

Immunohistochemical Expression of Her2/Neu Receptor in Human Colorectal Carcinoma (A Clinicopathological Study)

Kifah Hamdan AbdulGhafour

ABSTRACT:

BACKGROUND:

Colorectal adenocarcinoma is the most common type of gastrointestinal cancers in Iraq, and according to the Iraqi cancer registry (ICR) reports, the incidence of colorectal carcinoma was 2.99% of whole body malignancies (ICR 2010). In males, it's the 5th common cancer while in females it's the 4th most common cancer.

Her2/neu is an important oncogene in breast cancer, but its prevalence and significance in colorectal carcinoma have been documented.

OBJECTIVE:

To determine the frequency and the pattern of Her2/neu expression in colorectal adenocarcinoma by immunohistochemical technique and to correlate this expression with different clinicopathological parameters.

MATERIAL AND METHOD:

Twenty five cases of colorectal adenocarcinoma were studied, these cases were diagnosed in private laboratories in Baghdad/Iraq from November 2011 to march 2013. Clinicopathological parameters such as age, gender, pathological diagnosis, including the tumor site, size, lymph nodes status, grade and stage of tumor were taken from patients file. Sections of 4 µm stained by H&E stain and immunohistochemical stained for Her2/neu.

Using infiltrative ductal carcinoma of the breast as control positive, evaluation of Her2/neu expression by immunohistochemistry in all cases was performed.

RESULTS:

Fourteen (56%) of the cases were males, 11 (44%) case were females, with age distribution ranging from (24-89) years, with a mean age of 56.5 years. Tumor size ranges between 2.5-10 cm, with mean of 6.25 cm. Seven (28%) cases were localized in the cecum, 5 (20%) from each rectum, sigmoid and left colon, respectively and 3 (12%) involving more than one segment of the colon.

Histologically the tumor grade ranges from moderately differentiated in 23 (92%) cases and poorly differentiated in 2 (8%) cases. Regarding pathological staging (TNM system), 5 (20%) were stage T2, 17 (68%) were stage T3 and 3 (12%) were T4. Lymph node involvement found in 10(40%) of the cases and distant metastasis was found in 2 (12%) cases. Her2/neu expression was present in 4(16%) cases of 25 colorectal adenocarcinoma; there was no correlation with age, sex, histopathological grade, location, lymph nodes status and tumor invasion.

CONCLUSION:

Concerning data exists about the prevalence of her2/new expression in colorectal adenocarcinoma, there was no significant correlation between her2/neu over expression and tumor size, grade, localization of the primary tumor, lymph nodes status and depth of invasion.

KEYWORDS: colorectal adenocarcinoma, her2/neu

INTRODUCTION:

Her2/neu oncogene is a member of Tyrosine kinase family similar to epidermal growth factor receptor (EGFR), Her-1, Her-3, her-4 are located on chromosome 17q21 and encodes as 185kd

transmembrane protein that lacks a natural ligand. Her-2 activation initiates signal cascades including the MAPK (mitogen activated protein kinase) and 3- kinase pathways that are essential for cell proliferation and differentiation⁽¹⁾. Over expression of the Her 2/neu is detected in 25-35% of breast cancer.⁽²³⁾

College of Medicine/ Baghdad University
Department of Pathology.

HER2/NEU RECEPTOR IN COLORECTAL CARCINOMA

Over expression of Her 2/neu has been reported in many other epithelial malignancies including cancer of the lung ⁽⁴⁾, prostate ^(5,6), bladder ^(7,8), pancreas ⁽⁹⁾, esophagus ⁽¹⁰⁾, and stomach ⁽¹¹⁾.

Either her2/neu protein over expression or gene amplification is associated with approximately one fourth of all cases of gastrointestinal tract malignancies.¹²⁾

The success of anti – Her 2/neu therapy in breast cancer has led to evaluation of Her 2/neu expression in multiple tumors, colorectal cancer among them ⁽¹³⁺¹⁴⁾

Conflicting data exist about the prevalence of Her2/neu over expression in colorectal cancer, which ranges from 0 to 83% ⁽¹⁵⁾ as well as the relationship between Her 2/neu over expression and the clinicopathological features.

MATERIAL AND METHODS:

This study is retrospectively designed, a total of 25 cases of colorectal adenocarcinomas diagnosed in private pathology laboratories in Baghdad/ Iraq, during the period from November 2011 to march 2013, all cases were evaluated in terms of age, gender, pathological diagnosis, including tumor size, location, lymph nodes situation, stage and grade of tumor.

Two sections of 4um thickness were taken from paraffin blocks of the tumor, one section was stained with hematoxylin and eosin (H&E) and the other dewaxed and processed for immunohistochemical staining using Hercept

test kit (K5204 Dako/ Denmark) according to manufactures instructions.

Known positive breast cancer cases used as positive control.

The criteria for positive immunoreactions for Her2/ neu is a dark brown staining of the cell membrane, the proportion of the staining was assessed by counting the percentage of positive cells in 100 malignant cells using a high power objective lens (x40), each sample was scanned for five random fields ⁽¹⁶⁾.

The Her 2/neu scoring system follows that employed by S. China et al ⁽¹⁷⁾. this system correlate the positivity of Her2/neu in both surgical and biopsy specimen and scored the expression to negative, equivocal and positive.

RESULTS:

The 25 cases that are included in this study are divided into different subgroups according to each studied parameter. A comparison has been made among the subgroups in regards to over expression.

1) Her 2 /neu expression:

According to her2/neu scoring system (as mentioned previously), seventeen (68%) cases expressed a totally negative staining of Her2/neu marker, eight (32%) cases stained positive for the marker. Their score was as follows: two cases were +1, two cases were +2 and four cases +3. As shown in figure 1

Table 1: The distribution of cases according to Her2/neu score.

Her 2/neu expression score	Frequency	Percentage %
0	17	68%
+1	2	8%
+2	2	8%
+3	4	16%
Total	25	100%

2) Age: distribution:

Patients age ranges from 24-89 years with a mean 56.5 years, divided into two groups with a

cut-off point of 60 years, two cases (8%) of Her2/neu positive cases for each category. The difference is not statistically significant.

Table 2: The distribution of Her2/neu positive cases in relation to age.

Age group	Her2/neu negative	Her2/neu positive	Total %	P value
≤ 60 years	11 (44%)	2 (8%)	13 (52%)	P > 0.05 NS
>60 years	10 (40%)	2 (8%)	12 (48%)	
Total	21 (84%)	4 (16%)	25 (100%)	

HER2/NEU RECEPTOR IN COLORECTAL CARCINOMA

3) Gender distribution:

Eleven (44%) cases were females, 14 (56%) cases were males, all of the showed negative staining for Her2/neu marker. Two (8%) males

and 2 (8%) females with colorectal adenocarcinoma showed positive staining. There was no significant differences between the two groups.

Table 3: The distribution of Her2/neu positive cases in relation to the gender.

Sex	Her2/neu negative	Her2/neu positive	Total %	P value
Male	12 (48%)	2 (8%)	14 (56%)	P > 0.05 NS
Female	9 (36%)	2 (8%)	11 (44%)	
Total	21 (84%)	4 (16%)	25 (100%)	

4) Distribution according to the grades of the tumor:

Histologically the tumor grades range from a moderately differentiated in 23 (92%) cases and poorly differentiated in 2 (8%) cases as shown in table (4).

Three cases (12%) of the Her2/neu positive tumors were moderately differentiated, while only one (4%) case was poorly differentiated, analysis the data showed no significant correlation between the degree of differentiation and the frequency of over expression of Her2/neu marker (p value P > 0.05).

Table 4: The incidence and frequency of Her2/neu positive cases in relation to grade of the tumor.

Grade of tumor	Her2/neu negative	Her2/neu positive	Total %	P value
Moderately differentiated	20 (80%)	3 (12%)	23 (92%)	P > 0.05 NS
Poorly differentiated	1 (4%)	1 (4%)	2 (8%)	
Total	21 (84%)	4 (16%)	25 (100%)	

5) Topographic distribution of the tumor:

Seven (28%) cases located in the cecum, and the remaining cases were located in the rectum, sigmoid, left colon; five (20%) cases for each location and 3 (12%) located in more than one segment of the colon.

Three (12%) cases were demonstrating Her2/neu positivity located in the cecum and the remaining cases (4%) was in the rectum. No significant correlation between the site of the tumor and the expression of Her2/neu were found.

Table 5: The distribution of the studied cases according to the anatomical site of the colon and corresponded Her2/neu figures in each site.

Site	Her2/neu negative	Her2/neu positive	Total %	P value
Cecum	4 (16%)	3 (12%)	7 (28%)	P > 0.05 NS
Rectum	4 (16%)	1 (4%)	5 (20%)	
Sigmoid	5 (20%)	-	5 (20%)	
Left colon	5 (20%)	-	5 (20%)	
More than one site	3 (12%)	-	3 (12%)	
Total	21 (84%)	4 (16%)	25 (100%)	

6) Distribution according to the stage of the tumor:

In this study no cases were in T1 stage of the cancer, five (20%) cases were invading the muscularis propria T2, 17 (68%) cases were T3 and 3 (12%) cases were T4 (invading the serosa).

Expression of Her2/neu distributed among the cases were as follow: 1 (4%) case in T2, 1 (4%) case T3 and 2 (8%) cases T4. According to these findings, there was no statistical significant between depth of invasion and over expression of Her2/neu.

HER2/NEU RECEPTOR IN COLORECTAL CARCINOMA

Table 7: Distribution of Her2/neu over expression by the tumor in relation to depth of wall invasion.

Depth of invasion	Her2/neu negative	Her2/neu positive	Total %	P value
T1	-	-	-	P > 0.05 NS
T2	4 (16%)	1 (4%)	5 (20%)	
T3	16 (20%)	1 (4%)	17 (68%)	
T4	1 (4%)	2 (8%)	3 (12%)	
Total	21 (84%)	4 (16%)	25 (100%)	

7) Distribution according to the lymph nodes status (N of TNM).

Fifteen (60%) cases showed no nodal involvement, while 10 (40%) cases presented with nodal involvement, four (16%) cases were

N1, 6 (24%) cases were N2, two (8%) expressed Her2/neu were N1, the other 2 (8%) were expressed Her2/neu were N2. No significant association was found between the lymph node status and Her2/neu over expression.

Table 8: Frequency distribution of Her2/neu over expression in relation to lymph node status.

Lymph node	Her2/neu negative	Her2/neu positive	Total %	P value
N0	15 (60%)	-	15 (60%)	P > 0.05 NS
N1	2 (4%)	2 (8%)	4 (16%)	
N2	4 (16%)	2 (8%)	6 (24%)	
total	21 (84%)	4 (16%)	25 (100%)	

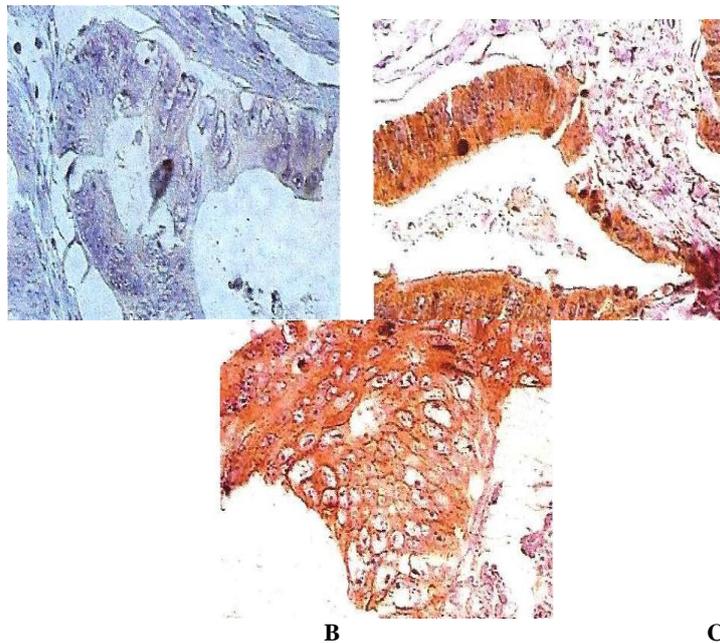


Figure 1: Colonic adenocarcinoma, moderately differentiated, (A) Her2 staining negative, (B) Her2 staining cytoplasmic and (C) her2 staining membranous and cytoplasmic, 40x.

DISCUSSION:

In this study, Her2/neu is found to be positive in four of 25 cases of colorectal carcinoma constituting 16%, Schuel et al⁽¹⁸⁾ examined 77 colorectal cancer, 56 (70%) were negative, strong membranous Her2/neu staining were only detected in 2 (3%) cases.

Qingguo Li et al⁽¹⁹⁾ examined 317 colon cancer samples for the presence of Her2/neu oncoprotein expression, only 7 samples were strongly membranous positive.

Similar results were described in the study of Kavangh et al⁽²⁰⁾, they found Her2/neu protein expression in 11% of their cases with two displaying strong positive membranous Immunohistochemistry. Park et al⁽²¹⁾ found over expression of Her2/neu in 12.5% cases.

Ross et al studied the Her2/neu oncogene tumors of the gastrointestinal tract, they found the wide range of Her2/new expression in esophageal, gastric and colon cancer. They concluded that either Her2/neu protein over expression or gene amplification is associated with one fourth of all gastrointestinal malignancies⁽¹²⁾

Our study colorectal carcinoma tends to be more common in elderly patients, the age ranges (24-89) years with mean 56.5 years, 14 (56%) male, 11 (44%) female, Gahaffalzadegan et al⁽²²⁾ found age range 28-82 years with mean age of 56 years and 50.7% males and 49.3% female cases.

Our study results indicate a low rate of Her2/neu positivity and no correlation with clinicopathological features be hampered by the small number of cases (25 cases), however these results are in agreement with some large patient series. B Schuell et al⁽¹⁸⁾ & Jesus et al⁽²³⁾ reported that Her2/neu expression was not correlated with age, sex, tumor differentiation, localization, of the primary tumor, and overall survival, they indicated that her2/new expression was unlikely to play a major role in the therapeutic management of colorectal cancer. Previous reports have demonstrated an association between Her2/neu positive expression and more aggressive colorectal tumors. Demirbas et al⁽²⁴⁾ reported an association between Her2/neu over expression and tumor size > 5 cm, differentiation grade and vascular lymphatic invasion.

Over expression of the Her2/neu receptors is detected in 25-35% of human breast cancer^(3,7), data about the prevalence of Her2/neu overexpression in colorectal cancer ranging from 0 to 83%^(12,16)

There are several positive reasons for discrepancies between results, the most likely

reason for the divergent finding of the antibodies is the different scoring system, the sensitivity of the antibodies used makes the comparison studies very challenging.

CONCLUSION:

Concerning data exist about the prevalence of her2/neu over expression in colorectal adenocarcinoma; there was no significant association between Her2/neu over expression and a set of clinicopathological parameters (age, gender, location of tumor, tumor grade and stage).

Moderate (2+) overexpression of Her2/neu was detected in a small proportion of colorectal cases & considering the low rate of Her2/neu overexpression, studies with larger sample sizes are essential to understanding the biologic role of Her2/neu in colorectal carcinoma.

REFERENCES:

- 1- Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell*. 2000;103:211-25.
- 2- Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*. 1987;235:177-82.
- 3- Slamon DJ, Godolphin W, Jones LA, et al: Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 1989;244:707-12.
- 4- Laptalo L., Lara P. N., Lau D. H., et al.: HER-2/neu screening in advanced non-small cell lung cancer (NSCLC): a California cancer consortium trial of trastuzumab and docetaxel, *Proc. Am. Soc. Clin. Oncol.* 2001;20: 335a.
- 5- Reese D. M., Small E. J., et al.: HER-2 expression in androgen-independent prostate cancer, *Proc. Am. Soc. Clin. Oncol.* 2000;19: 347a.
- 6- Estrada C. R., Coogan C. L., Kapur S., et al: HER-2/neu receptor protein over-expression in grade I, II and III bladder transitional cell carcinoma, *Proc. Am. Soc. Clin. Oncol.* 2001;20:199a.
- 7- Jimenez R. E., Grignon D. J., Vaishampayan U., et al.: Analysis of HER-2/neu overexpression in primary and metastatic transitional cell carcinoma of the bladder, *Proc. Am. Soc. Clin. Oncol.* 2000;19: 329a.
- 8- Safran H.: , HER-2/neu overexpression in pancreatic adenocarcinoma, *Proc. Am. Soc. Clin. Oncol.* 2000;19:317a.

HER2/NEU RECEPTOR IN COLORECTAL CARCINOMA

- 9- Safran H., Ramanathan R., Schwartz J., et al : Herceptin and Gemcitabine for metastatic pancreatic cancers that over express HER-2/neu, Proc. Am. Soc. Clin. Oncol.2001;20:130a.
- 10- Steinhoff M., Tantravahi U., King T., et al : HER-2/neu overexpression in adenocarcinoma of the distal esophagus, Proc. Am. Soc. Clin. Oncol.2001;20:150b.
- 11- Tsai J. Y., Aviv H., Benevenia J., et al; Prognostic factors in osteosarcoma—role of HER-2/neu and p53: an immunohistochemical (IHC) and fluorescence in situ hybridization (FISH) analysis, Proc. Am. Soc. Clin. Oncol.2001;20:295b.
- 12- Ross J. S., McKenna, 2 The HER-2/neu oncogene in tumors of the gastrointestinal tract, Cancer Invest.2001;19:554-68. [IVSL].
- 13- Caruso ML, Valentini AM: Immunohistochemical p53 overexpression correlated to c-erbB-2 and cathepsin D proteins in colorectal cancer. Anticancer Res 1996, 16:3813-3818.
- 14- Yang JL, Ow KT, Russell PJ, Ham JM, Crowe PJ: Higher expression of oncoproteins c-myc, c-erb B-2/neu, PCNA, and p53 in metastasizing colorectal cancer than in nonmetastasizing tumors. Ann Surg Oncol 1996;3:574-79.
- 15- Osako T, Miyahara M, Uchino S, Inomata M, Kitano S, Kobayashi M: Immunohistochemical study of c-erbB-2 protein in colorectal cancer and the correlation with patient survival. Oncology 1998;55:548-55.
- 16- McKay J. A., Loane J. F., Ross V. G., et al; C-erbB2 is not a major factor in the development of colorectal cancer, Br. J. Cancer,2002;86:568-73.
- 17- Uchino S, Tsuda H, Mahyama K. et al: over expression of c-erb-b-2 protein in gastric cancer ; its correlation with long term survival of patients. Cancer 1993; 72:3179-84.
- 18- B Schuell, T Gruenberger, W Scheithauer, et al. HER 2/neu protein expression in colorectal cancer *BMC Cancer* 2006;6:123. [IVSL].
- 19- Qingguo Li1, Daorong Wang, Jing Li, et al: Clinicopathological and prognostic significance of HER-2/neu and VEGF expression in colon carcinomas , *BMC Cancer* 2011;11:277.
- 20- Kavanagh DO, Chambers G, O'Grady L, et al : Is overexpression of HER-2 a predictor of prognosis in colorectal cancer? *BMC Cancer* 2009;9:1-6.
- 21- Park DI, Kang MS, Oh SJ, et al : HER-2/neu overexpression is an independent prognostic factor in colorectal cancer. *Int J Colorectal Dis* 2007;22:491-97.
- 22- Ghaffarzagdegan K, Sharifi N, Vosooghnyia H, et al : Her-2/neu expression in colon adenocarcinoma and its correlation with clinicopathologic variables. *IJBMS* 2006;1:64-69.
- 23- Jesus EC, Matos D, Artigiani R, et al : Assessment of staging, prognosis and mortality of colorectal cancer by tumor markers: receptor erbB-2 and cadherins. *Acta Cir Bras* 2005;20:422-27.
- 24- Demirbaş S, Sücüllü I, Yildirim S, et al : Influence of the c-erb B-2, nm23, bcl-2 and p53 protein markers on colorectal cancer. *Turk J Gastroenterol* 2006;17:13-19.