

HER2/NEU PROTEIN EXPRESSION IN COLORECTAL CANCERNimmy Andrews¹, Letha V², Sankar S³¹Resident, Department of Pathology, Government Medical College, Kottayam, Kerala, India.²Additional Professor, Department of Pathology, Government Medical College, Kottayam, Kerala, India.³Professor and HOD, Department of Pathology, Government Medical College, Kottayam, Kerala, India.**ABSTRACT****BACKGROUND**

Conflicting data exists about the prevalence of HER2/neu over expression in colorectal cancer ranging from 7% to 83%.¹ Here we are trying to clarify the extent of expression of HER2/neu in Colorectal cancer in our population. In contrast to breast cancer, in colorectal cancer, cytoplasmic HER2 could be associated with good prognosis. HER2 targeted drugs can restrain invasion and metastasis of colorectal cancer in which HER2 is overexpressed.

Aims and Objectives- To study the rate and pattern of HER2/neu status in surgically resected tissue in colorectal carcinoma. To describe the expression of HER2/neu in relation to age, sex, TNM stage, grade and primary location of colorectal carcinoma.

Setting- Department of Pathology, Government Medical College, Kottayam.

Design- Descriptive study, Cross sectional study.

MATERIALS AND METHODS

Descriptive study was conducted in 50 resected specimens of colorectal cancer received in department of Pathology, Government Medical College, Kottayam, during study period of 18 months (April 2017 to September 2018).

Analysis-SPSS software.

RESULTS

Mean age of the present study was 61.8. Majority of patients belong to 60-69 years age group (46%). Rectosigmoid region was most prone for CRC in this study constituting 21 cases (42%). 74 % cases were well differentiated type, but no cases of poorly differentiated type were seen in this study. HER2 was positive in 84% cases which was comparable with the study done by Asma Shabbir et al. 36% showed weak positivity, 40% showed moderate positivity and 8% showed strong positivity. 50% cases showed Score 3+. HER2 positivity showed significant association with location of malignancy- highest in rectosigmoid region (42%) and lowest in transverse colon (8%). Other clinicopathologic variables showed no significant association with HER2 positivity, intensity, pattern and scoring.

CONCLUSION

The present study was done to describe the rate and pattern of positivity of HER2 in colorectal carcinoma specimens and to describe the relation with various clinicopathologic variables. HER2 was positive in 84% cases in this study. Association between HER2 positivity and location of malignancy is found to be significant with a p value of 0.01. Highest was found in Rectosigmoid region and lowest was found in transverse colon, 42% and 8% respectively.

KEY WORDS

Colorectal Cancer, HER2, Resected Specimen.

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BACKGROUND

Colorectal carcinoma accounts for about 10% of new cancer diagnoses and 11% of deaths related to cancer. It is the fourth most common malignancy worldwide, with ~10 lakh new cases and 5 lakh deaths recorded each year.² Current estimates suggest that more than half of the patients with Colorectal cancer will either have liver metastases at presentation or subsequently develop metastasis. Among patients who undergo curative resection for Colon cancer, 10–20% will develop pulmonary metastasis and 10% of these patients will have isolated pulmonary lesions.³

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Despite advances in surgery and adjuvant therapy, colorectal cancer remains one of the leading causes of cancer-related deaths worldwide. Further improvements in understanding tumour biology and identifying oncogenic agents have led to the development of new therapeutic targets. Therefore, identification of biological markers for targeted therapy continues to be a high priority in human cancer treatment. Human Epidermal Growth Factor (HER2/neu) has strong therapeutic implications in certain cancers like breast cancer and gastric cancer.⁴ Literature on its frequency in colorectal cancer is scarce. Conflicting data exist about the prevalence of HER2/neu overexpression in colorectal cancer ranging from 0% to 83 %. In contrast to breast cancer, there is evidence that in colorectal cancer cytoplasmic HER2 could be associated with good prognosis. Also HER2 targeted drugs can restrain invasion and metastasis of Colorectal cancer in which HER2 is overexpressed.

MATERIALS AND METHODS**Study Design**

Descriptive study.

Study Setting

Department of Pathology, Government Medical College, Kottayam.

Study Period

April 2017 to September 2018

Study Population

50 cases of colorectal cancer specimens which are received in the Department of Pathology, Government medical college, Kottayam during my study period from April 2017 to September 2018

Sample Size

Sample size (n) = $4pq/d^2$

According to study done by Asma shabbir et al⁵ we got

P- Prevalence of Her-2 positivity in colorectal cancer in previous study = 78.9%

Fixing Relative Precision (d) as 15% of P, = $15 \times 78.9/100 = 11.8$

$q = 100 - p = 100 - 78.9 = 21.1$

Sample size (n) is calculated as

$4pq/d^2$

$(4 \times 78.9 \times 21.1) / 11.8^2 = 50.$

Inclusion Criteria

All cases of adenocarcinoma of Colon and rectum in surgically resected specimens

Exclusion Criteria

Small biopsies of colon and rectum with adenocarcinoma are excluded.

Study Tool

1. Clinical proforma.
2. Clean dry microscopic slide.
3. Haematoxylin and eosin stain.
4. HER2//Erb2 (EP3).
5. Microscope.
6. Formalin fixed paraffin embedded specimen.

Study Procedure

All specimens are received in formalin. These are processed, paraffin embedded, thin (5micronmeter) sections are taken and stained by Haematoxylin and Eosin and immunohistochemistry for HER2/neu. Immunohistochemical staining should be conducted as follows:

Standard Operating Procedure (Immunohistochemistry)**Principle**

Formalin fixation plus tissue processing in paraffin as well as even dehydration with ethanol can mask many antigenic sites and hinder antibody binding. Immunohistochemistry involves exposure of these antigenic sites by treating thin paraffin sections to high heat bathed in various solutions with controlled pH with the help of a pressure cooker/antigen retriever. The final result is interpreted by the development of brown Colour in tissue sections.

Materials/Microtome APES Coated Glass Slides (or Positively Charged Slides)

- Xylene.
- Ethanol Peroxide.
- Distilled water.
- Antigen retriever.
- 700-900 Watt Pressure Cooker.
- Slide racks.
- Glass dishes for buffers.
- Plastic, microwaveable rack containers.
- Humid/moisture chamber.
- Counterstain (Haematoxylin).
- Sodium Phosphate, Dibasic-500gms.
- Sodium Dihydrogen Phosphate, Monohydrate-500 gms.
- Mounting medium-DPX (Distrene Dibutyl phthalate Xylene).
- Cover slips (22x22mms).

Buffers**1. Tris Buffer Saline (TBS): pH 7.6(IHC Wash Buffer)**

- Tris-0.605 g.
- NaCl-8 gms.
- 1N HCl-4.4 ml pH-7.6.
- Distilled water-1 Litre.

Mix to dissolve. Adjust pH to 7.6 with 1N HCl and then store this solution at room temperature for 3 months or at 4C for longer storage.

2. Citrate Buffer (Antigen Retrieval Buffer) pH6.0

- Tri-sodium citrate (Dihydrate)-2.94 g.
- Distilled water-1000ml.
- 1Normal HCl-5ml.

Mix to dissolve. Adjust pH to 6.0 with 1N HCl and then store this solution at room temperature for 3 months or at 4C for longer storage.

3. Tris EDTA buffer (Antigen retrieval buffer)pH9.0

- Tris Base-1.21 g.
- EDTA (Ethylene Diamine Tetra Acetic acid)-0.37 g.
- Distilled water-1000 ml (100 ml to make 10x, 50ml to make 20x).
- Mix to dissolve. Adjust the pH to 9.0 with 1N NaOH, mix well.
- Store this solution at room temperature for 3 months or at 4°C for longer storage.

Procedure**(a). Preparation of Paraffin Slides**

1. Prepare sections between 3-4micrometer thickness on Poly-L-Lysine coated slides or silanized slides to keep the samples from detaching, especially during antigen retrieval approaches. Mark the sections carefully for antigens to be detected and keep the slides in a slide rack
2. Incubate the slides overnight at 60°C

OR

3. After air dry, bake the slides at 600C for 1hr in Hot air oven.
4. Dip slides in xylene (three times) for 10 minutes each to remove the paraffin.

5. Rehydrate the tissues by dipping the slides in absolute ethanol(100%), then in 90%ethanol(2changes, 5minutes each)
6. Keep in running tap water for 10minute.

(b). Antigen Retrieval

Place the slide rack in 300-400ml of citrate buffer or Tris EDTA buffer for 3 minutes at room temperature.

Place rest of the buffer and the slide rack in the pressure cooker or antigen retriever (Depending on the method used). Assemble the top and lid of the pressure cooker and lock into position; place the cooker onto a hot plate and cook for 10 minutes or for 40 minutes at high power in antigen retriever. When the cooking time is completed, remove the pressure cooker immediately and keep under running tap water. When the steam is completely dissipated, open the lid and very carefully remove the slides. Place slides in wash buffer for 5 minutes.

(c). Detection

1. Cover sections with Peroxide block for 10 minutes. Wash sections in TBS for 2x5 minutes.
2. Incubate with Power Block for 10 minutes.
3. Incubate with Primary antibody for 30 minutes. Wash in TBS for 2x5 minutes.
4. Incubate with super enhancer for 20 minutes. Wash in TBS for 2x5 minutes.
5. Incubate with SS Label for 30 minutes. Wash in TBS for 2x5 minutes.
6. Develop peroxidase activity with DAB (3, 3'-Di Amino Benzidine) working solution for 5.minutes. (Prepare DAB Working Solution by adding 50 microliters of DAB Chromogen to 1 ml of DAB buffer). Rinse slides in running water.
7. Counterstain with Haematoxylin. Rinse slides in water for 5 minutes for blueing.
8. Dehydrate, clear and mount sections with DPX.

Statistics

The Data was entered in Microsoft Excel and further Statistical Analysis was done using SPSS Software

1. Mean and frequency of-
 - Age.
 - Sex.
 - Anatomical location.
 - Grade.
 - HER2 positivity.
 - Intensity of HER2 positivity.
 - Score of HER2 positivity.
 - Pattern of HER2 positivity.
2. Relation between HER2/neu protein expression and other clinic pathologic variables were studied using chi-square testing and p value was calculated accordingly.

RESULTS

Descriptive study of frequency of HER2/neu positivity in 50 cases of CRC in resected specimens was done in department of Pathology, GOVT Medical College Kottayam during the study period of 18 months. Mean age of the present study was 61.8, minimum age was 26 years and maximum was 85.Majority of patients belong to 60-69age group (46%) Sex preponderance was equal in our study. Rectosigmoid region was most prone for CRC in this study constituting 21 cases (42%).74% cases were well differentiated type and 26% were moderately differentiated, but no cases of poorly differentiated type were received in the study period. Lymphovascular emboli was present in 15% cases in the present study. HER2/neu was positive in 84% cases which was comparable with the study of Asma Shabbir et al. 36% showed weak positivity, 40% showed moderate positivity and 8% showed strong positivity.50% cases showed score 3+, 24% showed score 2+ and 10% showed score 1+ for HER2/neu positivity. HER2/neu positivity showed significant association with location of malignancy-highest in Rectosigmoid region (42%) and lowest in Transverse Colon (8%)Other clinicopathologic variables showed no significant association with HER2/neu positivity, intensity, pattern and scoring.

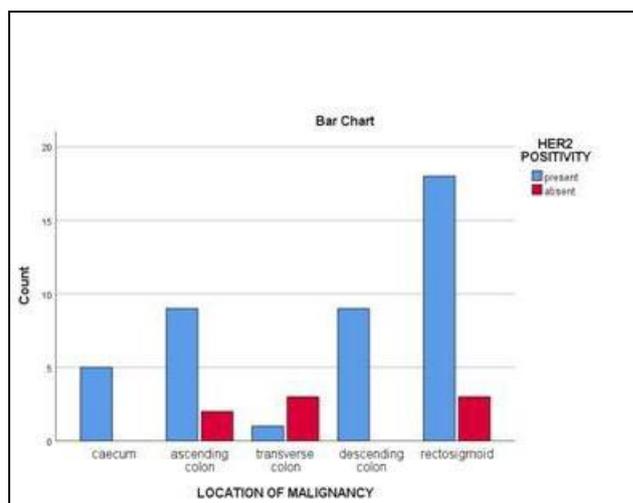


Figure 1. Association Between Location of Malignancy and HER2 Positivity

Location of Malignancy	HER2 Positivity		Total
	Present	Absent	
Caecum	5	0	5
Ascending Colon	9	2	11
Transverse Colon	1	3	4
Descending Colon	9	0	9
Rectosigmoid	18	3	21
Total	42	8	50

Table 1. Location of Malignancy and HER2 Positivity

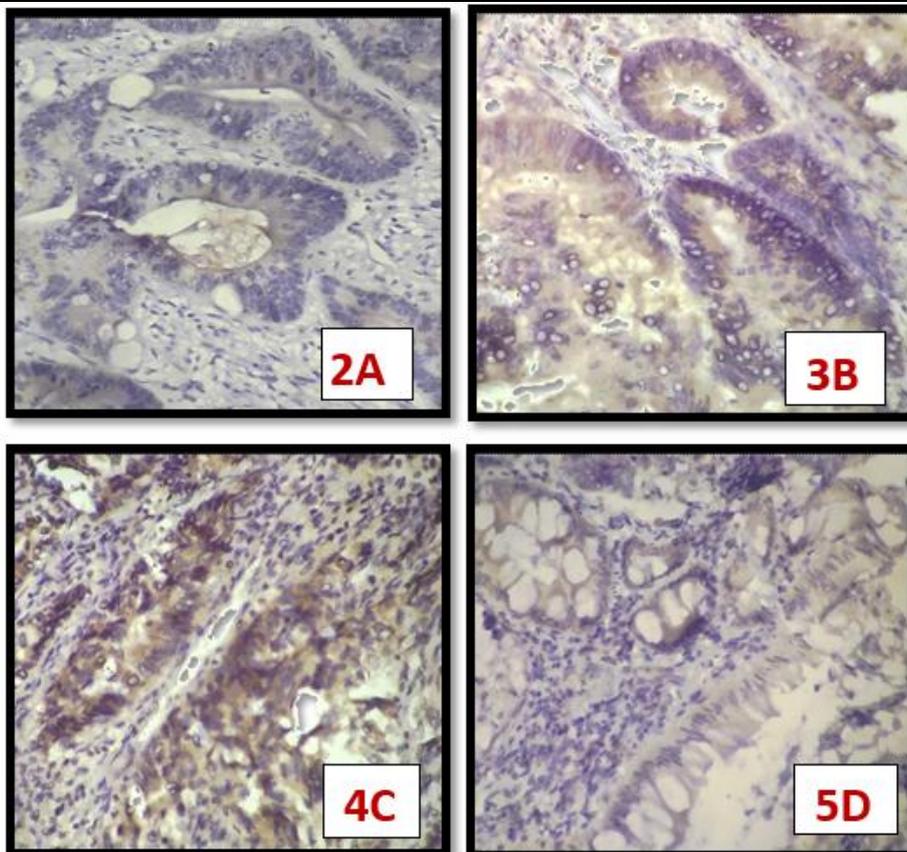


Figure 2A. Cytoplasmic Weak Positivity, Figure 3B. Cytoplasmic Moderate Positivity, Figure 4C. Cytoplasmic Strong Positivity, Figure 5D. Cytoplasmic and Membranous Positivity.

	HER2
Present Study	84%
Asma Shabbir et al	78.9%
Seo et al ⁶	65%
Suma et al ⁷	44.00%

Table 2. 84% cases are variably positive for HER2 in the present study. This finding is comparable with study of Asma Shabbir et al. 78.9% cases were positive for HER2 in their study

	1+	2+	3+
Present Study	10%	24%	50%
Asma Shabbir et al	30.6%	24%	45.3%

Table 5. According to the present study, HER2 was negative in 16% cases, and score of 3+ was seen in 50% cases. In the study of Asma Shabbir et al, 21.1% were negative for HER2 and score of 3+ was found in 45.3% cases

	Weak	Moderate	Strong
Present Study	36%	40%	8%
Asma Shabbir et al	25.3%	46.6%	28%
Suma et al	20.00%	12.00%	12.00%

Table 3. Comparing the intensity of HER2 positivity, our study showed 40% moderately positive cases, 36% are weakly positive and 8% are strongly positive whereas study of Asma Shabbir et al also showed more moderately positive cases

	Cytoplasmic	Membranous	Both
Present Study	54%	0%	30%
Asma Shabbir et al	48%	26.6%	25.3%
Seo et al	57.5%	0%	7.5%

Table 4. On comparing the pattern of HER2 staining, the present study showed 54% cytoplasmic staining and 30% both cytoplasmic and membranous staining. No cases of membranous staining were seen in our study. In the study of Asma Shabbir et al, 26.6 % showed membranous HER2/neu expression, 48 % cases showed cytoplasmic expression and 25.3% cases showed membranous + cytoplasmic expression

DISCUSSION

HER2 is a member of the epidermal growth factor family and is over expressed in malignancies of breast, ovarian, gastric, Colorectal, pancreatic and endometrial cancers⁴. HER2/neu expression can be either membranous or cytoplasmic with different clinical implications in different cancers. For example, cytoplasmic HER2/neu in breast cancer does occur but it is considered irrelevant because the monoclonal antibodies approved for its treatment, targets only membranous forms. But HER2/neu expression is membranous as well as cytoplasmic in colorectal adenocarcinoma with cytoplasmic expression favouring survival prognosis.

The present study was conducted in 50 cases of resected specimens of colorectal carcinoma in department of pathology, Govt. Medical College, Kottayam during April 2017 to September 2018. Immunostaining for HER2/neu was done in all cases and it is interpreted.

CONCLUSION

HER2/neu was positive in 84% cases in this study.

Association between HER2/neu positivity and location of colorectal carcinoma is found to be significant with a p value of 0.01 (<0.05). Highest positivity was found in rectosigmoid region and lowest was found in transverse colon, 42% and 8% respectively.

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