases belong to SCC grade II, among them 51.4% cases expressed 2+ staining, 21.6% cases express 1+ staining, 13.5% cases expressed 3+staining and rest of the cases expressed no definite membrane staining. 5 cases belong to SCC grade III. Among them 60% expressed 3+ staining and 40% cases expressed 2+ staining. Out of total 70 cases, 12 cases were diagnosed as adenocarcinoma. Among them 58.3 cases expressed 1+ staining and rest of the cases showed no definite membrane staining. . Out of total 70 cases, 4 cases belonged to adenosquamous carcinoma of which 75% cases expressed 1+staining and rest of the cases did not show any membrane staining. Statistically significant difference in staining pattern was observed in different types of cervical carcinoma (P=0.001) (table 5).It was observed that among 12 cases of SCC grade I, no cases were found to express Her2. 37 patients belonged to SCC grade II, among them 24 cases were positive for Her2 and 13 cases were negative for HER2. Among 5 cases of SCC grade III, all were positive for HER2 staining. Among 12 ADC cases, none of them were positive for HER2. In 4 adenosquamous cases, all were negative for HER2. A significant association was found between HER2 expression and different types of cervical carcinoma diagnosed by WHO classification (P=0.001) (Table 6).

Figure 1: Bar diagram shows age distribution of the patients (n=70)

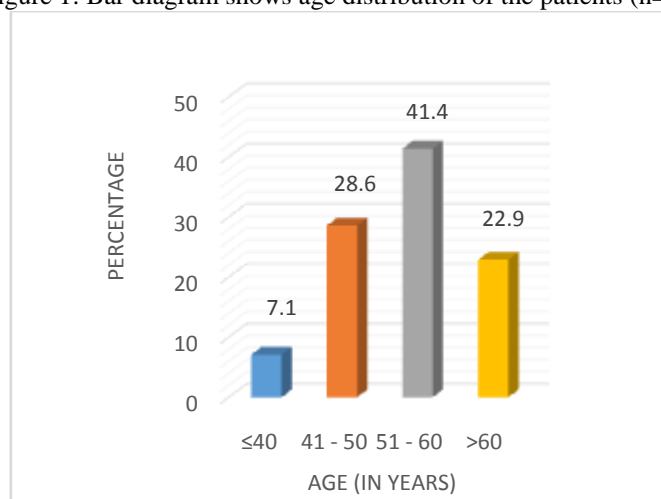


Table 1: Distribution of the patients according to age of menarche (n=70)

Age of menarche (years)	Number of patients	Percentage
≤9	4	5.7
11-12	40	57.1
13-14	20	28.6
>15	6	8.6
Mean±SD	12.23±1.49	
Range (min, max)	9,16	

Table 2: Distribution of the patients according to age at marriage (n=70)

Age at marriage (years)	Number of patients	Percentage
≤16	21	30.0
>16	49	70.0
Mean±SD	16.27±2.69	
Range (min, max)	13,21	

Figure 2: pie chart shows menstrual status of the patients

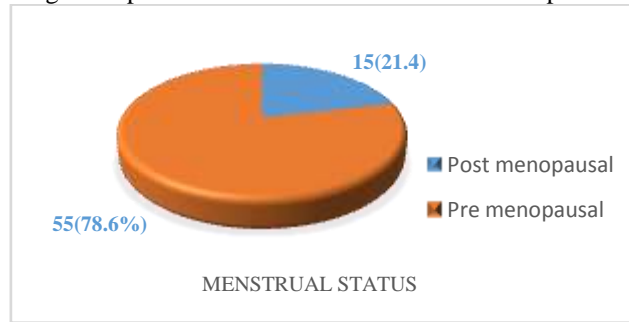


Figure 3: pie chart shows parity of the patients

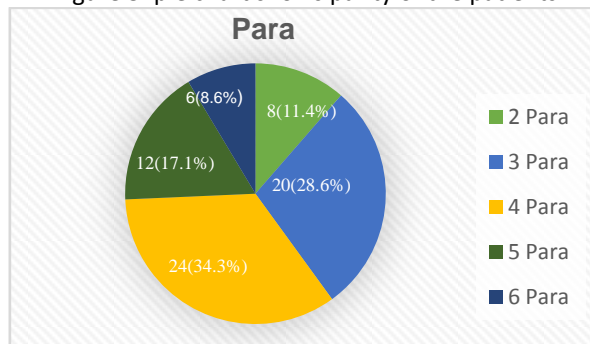


Table 3: History of exposure of patients (n=70)

Exposure history	Number of patients	Percentage
Yes	7	10.0
No	50	71.4
Not answered	13	18.6

Table 4: Types of specimen (n=70)

Specimen types	Number of patients	Percentage
Hysterectomy	58	82.9
Biopsy	12	17.1

Table 5: Expression of Her-2 Staining pattern in different Histopathological classification of cervical carcinoma according to WHO (n=70)

Her-2 Staining Pattern	Histopathological classification										P value
	SCC I (n=12)		SCC II (n=37)		SCC III (n=5)		Adeno carcinoma (n=12)		Adeno squamous (n=4)		
0	n	%	n	%	n	%	n	%	n	%	0.001s
1+	3	25.0	5	13.5	0	0.0	5	41.7	1	25.0	
2+	9	75.0	8	21.6	0	0.0	7	58.3	3	75.0	
3+	0	0.0	19	51.4	2	40.0	0	0.0	0	0.0	
s= significant (P<0.05) P value reached from chi square test											

Table 6: Association between cervical carcinoma by WHO with Her-2 expression (n=70)

Histopathological Classification by WHO	n	Her-2				P value
		Yes		No		
SCC grade I	12	n	%	n	%	0.001s
SCC grade II	37	0	0.0	12	100	
SCC grade III	5	24	64.86	13	35.13	
Adeno carcinoma	12	5	100	0	0.0	
Adenosquamous	4	0	0.0	12	100	
s= significant p value reached from chi square test						

4. Discussion

In this present study, it was observed that most of patients belonged to 51-60 years age group. The mean age was 53.96 ± 9.14 years. Age range 35 to 75 years. In another study, Mauer et al¹⁰ reported that most (85%) of the carcinoma cervix patients ranged from 31 to 60 years of age while youngest patient was 30 years and the oldest patient was 81 years old. Maibam and Singh¹¹ reported that 64 patients with cervical cancer, majority fall in the age group of 40+ years (88.9%). Sharma et al¹² found 36% cases in fifth decade of life indicating maximum incidence in premenopausal age group. Ray et al⁴ also found similar age group involved by cervical carcinoma. In contrast Makuza et al¹³ found mean age of the women presenting for screening of carcinoma cervix was 37.0 years and most of their participants fall between 30 and 35 year (47.7%), which was below the mean age observed in present study and also below the age range found in this study. Regarding age at menarche, it was observed that 57.1% patients belonged to 11-12 years of age group. The mean age of menarche was found 12.23 ± 1.49 years which ranged from 9 to 16 years. Boyd and Doll¹⁴ at England found age at menarche at 14 years. Present study found similar age range of menarche. In the present study, it was observed that 70.0% of patients got married when their ages were >16 years. The mean age of marriage was found 16.27 ± 2.69 years which ranged from 13 to 21 years. The average age of marriage of 288 married women with carcinoma of the cervix was found 22.7 years which was higher than that of present study¹⁴. In this current study, 78.6% of patients with carcinoma of cervix were premenopausal and 21.4% postmenopausal. Regarding parity of patients 34.3% patients were para four followed by 28.6% para three, and 17.1% Para five. Mauer et al¹⁰ documented very few cases of carcinoma cervix in females with low parity status compared to 35.9 % of cases with parity ≥ 05 and 75.4 % of cases having parity ≥ 3 , which was consistent with the current study. In this present study, it was observed that 10.0% patients had positive history of exposure. However no previous studies commented on this issue. In this current study, it was observed that 82.9% patients underwent hysterectomy and 17.1% biopsy. In the present study most of well differentiated SCC showed 1+, moderately differentiated showed 2+ and poorly differentiated showed 3+ membrane staining. Gupta et al¹⁵ showed a significantly higher expression of HER-2/neu in squamous cell carcinoma of cervix and found that 2 (+) and 3 (+) membrane staining of HER2/ neu in 33 (44%) of carcinoma cases and 1 (10%) of CIN cases. Brummet al¹⁶ reported a high rate (75%) of expression of HER2/neu in SCC carcinoma of cervix. An study by Sarwade et al¹⁷ showed that moderately and poorly differentiated squamous cell carcinomas showed 2 (+) and 3 (+) positivity respectively where as well differentiated carcinoma showed only (+) positivity. However because of small sample size, statistical correlation could not be established between HER-2/neu expression and different grades of SCC ($p > 0.05$). Joseph et al¹⁸ also did not find any correlation between HER-2/neu expression and grade of SCC. Present study finding were comparable to the findings showed by Sarwade et al¹⁷, Gupta et al¹⁵ and Brumm et al¹⁶ but contrast to Joseph et al¹⁸. In present study, regarding HER2 membrane staining in adenosquamous carcinoma out of the total 4 cases 3 cases show 1+ membrane staining and rest of the cases did not show any membrane positivity and in adenocarcinoma, out of the total 12 cases 7 cases showed 1+ membrane positivity and rest of the cases did not show any membrane positivity. Sharma et al¹² showed in their study that out of 3 cases adenocarcinoma, none of them was found to be strongly positive. All cases showed 1(+) membrane positivity i.e., mild positivity. Kihana et al¹⁹ studied 44 cases of cervical adenocarcinoma and found (+) and (++) positivity in 34 (77%) cases. Gupta et al¹⁵ found out 13 cases (60%) of adenocarcinoma, most of the cases showed 2 (+) positivity present study finding were similar to above studies. In this present study, it was observed that 37 patients were of SCC grade II when diagnosed by H&E stain, when evaluated by Her-2 among them 64.86% cases were Her2 positive and 35.13% were Her-2 negative. SCC grade I were 8 cases and all of them were Her2 negative. SCC grade III was 5 cases and all of them were Her2 positive. ADC and Adenocarcinoma were 12 and 4 cases respectively and all were Her2 negative. Sarwade et al¹⁷ in their study showed 52% of carcinoma cases expressed positive membrane staining for HER2/neu (13/25), which showed significant association. Study by Gupta. et al¹⁵ mentioned that out of 48 cases of SCC, HER2 positivity was found 26 cases of SCC which showed no significant ($p > 0.05$) association between HER2/neu positivity and grades of squamous cell carcinoma.

Limitations of the study

This was a single center study. Limited sample size with short duration. So, that the results of the study may not reflect the exact picture of the country.

5. Conclusion

The present study showed SCC is the most common type of cervical carcinoma (77.13%) followed by adenocarcinoma (17.14%) and adenosquamous carcinoma (5.7%). Regarding HER2 expression out of total 70 cases 29 cases were HER 2 positive- most of them showing 2 + membrane positivity. It was observed membrane positivity increased with increasing grades of tumor. A significant association was found between HER2 expression and different type of cervical carcinoma. HER2/neu amplification in cervical carcinoma

suggests the role of this gene in tumorigenesis and histological behavior of carcinoma cervix. As HER2/neu expression is present in a significant proportion of cases with carcinoma cervix.

6. Recommendations

It could have therapeutic implications instituting trastuzumab therapy in treatment protocol of cervical carcinoma. Further studies are needed attending this issue.

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