



## HER-2/NEU EXPRESSION IN COLORECTAL CARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGIC VARIABLES

### KEYWORDS

HER-2/NEU, Immunohistochemistry, colorectal adenocarcinoma.

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### ABSTRACT

**Background:** Colorectal cancer is one of the most common leading causes of cancer related mortality and account for approximately 9% of all human cancers. Immunohistochemical marker such as HER-2/neu can be used for the diagnosis and prognosis of colorectal adenocarcinoma.

**Aims and objectives:** To study the immunohistochemical overexpression of HER-2/ neu and its correlation with clinico pathologic variables and its role in targeted therapy.

**Materials and methods:** Histomorphological and immunohistochemical pattern of expression of HER-2/ neu were studied and analysed for 60 cases of colorectal adenocarcinoma specimens received over the period of two years.

**Results:** Out of total sixty cases studied 40 cases were HER-2/neu positive and 20 cases were HER-2/neu negative which constitutes 67% and 33% respectively.

**Conclusion:** The rate of HER-2/neu expression in colon carcinoma is high.

### INTRODUCTION

Colorectal cancer is the 3rd most common cancer across the globe.<sup>1</sup> A rise in incidence and mortality rates of colorectal cancers are illustrated in Asia.<sup>2</sup> Etiology appears to be multifactorial which includes genetics, environmental factors and dietary factors. Peak incidence is in the seventh decade and a male predominance is seen. Sigmoid colon and rectum are the most commonly affected sites.

Colorectal carcinomas may be fungating, intraluminal or ulcerating masses. Adenocarcinomas account for more than 90% of colorectal carcinomas. Most common histological pattern is classic adenocarcinoma.

#### Other subtypes are:

- Mucinous adenocarcinoma
- Signet ring cell carcinoma
- Medullary carcinoma
- Serrated carcinoma
- Adenosquamous carcinoma
- Small cell carcinomas or neuroendocrine carcinomas

The grading system for colorectal adenocarcinomas is based on the extent of well-formed glands either well differentiated (>95%), moderately differentiated (50-95%) or poorly differentiated (<50%).

Histomorphological assessment on hematoxylin-eosin (H&E) stained sections remains the most important diagnostic tool.

Immunohistochemistry can be used for the diagnosis and prognosis of colorectal adenocarcinomas. Various immunological markers for colon carcinoma are MUC1 and MUC3, P53, CK20, CEA, HER-2/neu, CD X2, tumour associated glycoprotein, cathepsin, HCG and PLAP.

Human epidermal growth factor receptor (HER-2/neu) over expression correlates with mitogenesis, malignant transformation, invasion and metastasis and correlates with poor prognosis. Human Epidermal Growth Factor (Her-2/neu) is a proto-oncogene located on chromosome 17q21 that encodes ErbB-2.<sup>3</sup> HER-2/neu

activity initiates signal cascades including MAPK (Mitogen activated protein kinase) and p13k (AKT 3 kinase) pathways that are essential for cell proliferation and differentiation. The purpose of this study is to analyze the histomorphological patterns and grades of colorectal carcinoma and to study immunohistochemical overexpression of HER-2/ neu and its correlation with clinico pathologic variables and its role in targeted therapy.

### MATERIALS AND METHODS

A Prospective study was carried out in the department of Pathology, tertiary care Hospital, Tamilnadu for a period of two years. 60 resected colorectal specimens received in the Department of Pathology were fixed adequately with neutral buffered formalin, processed and sections of four micron thickness were cut & stained with hematoxylin and eosin stains for histological typing and grading of lesions according to WHO classification. Sections on coated slides were subjected to immunohistochemical staining for Her2/neu immunoexpression.

A Two Step indirect technique of immunohistochemistry was done. Positive and Negative control for HER-2/neu was also run each time. Then immunohistochemical evaluation was done. Sections were examined under high power objective for positive immunoreactivity. Positivity is considered when the neoplastic cells have cytoplasmic, membranous or membranous and cytoplasmic golden brown staining. Semiquantitative scoring was carried out.

Grading of intensity of staining: 0-Absent, 1- Weak, 2-Moderate, 3-Strong.

Percentage of positive tumour cells: +1 --> 10-40% of cells are positive +2 --> 41-70% of cells are positive +3 --> >70% of cells are positive.

HER-2/neu was considered positive if >10% of cells have strong immunostaining or >50% of cells show moderate staining.

Patient age, gender and HER-2/neu status was determined and expressed.

Statistical analysis using SPSS software version was done and the

variables expressed as percentage of number (%). For statistical comparisons Chisquare tests was employed. p Value <0.05 was considered statistically significant.

**OBSERVATION AND RESULTS**

Sixty cases of colorectal carcinomas were included in the study. Patient and the tumour characteristics & HER-2/ neu expression in colorectal adenocarcinoma and its relation with clinicopathological variables were shown in table 1 & 2 respectively. HER-2/ neu pattern of staining was shown in table 3.

**Table: 1 Patient and tumor characteristics**

Characteristics	Cases	Percentage
<b>Age</b>		
31-50	14	23%
51 - 70	40	67%
> 70 yrs	6	10%
<b>Sex</b>		
Male	42	70%
Female	18	30%
<b>Location</b>		
Right colon	12	20%
Left colon	28	47%
Rectum	20	33%
<b>Types</b>		
Conventional	50	83.30%
Mucinous	8	13.40%
Signet ring cell	2	3.30%
<b>Conventional adenocarcinoma Grade(n=50)</b>		
Grade I	26	52%
Grade II	20	40%
Grade III	4	8%
<b>Her2/neu</b>		
Positive	40	67%
Negative	20	33%

**Table:2 Percentage and intensity of HER2/neu staining in colorectal adenocarcinoma**

Clinicopathological Variables	HER2/neu				
	-VE	+1	+2	+3	+VE (%)
<b>Age</b>					
31-50	6 (43%)	4	0	4	57%
51 - 70	14(35%)	8	6	12	65%
> 70 yrs	0	2	0	4	100%
<b>Sex</b>					
Male	20(48%)	8	6	8	52%
Female	0	6	0	12	100%
<b>Site</b>					
Right colon	2(17%)	2	2	6	83%
Left colon	12 (43%)	10	2	4	57%
Rectum	6(30%)	2	2	10	70%
<b>Types</b>					
Conventional	12 (24%)	14	6	18	76%
Mucinous	6(75%)	0	0	2	25%
Signet ring cell	2(100%)	0	0	0	0%
<b>Conventional adenocarcinoma Grade(n=50)</b>					
I	6 (23%)	8	2	10	77%
II	6 (30%)	6	2	6	70%
III	0	0	2	2	100%
<b>Lymphnode</b>					
Metastases	0	4	6	6	100%
Reactive	6 (43%)	6	0	2	57%
Nil	14 (47%)	4	0	12	53%

These results were analyzed using Chi-square analysis and found to be statistically significant with a p value <0.05 for age,sex,grade of

tumour and lymph node metastases. But for the location and types of tumour, Chi square analysis showed a statistically insignificant result. (p>0.05)

**Table:3 HER-2/ neu pattern of staining**

PATTERN	GRADE		
	I	II	III
CYT	18 (90%)	14( 100%)	0
MEMB&CYT	2 (10%)	0	4(100%)
Total	20	14	4

Out of 67% positive cases 31% showed +3 staining, 13% showed +2 staining and 23% showed +1 staining for HER-2/neu. The staining intensity in most cases was moderate (45%) to strong (19.5%) with weak staining in only one case (2.5%). 56.9% of cases showed cytoplasmic positivity and 10.1% showed membranous-cytoplasmic staining. No case showed pure membranous pattern of staining.

Figure: 1 shows subtypes of colorectal adenocarcinoma and figure:2 shows HER-2/ neu positivity in those cases.

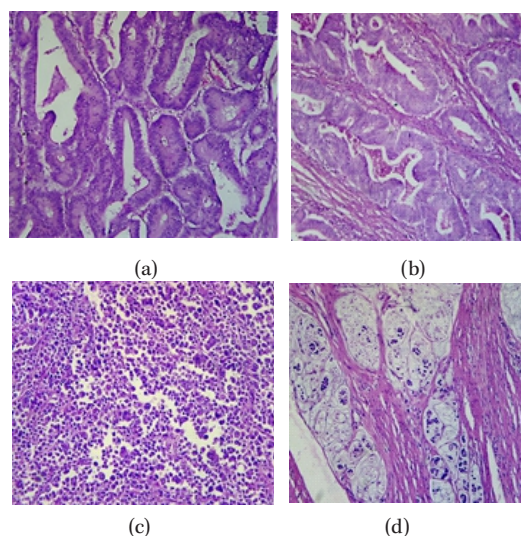


Fig:1. (a)Well differentiated adenocarcinoma of colon H & E(10X) (b)Moderately differentiated adenocarcinoma of colon H & E(10X) (c)Poorly differentiated adenocarcinoma of colon H & E(10X) (d) Mucinous adenocarcinoma of colon H & E(10X)

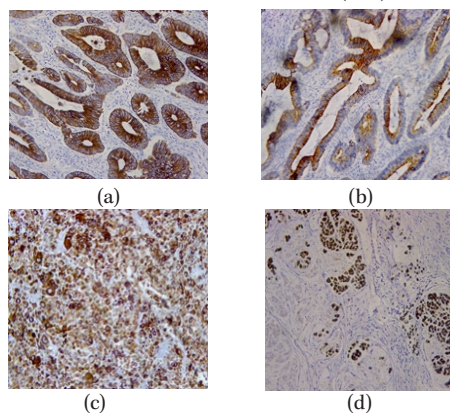


Fig:2.(a)HER-2/neu expression in Grade I adenocarcinoma (10X) (b)HER-2/neu expression in Grade II adenocarcinoma (10X) (c)HER-2/neu expression in Grade III adenocarcinoma (10X) (d)HER-2/neu expression in mucinous adenocarcinoma (10X)

**DISCUSSION**

Colorectal carcinomas are one among the most common human malignancies. Most of the tumours are diagnosed, classified and graded with H & E stained sections. Various immunological markers

are expressed in colorectal malignancies and these are studied to identify targeted therapy and to increase the survival of the patients. HER-2/neu overexpression indicates higher tumour grade and stage and a monoclonal antibody (Trastuzumab) directed against HER-2 has increased survival in many tumours. Hence HER-2/neu expression in colorectal carcinomas are being studied extensively. Overexpression of Her-2/neu in colorectal cancer shows a wide range of variability between 0-84% in different studies.<sup>4</sup>

In our study, HER-2/neu positivity was seen in 67% of the cases. HER-2/neu expression increased as age of the patients increased. Similar results were seen in study by Manmeet Kaur Gill et al (2011) in which HER-2/neu percentage positivity was increased as age increased with a p value of 0.002.<sup>5</sup>

HER-2/neu expression was higher in females (100%) compared to males. In a study by Gruenberger et al (2006) percentage positivity was more in males than in females which was in contrast to our study.<sup>6</sup>

Location and type of tumour had no correlation with HER-2/neu. These results were similar to other study by Mohammadi et al (2011) in which no statistically significant correlation was obtained between HER-2/neu expression and site of tumour.<sup>7</sup> Similar results were observed in a study by Kavanagh et al (2009) in which there was no statistically significant relation between HER-2/neu expression and histologic types.<sup>8</sup>

HER-2/neu staining was higher in grade III tumours (100%) when compared to 77% in grade I tumours showing a positive correlation of HER-2/neu with grade. This suggests as the tumour grade increases HER-2/neu positivity increases. These results were similar to studies by Manmeet Kaur Gill et al (2011) and Hay et al (2003) in which they found significant correlation between HER-2/neu and grade. They also observed that grade III tumours showed 100% HER-2/neu positivity.<sup>5</sup>

Membranous cytoplasmic staining was higher (100%) in grade III tumours. A study by Shoroq Mohammed Abas al (2014) also showed 100% membranous-cytoplasmic HER-2/neu positivity in grade III tumours more than grade I.<sup>9</sup> Similar results were also observed by Ghaffarzadegan et al (2006).<sup>10</sup>

There was 100% HER-2/neu positivity in metastatic nodes showing a positive correlation. This was similar to study by Manmeet Kaur Gill et al (2011) in which out of 40 cases in 20 cases lymph nodes were recovered and ten were metastatic and all the ten cases were HER-2-neu positive.<sup>5</sup>

## CONCLUSION

Our study concludes that the rate of HER-2/neu expression in colon carcinoma is high. Most cases had cytoplasmic staining but membranous - cytoplasmic staining was seen in higher grades. Hence targeted therapy could be helpful in patients with high grade and with lymph node or distant metastases. Because of many pitfalls in immunohistochemistry, further studies such as gene amplification studies involving larger number of patients are needed to assess HER-2/neu expression in colorectal carcinomas and to develop new targeted therapy.

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