HER2/neu Expression in Colorectal Cancers

Suma S[1], Shameem K Ummerali[2]

ABSTRACT

Introduction: Colorectal carcinoma ranks third among the most common leading causes of cancer-related mortality in the world. Her2/neu oncogene one is of four epidermal growth factor receptors. Clinically, amplification and/or overexpression of Her2/neu has been associated with poor prognosis in a number of tumor types such as breast and ovarian cancer. Conflicting data exist about the prevalence of HER-2/neu overexpression in colorectal cancer ranging from 0 to 83%. This study was done to check the expression of Her-2/neu in colorectal cancers.

Material and Methods: The study included 50 patients with a histological diagnosis of colorectal cancers at our department between 2014 January and June 2015. HER2/neu immunohistochemistry was done on this cases and scored similar to invasive breast carcinomas.IHCs were analysed with grades and stages of colon cancers**.**

Results: The mean age in this study was 60.04 years. Male female ratio was 1:1. Sixty percentage tumours were from colon and all the cases were adenocarcinoma NOS. Eighty percentage of cases were grade 1 tumours and stage B was the common stage noticed. Thirtytwo percentage of cases showed lymph node metastasis. Only 24% of cases showed Her2/neu positivity and maximum positive cases were from grade1 tumours. Maximum number of Her2/neu positivity was given by stage B tumours.

Conclusion: HER2/neu protein expression was observed in colorectal cancer but rarely in the therapeutic range (2+ and 3+).

Keywords: Colorectal cancer, Her2/neu, Immunohistochemistry

INTRODUCTION

Colorectal cancer (CRC) is one of the the leading cause of cancer death in men and women.[1] Chemotherapy has shown to be an efficient strategy for adjuvant therapy, but is still not capable of preventing recurrence in all patients. So there is numerous ongoing research for alternative compounds to be used as adjuvant therapy.[2] Compounds targeted against specific tumor proteins are under trial for many cancers. One of these targets is HER2/neu, which is primarily associated with breast cancer.HER2 is found to be up regulated in 20%–30% of all breast cancers. Trastuzumab, a drug against the HER2/neu protein, showed marked improvement in survival of breast and gastric cancer pateints.[3, 4] So FDA has approved the use of trastuzumab in HER2-positive metastatic gastric cancers.[5] Several studies evaluating HER2 in colorectal cancer resulted in a large debate because overexpression rates varied between zero and 84%.[6] This study was done to estimate prevalence of HER2/neu positivity in colorectal cancers.

MATERIAL AND METHODS

A descriptive study of all cases of adenocarcinoma colon or rectum during a period of 18 months from January 2014 to June 2015 was conducted in the Department of Pathology, Govt T D Medical college, Alappuzha.

50 cases of colorectal cancer cases satisfying inclusion criteria were selected randomly during the period of one and half years.

Inclusion criteria

Surgically resected specimens of colorectal carcinoma with a histological diagnosis of adenocarcinoma colon/rectum received in the Department of Pathology during the study period was included in the study.

Exclusion criteria

Those who received chemotherapy or radiotherapy prior Surgery and Colonoscopic biopsy

Patient data including name, age, IP number, history of adjuvant therapy, CT scan report and other investigations, if any, were collected from the Department of Surgery and the surgery requisition form

Histopathology

The tissue samples from primary tumour were fixed in 10% formalin for 24 hours before processing it to be embedded on paraffin blocks. Routine sections were taken and stained with Hematoxylin and Eosin. The cases with diagnosis of adenocarcinoma colon/rectum were recruited into the study. These cases were further graded using the following grading system.

Well differentiated- more than 95% glands formation

Moderately differentiated- 50-95% glands formation

Poorly differentiated- less than 50% glands with predominant solid areas

Immunohistochemistry

Representative 3µm sections were taken from each paraffin block and immunohistochemical staining with Her2/neu was performed for each case as follows:

- Sections were dewaxed and rehydrated, then placed in Tris EDTA
- Borate buffer for antigen retrieval.
- Antigen retrieval was done by heat method in pressure cooker for 10 minutes followed by cooling to room temperature.
- Sections were washed in water for 5 minutes.
- Endogenous peroxidase were abolished by submerging the slid word slide repetiion in quenching solution(30 ml 30% H2O2:300 ml distilled water) for 20 minutes and then washed in tap water for 5 minutes; rinsed in distilled water for 3 minutes and kept in Tris EDTA Borate buffer for 5 minutes.

[1]Senior Resident, [2]Additional Professor, Department of Pathology, Government T D Medical College, Alappuzha, Kerala, India

Corresponding author: Dr Suma S, Senior Resident, Department of Pathology, Government T D Medical College, Alappuzha pin 688005, India

How to cite this article: Suma S, Shameem K Ummerali. HER2/neu expression in colorectal cancers. International Journal of Contemporary Medical Research 2017;4(6):1240-1243.

- Sections were dried by wiping all around.
- Serum blocking solution were added and kept for 10 minutes.
- Sections were then blotted and primary antibody was added on to the sections and incubated for 1 hour in a moisture chamber; washed in PBS for 5 minutes × 3 times.
- Sections were treated with super enhancer and kept for 25 minutes.
- Secondary antibody was then added and incubated for 25 minutes followed by PBS wash for 2 minutes up to 3 times.

Chromogen Diamino Benzidine was added (constituted by mixing 1 ml buffer, 50 microlitre liquid DAB) and kept for 5 minutes; washed in PBS for 5 minutes.

- Sections were then rinsed in distilled water for 5 minutes; stained with nuclear countersrain Harris Hematoxylin by 10 dips and blued for 5 minutes.
- Sections were dehydrated, cleared and mounted with DPX.

Invasive breast carcinoma sections were used as positive controls. Negative controls were created by omission of primary antibody and replacement with phosphate buffered saline.The 4 tiered scoring system for breast carcinoma was utilized

Score 0-undetectable or membrane staining <10%.

Score1+-faint membrane staining >10%.

score2+- weak to moderate complete membrane staining >10%.

Score3+-moderate to strong complete membrane staining in >30%

Score o/1+ -negative HER2neu expression.

Score 2+/3+ - positive HER2neu expression

Staging of tumour done with Modified Dukes staging system

Stage A- limited to the mucosa

Stage B – tumours extends through the wall

Stage C1-only regional lymph nodes were positive

Stage C2- node at the point of mesenteric blood vessel ligature were involved 22

Stage D- presence of distant metastasis.

Study was conducted on specimen coming routinely to Pathology Department. Consent was routinely taken. The extra tissue section studies involved no expense to the patient. No other ethical issues involved.

RESULTS

A total of 50 colorectal carcinoma cases were included in the study. The median age of our patients, of whom 25 were male, was 60.04 years (range 35–85). A peak incidence was seen in the 5th to 6th decades of life. The lesions were present in the colon in 30 cases and in the rectum in 20 cases. All the cases were conventional adenocarcinomas, out of which 40cases were of well differentiated (grade1) tumuor, 8 cases were of moderately differentiated (grade2) tumour and 2 cases were of poorly differentiated (grade3) tumour. The staging was done by Modified Dukes staging system and majority of patients were included in the stage B (62%). No cases were included in the Stage A. 24% of cases were included in the stage C and 14% cases were in the stage D [table 1].

Immunohistochemical staining for Her-2/neu was performed on all the 50 cases of colorectal carcinoma, the scoring was done and the results were interpreted in terms of the intensity, pattern and the percentage of Her-2/neu staining. There were 22 (44%) Her-2/neu positive cases and 28 (56%) Her-2/neu negative cases. 12% showed 3+ staining, 12% showed 2+ staining and 20% of the cases showed 1+positivity [table2]. Majority of the cases show membranous and cytoplasmic staining. No cases showed pure membranous staining.Her2/neu scoring was done similar to breast carcinoma. Score 0 and 1+ were taken as negative and Scores 2+ and 3+ were taken as positive. Of the total 40 cases of grade I conventional adenocarcinoma, 11 cases showed positivity, whereas in grade II, out of 8 cases, none of the cases showed positivity and there were only two cases in gradeIII, one of which showed 2+ positivity.

Eighteen percentage of stage B cases showed Her2/neu positivity. Only 4% and 2% cases respectively from stage C and stage D cases showed positivity [table-3].

DISCUSSION

Colon cancer accounts for 15% of all the diagnosed cancers and its incidence increases annually by 2%.[7] HER2/neu expression in colon cancer is a marker for poor prognosis. Moreover, it is used to predict the patient response to adjuvant chemotherapy in colorectal pateints.[8,9]

HER2/neu expression was very well studied in breast cancer. About 25-35% of breast cancer pateints showed HER2/neu expression. Most of the previous studies showed wide range of HER2/neu expression in colorectal cancers.[10]

In this study thirty eight cases (76%) were Her2/neu negative and twelve cases (24%) were Her2 positive. One of the important features of Her2/neu staining in colorectal carcinoma in our study was the pattern of staining. There was 44% of positive staining cases (including 1+ cases), out of which 14 cases showed cytoplasmic staining and 8 cases showed cytoplasmic and membranous pattern of staining. None of the cases showed a pure membranous pattern of staining. The intensity of staining in most of the cases varies from weak to moderate. Although

Gender	
Male	25
Female	25
Age	
Median	60.04years
Range	(35-85)
Tumor location	
colon	30
Rectum	20
Both	0
Dukes stage	
A	0 (0%)
B	31 (62%)
C	13 (24%)
D	6 (14%)
Tumor grade	
Well	40 (80%)
Moderate	8 (16%)
Poor	2 (4%)

Table-1: Clinicopathologic characterestics of colon cancer

Grade	Her2/neu negative	Her2/neu positive
Grade 1	29 (58%)	11 (22%)
Grade 2	8 (16%)	0
Grade 3	1 (2%)	1 (2%)
Total	38 (76%)	12 (24%)

Table-2: Her2/neu expression according to grade

Stage	Her2/neu negative	Her2/neu positive
A	0	0
B	22 (44%)	9 (18%)
C	11 (22%)	2 (4%)
D	5 (10%)	1 (2%)

Table-3: Her2/neu expression according to stage

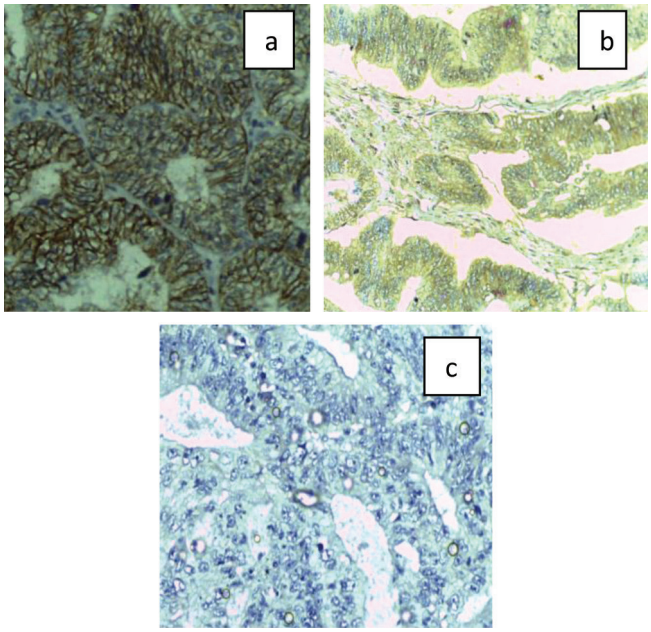

Figure-1: a) Colon adenocarcinoma Her2/neu membranous saining, 10x. b) colon adenocarcinoma Her2/neu cytoplasmic staining.10x c) colon adenocarcinoma Her2/neu negative staining 10x.

cytoplasmic staining of the tumour cells was observed, but only membranous staining was considered to be specific.

Similar study done by Ghaffarzadegan et al.[11] on 69 cases of colon adenocarcinoma showed 41 (59.4%) HER2 positive and 28 (40.6%) HER2 negative cases. Twenty seven (65.9%) cases had cytoplasmic and 14 (34.1%) cases had membranous (predominant) and cytoplasmic staining.None of the cases showed pure membranous staining. Half et al[12] discovered membranous overexpression in only five out of 96 colorectal tumours (5%), while they determined cytoplasmic over expression in 61 tumours (63%). Four out of five membranous HER2-overexpressing tumours showed amplification with FISH, while cytoplasmic HER2-overexpressing tumours failed to show amplification with FISH technique.RT-PCR showed a higher mRNA expression in membranous overexpressing tumours compared to cytoplasmic overexpressing tumours.

The present study showed the well differentiated carcinoma was more encountered than others representing 80% of cases. This was compared to the study by Ghaffarzadegan et al[11] that 75% were well differentiated. While Dalal A Elwy et al.[13] found that commonest grade was moderately differentiated representing 75%. In this study most of Her2/neu positivity was noted in well differentiated tumours. Out of 2 poorly differentiated carcinoma cases one showed 2+ positivity. All the cases moderately differentiated tumours were Her2/neu negative.

Thirty one cases (62%) of this study were stage B, 26% cases were stage C and 12% cases were Stage D. This was comparable to study by Ghaffarzadegan et al[11] and they found that stage B was the most common (47%).

According to the literature, Her2/neu expression in colorectal carcinomas were variable. Wide range of HER2/neu expression may be due to different IHC procedures and HER2/neu scoring system used.

Gene amplification technique like FISH or PCR may be needed to know the exact expression of Her2/neu in colorectal carcinomas

CONCLUSION

In our study we analyzed 50 specimens of malignant colorectal cancer lesions. In only 24% the cases showed HER2/neu protein expression in the therapeutic range (2+ and 3+), 76% were Her-2/neu negative. Most of the HER2/neu positivity was given by well differentiated tumors. Studies with more number of cases and gene amplification studies like FISH or PCR is essential to know the role of oncogenesis of HER2/neu in colorectal cancers.

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Source of Support: Nil; **Conflict of Interest:** None

Submitted: 14-05-2017; **Accepted:** 09-06-2017; **Published:** 27-06-2017