# Pathological study of colonic cancer by application of human epidermal growth factor receptor type II (Her-2\ neu)

**Liwaa H. Al-Kelabi, FIBMS (Path.)**
Department of Pathology and Forensic Medicine, Faculty of Medicine, Kufa University, Kufa, P.O. Box 18, Iraq.
HER-2/neu, colonic cancer

## (دراسة مرضية لسرطان القولون بتطبيق عامل النمو البشري النوع الثاني Her-2\neu)

**الخلاصة**

**الخلفية:** هذه الدراسة تهدف الى تقييم مغزى التعبير المفرط لعامل النمو البشري (HER-2/neu) في سرطان القولون بواسطة دراسة الصبغة المناعية النسيجية الكيميائية وعلاقته بعوامل التكهن وعوامل تقدم الورم المرضية والسريرية.

**الطريقة:** تم الحصول على ٤٠ عينة مثبتة بالفورمالين ومطمورة بالبارافين لمرضى مصابين بسرطان القولون. مجموعة من ١٠ حالات حميدة استخدمت للمقارنة. كذلك استخدمت ١٠ عينة لنسيج القولون الطبيعي كمجوعة قياسية. استخدمت طريقةLSAB لتحديد التعبير المناعي النسيجي لـ(HER-2/neu)

**النتائج:** تضمنت هذه الدراسة أربعين حالة مرضية لسرطان القولون،كانت أعمار المرضى تتراوح بين ٢١-٧٠ سنة وكان المعدل العمري ٤٨.٨ سنة .

أثبتت الدراسة أن هناك فرق ذا مغزى بين التعبير الزائد ل (HER-2/neu ) و بين درجة تمايزالورم ($p < 0.05$) value.كما لم يثبت وجود أي علاقة بين التعبير الزائد ل(HER-2/neu ) وبقية العوامل المرضية السريرية ألاخرى (عمر المريض، مرحلة الورم) ($p>0.05$) value .

كما لم تثبت الدراسة أن هناك فرق ذا مغزى بين التعبير الزائد ل(HER-2/neu) للحالات الحميدة بالمقارنة مع الحالات السرطانية ($p$ value$>0.05$).

**الاستنتاج :** بالاعتماد على نتائج هذه الدراسة نستنتج ان التعبير الزائد ل (HER-2/neu) كان موجودا في كل من الحالات الحميدة والحالات السرطانية كما لا يوجد فرق ذا مغزى للتعبير الزائد ل (HER-2/neu ) بالنسبة لعمر المريض ومرحلة الخبيث بينما يوجد فرق ذا مغزى بالمقارنة مع درجة تمايز الورم، وبالتالي يمكن لنا أن نستنتج أن HER-2/neu يلعب دور مهم في تولد نشأة خبيث القولون. ويمكن ألتوجيه بأن تعطيل HER-2/neu ربما يكون هدف لإعاقة عملية تطور السرطان وبالتالي يطور فعالية العلاج المضاد للسرطان.

**Abstract**

**Background:** This study aimed to assess the significance of human epidermal growth factor (HER-2/neu) protein overexpression and its possible correlation with clinicopathological parameters of patients with colonic cancer.

**Methods:**

FFPE (Formalin fixed, paraffin-embedded blocks) from 40 patients with colorectal carcinoma were included in this study. A group of 10 patients with benign lesions (polyp) were included as a comparative group and 10 normal colonic tissue sections were included as control group. Formalin fixed, paraffin-embedded blocks from 40 patients with colorectal cancer were included in this study. HER-2/ neu immunohistochemistry was performed using the HercepTest Kit (Dako, Glostrup, Denmark) according to the manufacturer's recommendations.

**Results:**
A total of 40 malignant cases were included.HER2/neu was considered as positive in(26 cases) 65% of colorectal carcinoma and a total of 10 benign colorectal lesions it was considered as positive in (7 cases)70% of it. So there is no significant difference from that of malignant cases (P>0.05). We did not find any significant difference between overexpression of HER-2/neu in relation to age and tumor stage (P>0.05), but there was significant difference in relation to tumor stage (P<0.05).

**Conclusion:**
Based upon the findings of this study, it can be concluded that HER-2/neu overexpression was observed in both colorectal cancer and benign colorectal lesions(polyps),no significant association with age, gender, tumor stage with significant difference in relation to tumor differentiation ,that mean HER-2/neu overexpression play an important role in the pathogenesis of colorectal cancer , which improve that the blocking of HER-2/neu may be a target for blocking the evolution and progression and hence improving the efficacy of anti-cancer therapy against this cancer.

**Background:**
Colorectal carcinoma (CRC) is the second leading cause of cancer death in the U.S [(1)]. The majority of these CRCs arise sporadically through a multistep process (a multi-step process which is characterized by the accumulation of genetic alterations), with the adenoma as the recognized intermediate step in their development in an adenoma-carcinoma sequence [(1,2)]. In recent decades researches have tried to identify biological markers in order to individualize chemotherapy and predict clinical outcome and tumor sensitivity(3).One of these markers is Her-2/neu protein which is one of the Her family receptors located on chromosome 17q 21,so it is membrane bound G-protein receptors that when activated done multiple signal transduction pathways regulating cellular growth ,In addition to that which is involved in normal cell proliferation and growth of tissue ,it is related to EGFR(epidermal growth factor receptor)this marker is studied well in the subject of breast carcinoma, in which both amplification and over expression correlate with the over all cause of diseases and poor prognosis and also represent as evaluated factor of poor response to both chemotherapy and endocrine therapy[(4)]. Clinically, c-erbB-2 amplification and/or over expression has been associated with poor prognosis in a number of tumor types such as breast and ovarian cancer[(5,6)]. Pathologic specimens from the National Surgical Adjuvant Breast and Bowel Project protocol B-06 were reviewed and correlated with patients' outcome. Overall survival was decreased in all HER-2/neu-positive patients, and those patients having HER-2/neu over expression with a good nuclear grade had a five-fold increase in mortality rate[(7)]. Over expression of the HER-2/neu receptor is detected in 25–35% of human breast cancer patients[(5,6)]. Treatment of these patients with Herceptin®, an anti-HER-2 monoclonal antibody, has been shown to reduce tumor volume, to augment the effects of chemotherapy and to increase survival in primary and metastatic breast cancer[(8,9)]. The success of HER-2/neu directed therapy in breast cancer has lead to evaluations of protein expression and gene amplification in multiple tumour types, colorectal cancer among others. Herceptin® has been shown to inhibit colony formation of the HCA-7 colon cancer cell line and HCA-7 tumor xenografts [(10)].
The aim of this study was to determine the incidence of Her-2 positivity in both colorectal cancer and benign colorectal tumour(polypes) ,further more I determine the

relationship of Her-2/neu expression and patient sex,age and also the relation with tumour stage and grade.

**Materials and Methods:**

This retrospective study involved 40 specimens of malignant colon lesions of patients who underwent elective surgery for colorectal Tumors.A group of 10 patients with benign lesions (polyp) were included as a comparative group, and Ten cases of normal colonic tissue from patients presented with colonic biopsy, other than tumor(inflammatory colonic conditions) were selected and regarded as a control group. Each step was done for the comparative and control groups in parallel with the study group.Tumour were staged according to TNM system and the Dukes classification[11,12]. HER-2/neu immunohistochemistry was performed using the Hercep-Test Kit (Dako, Glostrup, Denmark) according to the manufacturer's recommendations. 2 μm tissue sections were deparaffinized, rehydrated and placed in DAKO Epitope Retireval Solution for 40 minutes at 90°C, followed by cooling for 20 minutes at room temperature and treatment with peroxidase-blocking reagent. Afterwards slides were rinsed and incubated with the primary antibodies against HER-2/neu for 30 minutes followed by rinsing the slides and incubation with the DAKO Visualization Reagent for 30 minutes. After washing, the slides were incubated in diaminobenzidine for 10 minutes, counterstained with hematoxylin, de-hydrated and cover slipped. Evaluation of the results was done according to the criteria as recommended by the manufacturer using the scores from 0 to 3+. Score 0 is defended as no staining at all or membrane staining in < 10% of tumour cells. Score 1+is defined as faint/barely perceptable membrane staining in > 10% of tumour cells. The cells are only sarined in part of the membrane. 2+is defined as weak to moderate staining of the entire membrane in > 10% of the tumour cells. And 3+ is defined as strong staining of the entire membrane in > 10%. Score of 0 indicates a negative tumour, while scores of 1+, 2+ and 3+ were regarded as positive expression of c-erbB-2. Expression of Her-2/neu was assessed with respect to age of patient, Dukes' stage and degree of differentiation using the chi-squared test using SSPS 17 software..

**Results:**

A total of 40 patients with colorectal cancer were studied. For detailed patients' characteristics see Table 1. The median age of our patients, of whom ( 30 ) were male, was (48.8) years. The primary tumour site was the colon in ( rectosigmoid region) .

**Table 1. Patients characteristic**

| Variable | No. |
|---|---|
| Gender<br>Male<br>Female | <br>30<br>10 |
| Age<br><50 years<br>≥50 years | <br>15<br>25 |
| Histological grade<br>I<br>II<br>III | <br>15<br>18<br>7 |
| Stage of tumor<br>I<br>II<br>III | <br>1<br>9<br>30 |

**Her-2/neu expression and clinicopathological parameters:**

The expression of the c-erbB-2 protein was evaluated with respect to the patient's clinicopathological data. HER-2/neu immuno-expression was positive in (70%) of benign lesions and non of the control group conditions show HER-2/neu immuno-expression, while (65%)of malignant cases were positive for HER-2/neu without significant difference(p value >0.05)
(Table 2).

Over-expression of HER-2/neu was detected in (65%) in those patients <50 years old in comparison to (80%) in those who are ≥to 50 years old without significant difference (p value >0.05) .

Regarding tumor grade HER-2/neu immuno-expression was positive in (60%) of grade I , (83.3%) of grade II and (28.5%) of grade III with significant difference(p value <0.05),Fig(1)(d,e). Furthermore Over-expression of HER-2/neu was detected in (55.5%) of stage II and without significant difference (p value >0.05) (Table 2).

**Table 2. HER-2/ neu (C-ErbB-2) over-expression andclinicopathological characteristics.**

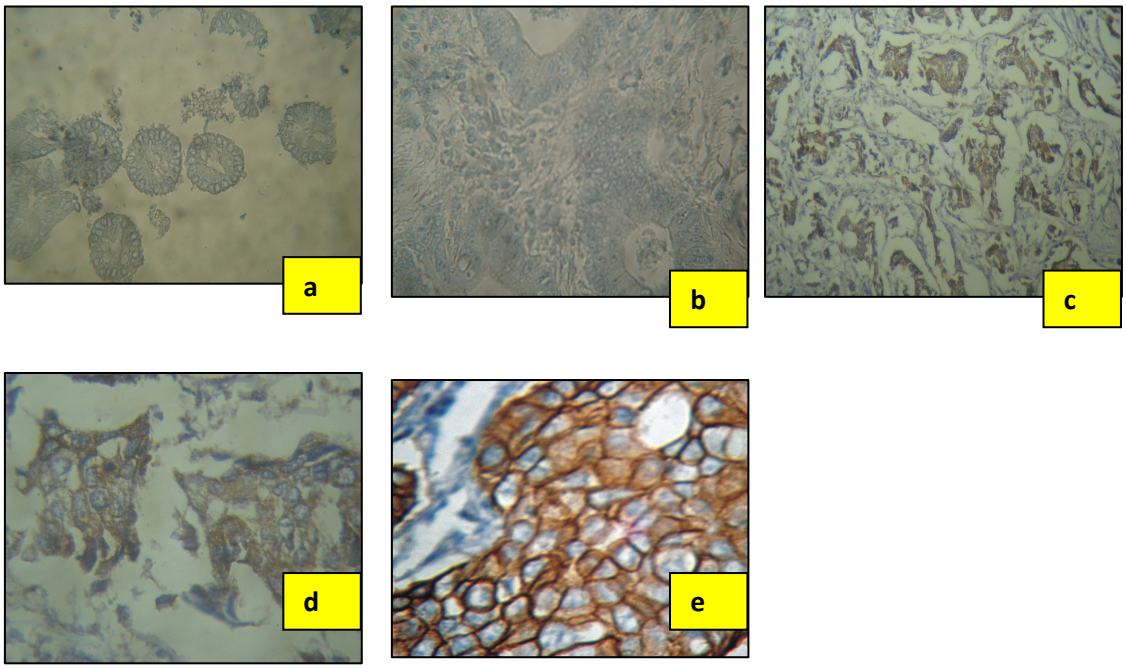
| Parameters | Total No. of patients | c-ErbB-2 protein overexpression ( Negative) | (positive ) | P value |
|---|---|---|---|---|
| Normal tissue | 10 | 10(100%) | 0 | |
| Benign lesions | 10 | 3(30%) | 7(70%) | >0.05 |
| Malignant cases | 40 | 14(35%) | 26(65%) | |

**Age group(years)**

| | | | | |
|---|---|---|---|---|
| <50 years | 15 | 7(46.6%) | 8(53.3%) | >0.05 |
| ≥50 years | 25 | 6(24%) | 19(76%) | |
| **Grade** | | | | |
| I | 15 | 6(40%) | 9(60%) | <0.05 |
| II | 18 | 3 (16.6%) | 15 (83.3%) | |
| III | 7 | 5 (71.4%) | 2(28.5%) | |

**Tumor stage**

| | | | | |
|---|---|---|---|---|
| Stage I | 1 | 1(100%) | 0(-) | >0.05 |
| Stage II | 9 | 4(44.5%) | 5(55.5%) | |
| Stage III | 30 | 8(26.6%) | 22(73.3%) | |




**Fig(1):shows:**

| |
|---|
| **a)**colonic polyp, score zero HER2/neu,IHCX20**.** |
| **(b)**colorectal cancer gradeI,score zero HER2/neu overexpression,IHCX40**.** |
| **(c)** colorectal cancer grade II ,score +1 HER2/neu overexpression,IHCX20**.** |
| **(d)** colorectal cancer grade II ,score +2HER2/neu overexpression,IHCX40**.** |
| **(e)** colorectal cancer grade III,score +3HER2/neu overexpression,IHCX40**.** |

**Discussion:**

The ErbB signaling network is known to influence a wide range of cellular processes, including proliferation, motility, and survival[13]). It is known that overexpression of EGFR often portends a worse prognosis [14,15]. Overexpression of the HER-2/neu receptor is detected in 25–35% of human breast cancer[5,6], but the level and incidence of HER-2/neu overexpression in primary colon tumours appears to be different than those observed in breast cancer. Conflicting data exist about the prevalence of HER-2/neu overexpression in colorectal cancer which ranges from 0 to 83 % [16,17]. In our study, we examined 40 colorectal cancer tumor samples for the presence of Her-2/neu oncoprotein by immunhistochemistry. No correlation could be found between clinicopathological features and the HER-2/neu overexpression,regarding the age and the stage of the disease . Similar results are described in the study of Nathason et al[18] . In contrast to these results are four studies who did report an association between Her-2/neu overexpression and advanced stage[19,20,21,22].Our study also show significant difference between HER-2/neu overexpression and the grade of the tumor ,this finding is agreed with Saeki T,etal [22]. Our study results indicating a very low rate of HER-2-/neu positivity and no correlation with clinicopathological features might be hampered by the small number of cases 40 cases, However, the results are in agreement with same other larger patient series. In contrast to these data are the above mentioned four publications. The most likely reason for this divergency is the technical variability in the performance of immunhistochemistry. Another reason may be due to the fact, that different antibodies have been used, stressing the importance of using standardized test systems most notably in case of therapeutic relevance of the results. The c-erbB-e protein expression was observed in colorectal cancer but rarely in the therapeutic range (2+ and 3+). As known from studies in Her-2/neu metastatic breast cancer, Herceptin®, a HER-2 neutralizing antibody, is only effective in the therapeutic range. In a study by Ramanathan et al[23]. Her-2/neu positive patients with advanced colorectal cancer should receive trastuzumab (Herceptin® and irinotecan treatment. Of 138 screened patients Her-2/neu overexpression was only detected in 11 (8%; 2+ in 5 and 3+ in 6 patients), therefore the study was prematurely closed.

Furthermore we recommend for further researches should be done to detect both the HER-2/neu overexpression and gene amplification by specific genetic methods like FISH technique or others to become more specified and accurate in detection of this marker in patient with colorectal cancer.

**References:**


1. Kinzler,K.W. and Vogelstein B. (1996) Lessons from hereditary colorectal cancer. Cell, 87, 159±170.
2. Shields,J.M., Pruitt,K., McFall,A., Shaub,A. and Der,C.J. (2000) Understanding ras: `it ain't over 'til it's over' Trends Cell. Biol., 10, 147±154.
3. Saltz LB, Meropol NJ, Loehrer PJ, Needle MN, Kopit J, Mayer RJ: Phase II Trial of cetuximab in patients with refractory colorectal cancer that express the epidermal growth factor receptor. J Clin Oncol 2004, 22:1201-1208.
4. Ross JS, McKenna BJ: The Her-2/neu oncogene in tumors of the gastrointestinal tract. Cancer Invest 2001, 19:554-68.
5. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL: Human breast cancer: correlation of relapse and survival with amplification of HER-2/neu oncogenes. Science 1987, 234:177-182.
6. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SA, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A, Press MF: Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989, 244:707-712.
7. Paik S, Hazan R, Fisher ER, Sass RE, Fisher B, Realmond C, Schlessinger J, Lippman ME, King CR: Pathologic findings from the National Surgical Adjuvant Breast and Bowel Project: prognostic significance of erbB-2 protein in primary breast cancer.J Clin Oncol 1990, 8:103-112.
8. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L: Use of chemotherapy plus monoclonal antibody against HER2 for metastatic breast cancer that overexpress HER2. N Engl J Med 2001, 344:783-92.
9. Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Salamon DJ, Murphy M, Novotny WF, Burchmore M, Shak S, Stewart SJ et, Press M: Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol 2000, 20:719-26.
10. Mann M, Sheng H, Shao J, Williams CS, Pisacane PI, Sliwkowski MX, DuBois RN: Targeting cyclooxygenase 2 and HER-2/neu pathways inhibits colorectal carcinoma growth. Gastroenterology 2001, 120:1713-9
11. Beahrs OH: Staging of cancer of the colon and rectum. Cancer 1992, 70:1393-6.
12. Hermanek P, Altendorf A: Pathol Res Pract. 1981, 173(1–2):1-11. PubMed Abstract.

13. Kirschbaum MH, Yarden Y: The ErbB/Her family of receptor tyrosine kinase a potential target for chemoprevention of epithelial neoplasm.J Cell Biochem Suppl 2000, 34:52-60.
14. Baselga J, Mendelsohn J: The epidermal growth factor receptor as a target for therapy in breast carcinoma.Breast Cancer Res Treat 1994, 29:127-138.
15. Baselga J, Mendelsohn J: Receptor blockade with monoclonal antibodies as anti-cancer therapy.Pharmacol Ther 1994, 64:127-54.
16. Ross JS, McKenna BJ: The Her-2/neu oncogene in tumors of the gastrointestinal tract.Cancer Invest 2001, 19:554-68.
17. McKay JA, Loane JF, Ross VG, Ameyaw MM, Murray GI, Cassidy J, McLeod HL: C-erbB-2 is not a major factor in the development of colorectal cancer. Br J Cancer 2002, 86:568-573.
18. Nathason DR, Culliford AT, Shia J, Chen B, D'Alessio MD, Zeng Z, Nash G, Gerald W, Barany F, Paty P: HER-2/neu expression and gene amplification in colon cancer. Int J Cancer 2003, 105:796-802.
19. Osako T, Miyahara M, Uchino , Inomata M, Kitano S, Kobayashi M: Immunohistochemical study of c-erbB-2 protein in colorectal cancer and the correlation with patients survival. Oncology 1998, 55:548-55.
20. Kapitanovic S, Radosevic S, Kapitanovic M, Andelinovic S, Ferencic Z, Tavassoli M, Primorac D, Sonicki Z, Spaventi S, Pavelic K, Spaventi R: The expression of p185(HER-2/neu) correlates with the stage of disease and survival in colorectal cancer. Gastroenterology 1997, 112:1103-13.
21. Lazaris AC, Theodoroporlos GE, Anastassopulos, Nakopoulou L, Panoussopoulus D, Papadimitriou K: Prognostic significance of p53 and c-erbB-2 immunohistochemical evaluation in colorectal adenocarcinoma.Histol Histopathol 1995, 10:661-8.
22. Saeki T, Salomon DS, Johnson GR, Gullick WJ, Mandai K, Yamagami K, Moriwaki S, Tanada M, Takashima S, Tahara E: Association of epidermal growth factor-related peptides and type I receptor tyrosine receptors with prognosis of human colorectal carcinomas.Jpn J Clin Oncol 1995, 25:240-9.
23. Ramanathan RK, Hwang JJ, Zamboni WC, Sinicrope FA, Safran H, Wong MK, Earle M, Brufsky A, Evans T, Troetschel M, Ealko C, Day R, Chen HX, Finkelstein S: Low expression of HER-2/neu in advanced colorectal cancer limits the usefulness of trastuzumab (Herceptin) and irinotecan as therapy. A phase II trail.Cancer Invest 2004, 22(6):858-65.