Original Article

Immunohistochemical expression of human epidermal growth factor receptor 2 (HER2/neu) in colorectal carcinoma: experience in a tertiary care hospital of Bangladesh

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ABSTRACT

Background: *Among various immunological markers of colorectal carcinoma, the human epidermal growth factor receptor 2 (HER2/neu) is a significant one. Overexpression of HER2/neu in breast cancer has already been established as a poor prognostic marker and trastuzumab (a monoclonal antibody against HER2/neu) has been shown to increase the patient survival. The success of anti-HER2 (Herceptin) therapy in breast cancer has led to the evaluation of HER2/neu expression in colorectal and other cancers. The aim of the study was to determine the expression of HER2/neu in colorectal carcinoma.*

Methods: *This cross-sectional study included 97 diagnosed cases of colorectal carcinoma and was conducted in the Department of Pathology, BIRDEM General Hospital, Dhaka from September 2018 to August 2020. Sections were taken from paraffin blocks for routine hematoxylin and eosin (H&E) stain and immunohistochemical stain with HER2/neu antibody. Relevant clinical and microscopic data including age, sex, tumor site and tumor size were collected and recorded.*

Results: *HER2/neu expression was observed in 47.4% (46) cases in this study. Among which 28.9% cases were found strongly positive (Score 3+) and 18.6% cases were found weakly positive (Score 2+). The mean age of colorectal carcinoma patient was 50.54±14.32 years and the highest expression of HER2/neu was found in 21-30 years age group. The tumor was mostly (66.0%) located in the colon whereas HER2/neu expression was more in rectum (54.5%). The tumor size was ≥5 cm in 64.9% cases while positive expression of HER2/neu was more in tumors <5 cm in size (50.0%). No statistically significant correlation of HER2/neu expression was found with age, sex, tumor site and size.*

Conclusion: *Expression of HER2/neu has been observed in nearly half of colorectal carcinoma patients with strongly positive in almost 29% cases.*

Key words*: colorectal carcinoma, HER2/neu expression, immunohistochemistry.*

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INTRODUCTION

Colorectal cancer has been considered as one of the most common malignancies all over the world. It is the 3rd most common cancer after lung and breast cancers and the 2nd leading cause (10% of all cancer deaths) of death from cancer.[1] The prognosis of colorectal carcinoma is related to a number of clinicopathologic parameters and molecular markers. The epidermal growth factor receptor (EGFR) is an established marker for prognosis of colorectal carcinoma. [2] The HER2/neu (EGFR2/ErbB2/CD340/P185) is the most potent oncogenic member of EGFR family. It is located on chromosome 17_q12 and encodes a 185 kD transmembrane protein with tyrosine kinase activity that functions as a growth factor receptor. The activation of

this receptor initiates a variety of signaling pathways that results in cell proliferation, survival, differentiation, angiogenesis and invasion. [3] In a variety of neoplastic cells, gene amplification and protein overexpression of HER2/neu is found leading to dysregulation of receptor-initiated pathways. As a result, there is abnormality in cell growth, progression and migration.[4]

In spite of earlier diagnosis and use of new treatment modalities, the mortality in colon cancer remains high. Investigators have focused on biological markers that could serve as prognostic and predictive factors as well as target for therapy. Tumors with HER2/neu overexpression are associated with higher postoperative recurrence rate leading to poor survival.[5] As a result, the role of HER2/neu is rising as a biomarker for prognostic & therapeutic target. Use of Herceptin (anti-HER2) therapy has been shown to prolong the survival in patients with HER2/neu overexpressing breast cancer.[6] Diverse rates of overexpression or amplification of HER2/neu has been reported in different population groups and different countries in colorectal carcinomas. Patients with colorectal carcinoma showing significant HER2 expression could further undergo targeted therapy by Herceptin which might prolong the survival of the patient. The aim of this study was to see the expression of HER2/neu in colorectal carcinoma in our population.

METHODS

This cross-sectional study was conducted in the Department of Pathology, BIRDEM General Hospital, Dhaka from September, 2018 to August, 2020. The study had been approved by hospital ethical committee. It included 97 patients of colorectal carcinoma who underwent surgical resection of colon. Patient who received chemotherapy/radiotherapy prior to surgery were excluded from the study. All surgical specimens were properly grossed and paraffin blocks and H&E-stained glass slides were prepared as per standard guidelines.

Two sections of 4-micrometer thickness were taken from each paraffin block. One section was stained for hematoxylin and eosin (H&E). The other section was taken on poly-L lysine coated slides and stained with monoclonal rabbit anti-human c-erbB2 oncoprotein (Code No. A0485). 1:300 dilution was used. This method was carried out manually following the avidin-biotin-peroxidase staining method. Positive controls were taken from HER2/neu positive breast carcinomas and negative controls were also taken.

Among 97 study cases, relevant data with attention to age, sex, site and size of tumor were recorded. Histopathological diagnosis was done on routine hematoxylin and eosin-stained sections. Scoring of expression of HER2/neu was done by a four-point scale described below.[7] The results were recorded and analyzed.

The percentage of stained cells was assessed by using a 4-point scale [7]:

Staining pattern	Score	HER2/neu protein overexpression assessment
No staining is observed or membrane staining is observed in less than 10% of the tumor cells.	0	Negative
A faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane.	1+	Negative
A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells.	2+	Weakly positive
A strong complete membrane staining is observed in more than 30% (formerly 10%) of the tumor cells.	3+	Strongly positive

RESULTS

The age of the present study population ranged from 20- 82 years with a mean age of 50.54 ± 14.32 years. Maximum positivity of HER2/neu was found in 21-30 years of age group. However, no significant correlation was found between positive expression of HER2/neu and different age groups. Male were more predominant than female with male to female ratio of 1.5:1. HER2/neu expression was found more in female patients without any statistically significant correlation (Table I).

In this study, out of 97 cases, 46 (47.4%) cases were found positive in term of expression of HER2/neu. Among which 28 (28.9%) cases were found strongly positive (Score 3+) and 18 (18.6%) cases were found weakly positive (Score 2+) (Figure- 1-3) (Table II). Remaining 51 (52.6%) cases were found negative. Among these, 22 (22.7%) cases showed score 1+ and 29 (29.9%) cases showed score 0 (Figure- 04) (Table II).

Site of tumor is categorized in this study into two groups, colon and rectum. Though colon was commonest site, HER2/neu positivity was more in rectal tumors that was 54.5%. However, there was no statistically significant correlation (Table III).

In the present study, the size of tumor ranged from 1.5-22.0 cm. Positive expression of HER2/neu was found more in tumors smaller than 5 cm. However, the data showed no significant correlation between expression of HER2/neu and size of tumor (Table IV).

Table I. Demography of study population with expression of HER2/neu (N=97)

Demography of study population	No. of cases	Her2/neu positive	Her2/neu negative	P value
Age (years)				
11-20	1 (1.0%)	0(0%)	1 (100%)	
21-30	7 (7.2%)	4 (57.1%)	3 (42.9%)	
31-40	20 (20.6%)	9 (45%)	11 (55%)	
41-50	25 (25.8%)	13 (52%)	12 (48%)	
51-60	21 (21.6%)	10 (47.6%)	11 (52.4%)	
61-70	14 (14.4%)	7 (50%)	7 (50%)	
≥71	9 (9.3%)	3 (33.3%)	6 (66.7%)	
Total	97 (100%)	46 (47.4%)	51 (52.6%)	.707^{ns}
Mean ± SD (Min- max)		50.54 ± 14.32 (20-82)		
Sex				
Male	58 (60%)	26 (44.8%)	32 (55.2%)	
Female	39 (40%)	20 (51.3%)	19 (48.7%)	
Total	97 (100%)	46 (47.4%)	51 (52.6%)	.532 ^{ns}

Table II. Immunohistochemical expression of HER2/neu in colorectal carcinoma (N=97)

Expression of HER2/neu	No of Cases	Percentage
Positive	46	47.4%
	3+ 28	3+ 28.9%
	2+ 18	2+ 18.6%
Negative	51	52.6%
	1+ 22	1+ 22.7%
	0 29	0 29.9%
Total	97	100%

Table III. HER2/neu overexpression in different sites of tumor (N=97)

Site of tumor	No. of cases	Her2/neu positive	Her2/neu negative	P value
Colon	64 (66.0%)	28 (43.7%)	36 (56.3%)	
Rectum	33 (34.0%)	18 (54.5%)	15 (45.5%)	.313 ns
Total	97 (100%)	46 (47.4%)	51 (52.6%)	

Table IV. HER2/neu overexpression in different sizes of tumor (N=97)

Size of tumor	No. of cases	Her2/neu positive	Her2/neu negative	P value
< 5 cm	34 (35.1%)	17 (50%)	17 (50%)	
≥5 cm	63 (64.9%)	29 (46%)	34 (54%)	
Total	97 (100%)	46 (47.4%)	51 (52.6%)	.709 ns
Mean ± SD		5.85 ± 2.83		
Median		5.50		
Range of size (Min. – Max.)		1.5 -22		

Table V. Expression of HER2/neu in colorectal carcinoma in different studies

Author	Year	Country	Study Population	HER-2 + ve cases (%)
Current Study	2020	Bangladesh	97	47.4%
Park et al	2007	South Korea	137	47.4%
Jesus et al	2005	Brazil	117	48.1%
Heidari et al	2017	Iran	137	54.0%
Torabizadeh et al	2016	Iran	50	40.0%
Mckay et al	2002	UK	249	81.5%
Sayadnejad et al	2017	Iran	50	60.0%
Demirbas et al	2006	Istanbul	124	44.3%
Abdul et al	2014	Iraq	25	24.0%
Kavanagh	2009	Ireland	106	11.0%
Kountourakis et al	2006	Greece	106	5.6%
Pappas et al	2013	Greece	51	3.9%
Heppner et al	2014	Germany	1645	1.6%

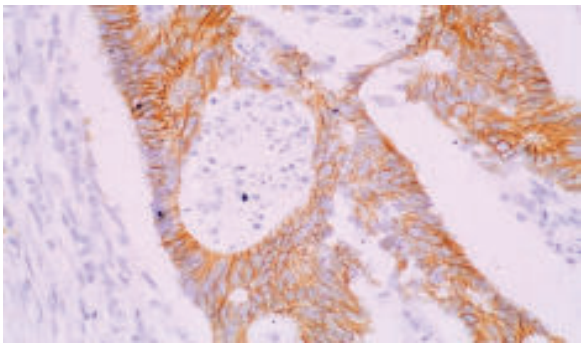

Figure 1. Photomicrograph of moderately differentiated colonic adenocarcinoma, showing positive HER2 expression (score-3+) (IHC40x)

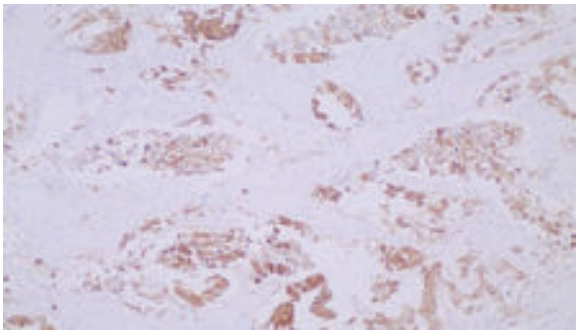

Figure 2. Photomicrograph of moderately differentiated colonic adenocarcinoma, showing positive HER2 expression (score-3+) (IHC 10x)

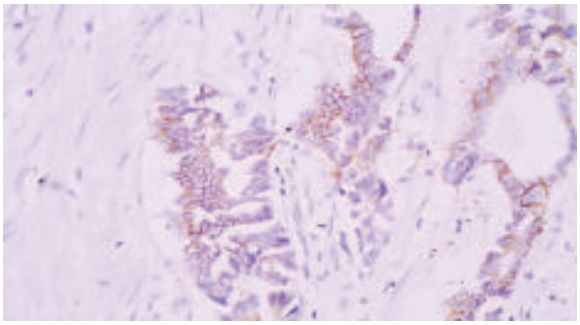

Figure 3. Photomicrograph of moderately differentiated colonic adenocarcinoma, showing positive HER2 expression (score-2+) (IHC40x)

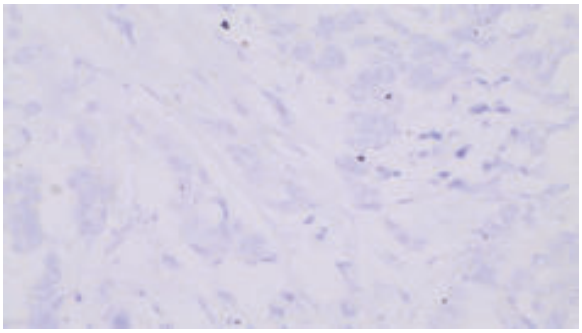

Figure 4. Photomicrograph of poorly differentiated colonic adenocarcinoma (signet ring cell type), showing negative HER2 expression (score-0) (IHC 40x)

DISCUSSION

Colorectal carcinoma is a heterogeneous disease. Combination of various cytotoxic agents with monoclonal antibodies directed against EGFR (cetuximab, panitumumab) and VEGF (bevacizumab) has improved the survival in metastatic colon cancer. [8] Trastuzumab (Herceptin), being a member of cetuximab group, could be used as targeted therapy in HER2/neu overexpressing colorectal carcinoma. This might prolong the survival and could be used as a prognostic factor.

The age range in the present study population was from 20 to 82 years with a mean age of 50.54±14.32 years. This finding was similar to the studies performed in India, Austria and Iraq in which the mean ages were 53.9±16.7 years (ranging from 19-88), 52.5 years (ranging from 30-83 years) and 56.5 years (ranging from 24-89 years) respectively.[9-11] Higher mean ages of colorectal carcinoma were observed in studies performed in Iran (60.2±13.9 years) and Greece (70.9±9.3 years).[6, 12] The variation in age range may be due to genetic causes, differences in lifestyle, geographical, racial and ethnic variations. In this study, the expression of HER2/neu was observed in different age groups of patients. Maximum positivity (57.1%) of HER2/neu was present in 21-30 years age group. However, no significant correlation was found which is similar to the studies performed in UK, Iran and Ireland.[4, 12-14].

In this study, the incidence of colorectal carcinoma was predominant in male population being 60.0%. This data was consistent with other studies performed in India (60% male), Iraq (56.0% male) and Iran (54.0% male). [4,9,11] HER2/neu positivity was more in female (51.3%) than male (44.8%) which was similar to the study performed in Iran.[4] However no significant correlation was found between the expression of HER2/neu and sex which was similar to the studies performed by Pappas and Park.[5,6]

In the present study, out of 97 cases, 46 (47.4%) cases were HER2/neu positive among which, 28 (28.9%) cases showed a scoring of 3+ and 18 (18.6%) cases showed a scoring of 2+. However, 51 (52.6%) cases were negative with a scoring of 1+ in 22 cases and 0 in 29 cases. According to ASCO guidelines, Her2/neu score 2+ cases are considered equivocal and are subjected to undergo FISH for further categorization. But due to unavailability of FISH in our institution, we have considered Her2/neu 2+ cases as weakly positive and calculated accordingly. [7]

Variable expression of HER2/neu in colorectal carcinoma has been observed in different studies. [4-6,11-19] (Table V). The current study was consistent with the studies performed in South Korea, Brazil and Istanbul. [5, 15, 16] The reasons for this variable expression may be due to lack of standardization of scoring system, differences in techniques in individual labs and diversity of the primary antibody. [6, 12]

In the current study, 66.0% tumors were located in colon and 34.0% in rectum. This result was similar to the studies performed in India (C/R-60%/40%) and Austria (C/R-58%/38%). [10, 20] On the contrary, Pappas and his colleagues performed a study in Greece which revealed higher prevalence of tumor in rectum (57.1%) than colon (42.9%). [6] In this study, 54.5% of rectal tumors and 43.7% colonic tumors showed HER2/neu positivity. HER2/neu expression was more prevalent in rectal tumors. This finding was similar with other studies done in South Korea and Germany. [5, 17] No significant correlation of HER2/neu expression with location of tumor was found.

The size of tumor ranged from 1.5–22.0 cm in maximum dimension with the median of 5.5 cm. This result was similar to the study performed in South Korea. They reported the median tumor size to be 5.4 cm (ranging from 2.0 – 27.0 cm). [21] HER2/neu positivity was 50% among tumors having a size of <5 cm and 46% of tumors having a size of ≥5 cm. However, no significant correlation was found between the expression of HER2/neu and tumor size. Similar results were reported in studies of South Korea and Ireland. [5, 14]

Conclusion

In conclusion, positive expression of HER2/neu has been observed in 47.4% cases of colorectal carcinoma with a score of 3+ in 28.9% cases in this study. This may be used as a potential therapeutic target in the treatment of colorectal carcinoma with a hope to increase the survival of the patient.

Limitation and recommendation

Cases with HER2/neu score: 2+ could not be grouped properly due to unavailability of FISH (Fluorescence in situ hybridization) and CISH (Chromogenic in situ hybridization). Further multicenter studies can be carried out including large number of patients to see actual expression of HER2/neu in our population.

Authors' contribution: SS, NA, MA planned the study. SS drafted manuscript. All authors read and approved final manscript.

Conflicts of interest: Nothing to declare.

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