EUROPEAN ACADEMIC RESEARCH
Vol. III, Issue 7/ October 2015

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Detection of Human Epidermal Growth Factor Receptor 2 in Colorectal Tumors

ABU ELGASIM ABASS AWAD ELKAREEM
Assistant Professor in Histopathology and Cytology
Sudan University of Science and Technology
Sudan

Abstract:

This study aimed to detect the expression of Human Epidermal Growth Factor Receptor 2 (HER2) in colorectal tumors samples using immunohistochemistry. Thirty formalin fixed paraffin block (FFPB) embedded samples were collected from patients samples previously diagnosed as colorectal tumors (20 samples were malignant colorectal samples and the remaining 10 samples were benign colorectal samples (polyps)). The FFPB were cut by rotary microtome, and then stained by immunohistochemical method (new indirect method) for detection of HER2. The data obtained was analyzed using SPSS computer program. The age of the patients ranged between 17 to 80 years with mean age of 49 years. The study revealed that most patients were older than 40 years representing 22 (73.3%) and the remaining 8 (26.7%) were younger than 40 years. Concerning sex, the study revealed that the majority of patients were males representing 17 (56.7%) and the remaining 13 (43.3%) were females. HER2 expression showed positive expression among malignant colorectal tumors in 9 (30.0%) samples and negative expression in 11 (36.7%) samples, while all benign colorectal tumors showed negative expression for HER2, this result showed significant statistical association (P= 0.009). The relation between HER2 expression and differentiation of tumors, the study revealed that HER2 expression showed positive result in well differentiated tumor in 2 (10.0%) samples and negative result in 5 (25.0%) samples, moderately differentiated tumors showed positive

result in 7 (35.0%) samples and negative result in 6 (30.0%) samples, this result showed insignificant statistical association (P= 0.125).

Conclusion: *The study concluded that the HER2 expression is associated with malignant colorectal tumors, with no association with the grade of cancer.*

Key words: colorectal adenocarcinoma, polyps, HER2.

INTRODUCTION:

Cancer of the colon is the disease characterized by the development of malignant cells in the lining or epithelium of the first and longest portion of the large intestine. Malignant cells have lost normal control mechanisms governing growth. These cells may invade surrounding local tissue, or they may spread throughout the body and invade other organ systems [(1)]. Colorectal (including anal) cancer is the third most common cancer in the world. An estimated 1.24 million people worldwide were diagnosed with colorectal cancer in 2008, accounting for 10% of the total [(2)]. Colorectal cancer continues to be one of the predominant cancers in the western world and second most common cause of death in the United States [(3)]. The risk of developing colorectal cancer increases with advancing age; more than 90% of cases occur in people aged 50 or older [(4)]. Other risk factors include having inflammatory bowel disease, personal or family history of colorectal cancer, genetic syndrome, lack of regular physical activity, low fruit and vegetable intake, a low-fiber and high-fat diet, overweight and obesity, alcohol consumption and tobacco use [(5)]. Diagnosis of colorectal cancer is via sampling of areas of the colon suspicious for possible tumor development typically done during colonoscopy or sigmoidoscopy, depending on the location of the lesion. The extent of the disease is then usually determined by a CT scan of the chest, abdomen and pelvis. There are other potential imaging tests such as MRI, which

may be used in certain cases. Colon cancer staging is done next and based on the TNM system which is determined by how much the initial tumor has spread, if and where lymph nodes are involved, and the extent of metastatic disease [6]. Treatment depends on many things, including stage of the cancer, treatments may include, surgery (most often a colostomy) to remove cancer cells, chemotherapy to kill cancer cells and radiation therapy to destroy cancerous tissue [7]. HER2 is a member of the human epidermal growth factor receptor (HER/EGFR/ERBB) family [8]. HER2 is encoded by ERBB2, a known proto-oncogene located at the long arm of human chromosome 17 (17q12). HER2 is named because it has a similar structure to human epidermal growth factor receptor, or HER1. Neu is so named because it was derived from a rodent glioblastoma cell line, a type of neural tumor. Gene cloning showed that HER2, Neu, and ErbB-2 are all encoded by the same gene [9]. Activation of *HER2* plays a key role in cell proliferation, cell differentiation, inhibition of apoptosis, and tumor progression [10-12].

MATERIALS AND METHODS:

Sample collection:

Paraffin embedded tissue blocks previously diagnosed as colorectal tumors were collected for this study.

Slides preparation:

One section of 4µm thickness were obtained from each formalin fixed paraffin embedded tissue using a rotary microtome for immunohistochemistry which is then taken in thermal coated slides and dried in hot plate oven at 80ºC for one hour.

Immunohistochemical staining:

Sections were brought to water and retrieved using water bath retrieval technique at 97ºC, then treated with hydrogen

peroxide solution for 15 minutes, then washed in phosphate buffer saline (pH 7.4) for 5 minutes, then treated with anti HER2 primary antibodies for 30 minutes, then rinsed in phosphate buffer saline, then treated with secondary polymer conjugate for 30 minutes, then rinsed in phosphate buffer saline, then treated with DAB for 7 minutes, then washed in phosphate buffer saline for 5 minutes, then counterstained in Mayer's haematoxylin for 1 minute, then washed in water and blued in 0.05% ammoniated water for 16 second, then washed in tap water, then dehydrated through ascending of ethanol (50%, 70%, 90%, 100%) 2 minutes for each then cleared in 2 change of xylene 2 minutes for each, and mounted in DPX mounting media [13].

Result Interpretation:

Results obtained were detected by researcher and confirmed by experienced histopathologist. Negative and positive controls were used for evaluation of the test sections.

Statistical analysis:

All information about the study population was entered a computer as well as obtained results. The data was analyzed using SPSS computer program.

RESULTS:

A total of 30 samples from patients diagnosed as colorectal tumor were investigated by immunohistochemical method, 20 of them were malignant colorectal tumors representing (66.7%), and the remaining 10 (33.3%), were benign Table (1). The age of the study population ranged 17 to 80 years old with mean age of 50 years. Most patients were older than 40 years representing 22 (73.3%) and the remaining 8 (26.7%) were younger than 40 years Table (2). Most patients were male representing 17 (56.7%) and the remaining 13 (43.3%) were

female Table (3). Malignant colorectal tumors revealed positive expression of HER2 in 9 (30.0%) samples and negative expansion of HER2 in 11 (36.7%) samples, while all benign colorectal samples showed negative expansion of HER2 (P=0.009) Table (4). Well differentiated tumors showed positive expression in 2 (10%) samples and negative expression of HER2 in 5 (25%) samples and moderately differentiated tumor showed positive expression of HER2 in 7 (35%) samples and negative expression of HER2 in 6 (30%) samples (P= 0.125) Table (5).

Table (1) Distribution of sample among the study population

Sample	Frequency	Percent
Malignant	20	66.7%
Benign	10	33.3%
Total	30	100%

Table (2) Distribution of age among the study population

Age group (year)	Frequency	Percent
Less than 40	22	73.3%
Older than 40	8	26.7%
Total	30	100%

Table (3) Distribution of sex among the study population

Sex	Frequency	Percent
Male	17	56.7%
Female	13	43.3%
Total	30	100%

Table (4) Immunohistochemical expression of HER2 among the study samples

Sample	HER2		Total
	Positive	Negative	
	N (%)	N (%)	N (%)
Malignant	9 (30%)	11 (36.7%)	20 (66.7%)
Benign	0 (0.0%)	10 (33.3%)	10 (33.3%)
Total	9 (30%)	21(70%)	30 (100%)

P value 0.009

Table (5) Correlation between HER2 expressions and cancer grade

Cancer differentiation	**HER2**		**Total**
	Positive	Negative	
	N (%)	N (%)	N (%)
Well differentiated tumor	2 (10%)	5 (25%)	7 (35%)
Moderately differentiated tumor	7 (35%)	6 (30%)	13 (65%)
Total	9 (45%)	11 (55%)	20 (100%)

P value 0.125

DISCUSSION:

Colorectal cancer, commonly known as colon cancer or bowel cancer is the third most commonly diagnosed cancer starts in a small area but can spread to other parts of the body to form metastatic tumors [(14)]. In this study the age of the study population ranged between 17 to 80 years with mean age of 49 years. Most patients were older than 40 years representing 22 (73.3%) and the remaining 8 (26.7%) were younger than 40 years. This proved that individuals older than 40 years are more susceptible for colorectal tumor due to acidosis (low degree of BH). This study compatible with Abeloff, *et al.,* [(1)], who reported that the condition is rare in people under 40 years and the majority of cases diagnosed in age over 55 years old. Also the study is consistent with the study of Marphy *et al.,* [(15)], who reported that the colorectal cancer appear mainly after the age of 50 years. Regarding sex, males are more affected by colorectal cancer than females. This attributed to increase smoking and consumption of alcohol in males than females. This result supported by Marphy *et al.,* [(15)] and Pischon *et al.,* [(16)], they reported that the incidence of colorectal cancer in appear in males higher than females. The description of tumor grade revealed that the most colorectal cancer patients are moderately differentiated tumor patients then in second place well differentiated tumor patients and poor differentiated tumor was not observed, this study compatible with Compton *et al.,* [(17)], who reported that the most colorectal adenocarcinomas

(70%) are diagnosed as moderately differentiated tumor, well and poorly differentiated carcinomas account for 20% and 10%, respectively. HER2 gene amplification was more frequently observed in CRCs [18]. Malignant colorectal tumors revealed positive expression of HER2 in 9/20 malignant samples compared to 0/10 in benign samples, this result show significant statistical association (P=0.009). This result supported by Nathanson *et al.,* ([19], they reported that HER2 protein overexpression suggests that this oncogene plays an infrequent role in the development and progression of colon cancer.

HER2/neu overexpression is observed in different grades of colorectal adenocarcinomas [20]. Based on this study, well differentiated tumors showed positive result of HER2 in 2/7 samples and negative result in 5/7 samples, moderately differentiated tumors showed positive result in 7/13 samples and negative result in 6/13 samples, this result showed statistical insignificant association (P=0.125). This study is compatible with the study of Delektorskaia *et al.,* [21], who reported that no correlation between HER-2/neu staining and grades of tumors.

CONCLUSION:

The study concluded that the HER2 expression is found to be positive in the malignant forms of colorectal samples while benign forms showed negative results, this expression showed no association with the grade of cancer.

REFERENCES:

1-Abeloff MD, Armitage JD, Niederhuber JE, Kastan MB, Mckenna WG. *Abeloffs Clinical Oncology,* 4th ed. Philadelphia, Pa: Elsevier.2013;2215-2234.

2-Ferlay J, Shin HR, Bray F. Estimates of worldwide burden of cancer. 2010; 127:2893-2917.
3-Rim SH, Seeff L, Ahmed F, King JB, Coughlin S. Colorectal cancer incidence in the United States, 1999-2004: an updated analysis of data from National Program of Cancer Registries and the Surveillance, Epidemiology, and End Results Program. *Cancer*. 2009; 115: 1967-1976.
4-Ries LAG, Melbert D, Krapcho M. SEER cancer statistics review, Bethesda, MD: 2008
5- Curry S, Byers T, Hewitt M. Fulfilling the Potential of Cancer Prevention and Early Detection. Washington, DC: National Academies Press. 2003.
6-Cunningham D, Atkin W, Lenz HJ, Lynch HT, Minsky B, Nordlinger B, Starling N. Colorectal Cancer. New England Journal of medicine. 2010;359: 1757-65.
7- Atkin WS, Edwards R, Kralj-Hans I, Wooldrage K, Hart AR, Northover JM, Parkin DM, Wardle J, Duffy SW, Cuzick J. UK Flexible Sigmoidoscopy Trial Investigators. Once-only flexible sigmoidoscopy screening in prevention of colorectal cancer: a multicentre randomised controlled trial. *Lancet*. 2010; 375(9726):1624-33.
8-Mitri Z, Constantine T, O'Regan R. The HER2 Receptor in Breast Cancer: Pathophysiology, Clinical Use, and New Advances in Therapy. *Chemother Res Pract* 2012: 743193.
9-Coussens L, Yang-Feng TL, Liao YC, Chen E, Gray A, McGrath J, Seeburg PH, Libermann TA, Schlessinger J, Francke U. Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. *Science. 1985;*230 (4730): 1132–9.
10- Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol*. 2001; 2: 127–137
11- Gschwind A, Fischer OM, Ullrich A. The discovery of receptor tyrosine kinases: targets for cancer therapy. Nat Rev Cancer. 2004;4: 361–370

12- Lee HE, Park KU, Yoo SB, Nam SK, Park J, Kim HH, Lee HS. Clinical significance of intratumoral HER2 heterogeneity in gastric cancer. *Eur J Cancer*. 2013;49: 1448–1457

13-Bancroft JD, Marilyn G. *Theory and practice of histological techniques*. 5th ed. London: Churchill Livingstone. 2002;125.

14-Boyle P, Langman JS. ABC of colorectal cancer: Epidemiology. *BMJ*. 2000; 321(7264).

15-Murphy G, Susan, Devesa S, Amanda, Cross J, Peter, Inskip D, Katherine A, McGlynn, and Michael B. Sex Disparities in Colorectal Cancer Incidence by Anatomic Subsite, Race and Age. *Int J Cancer*. 2001;128: 1668–1675.

16-Pischon T, Lahmann PH, Boeing H. Body size and risk of colorectal cancer. *Journal of national cancer*. 2006*;* 98:920-931.

17-Compton CC, Fielding LP, Burgart LJ, et al. Prognostic factors in colorectal cancer. College of American Pathologists Consensus Statement 1999. *Arch Pathol Lab Med*. 2000;124:979-94

18-Conradi LC, Styczen H, Sprenger T, Wolff HA, Rodel C. Frequency of HER-2 positivity in rectal cancer. *Am J Surg Pathol*. 2013*;*37: 522–531

19- Nathanosn DR, Alfred T, Culliford IV, Jinru S, Beiyun C, Matthew DA, Zhao-Shi Z, Garrett MN, William G, Francis B, and Philip BP. HER 2/neu EXPRESSION AND GENE AMPLIFICATION IN COLON CANCER *Int. J. Cancer*. 2003; 105, 796–802

20-Schuell B, Gruenberger T, Scheithauer W, Zielinski Ch, Wrba F. HER 2/neu protein expression in colorectal cancer. *BMC Cancer*. 2006; 6:123.

21-Delektorskaia VV, Perevoshchikov AG, Kushlinskii NE. Expression of nm23 and c-erbB-2 proteins in cells of primary colorectal cancer and its metastases, *Arkh. Patol*. 2003;65:11-5