İSTANBUL UNIVERSITY PRESS

RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ
Journal of Advanced Research in Health Sciences /
Sağlık Bilimlerinde İleri Araştırmalar Dergisi 2024
DOI: 10.26650/JARHS2024-1366466

C-ERB B2 EXPRESSION IN COLORECTAL CARCINOMA; ITS RELATIONSHIP WITH TUMOR GRADE AND OTHER PROGNOSTIC FACTORS

KOLOREKTAL KARSİNOMLARDA C-ERB B2 EKSPRESYONUNUN TÜMÖR DERECESİ VE DİĞER PROGNOSTİK FAKTÖRLERLE İLİŞKİSİ

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Citation/Atıf: Şengül Şimşek N, Yaldız M. C-ERB B2 expression in colorectal carcinoma; its relationship with tumor grade and other prognostic factors. Journal of Advanced Research in Health Sciences 2024;7(2):81-86. https://doi.org/10.26650/JARHS2024-1366466

ABSTRACT

Objective: The multidisciplinary management of colorectal carcinoma (CRC) is based on tumor stage, grade, and presence of vascular invasion. In this approach, information derived from the molecular characteristics of the tumor may be missed. There is a need to determine new prognostic parameters.

Human epidermal growth factor 2 (c-erb B2) protein overexpression is implicated in the development of various tumor types, while the data on CRC is ambiguous. This study aimed at determining the frequency of c-erb B2 overexpression in CRC, checking it against prognostic parameters, as it has a special role in breast and gastric cancers.

Material and Methods: A total of 71 colectomy specimens diagnosed as CRC at the Mustafa Kemal University Medical Faculty, Department of Pathology between January 2007, and December 2012, were included in this study. The c-erb B2 overexpression of tumor cells was detected by using immunohistochemistry (IHC).

Results: Among the 71 CRC cases, 34 (47.9%) of them were c-erb B2 score 0, 22 (31%) of them were score 1, 10 (14.1%) of them were score 2, 5 (7%) of them were score 3.

Conclusion: The overexpression of c-erb B2 protein was detected in 21.1% of CRC cases whereas no significant relationship with prognostic parameters was determined.

Keywords: ERBB-2 protein, colorectal neoplasms, prognosis

ÖZ

Amaç: Kolorektal karsinomlarda multidisipliner yaklaşımda tümör evresi ve derecesi ile vasküler invazyon varlığı esas alınmaktadır. Bu yaklaşımda tümörün moleküler karakteristik bilgileri atlanabilmektedir. Bu tümörler için yeni prognostik parametrelerin belirlenmesine ihtiyaç duyulmaktadır. Birçok tümör tipinin gelişiminde c-erb B2 proteini aşırı ekspresyonu gösterilmiştir, ancak kolorektal karsinomlarda bu konudaki sonuçlar net değildir. Bu çalışmada meme ve mide kanserinde özel bir rolü olan insan kaynaklı epidermal büyüme faktörü 2 (c-erb B2) proteini aşırı ekspresyonunun kolorektal karsinomlardaki frekansını belirlemek ve prognostik parametrelerle ilişkisini araştırmak hedeflenmiştir.

Gereç ve Yöntemler: Ocak 2007 ile Aralık 2012 tarihleri arasında Mustafa Kemal Üniversitesi Tıp Fakültesi Patoloji Bölümü'nde kolorektal karsinom tanısı alan 71 kolektomi örneği bu çalışmaya dahil edilmiştir. Tümör hücrelerinin c-erb B2 protein ekspresyonu, immünohistokimyasal yöntemle araştırılmıştır.

Bulgular: Yetmiş bir kolorektal karsinom olgusundan 34 (%47,9) tanesi c-erb B2 skor 0, 22 (%31) tanesi skor 1, 10 (%14,1) tanesi skor 2, 5 (%7) tanesi skor 3 olarak değerlendirilmiştir.

Sonuç: Kolorektal karsinom olgularının %21,1'inde c-erb B2 proteini aşırı ekspresyonu tespit edilmiştir; ancak prognostik parametrelerle anlamlı bir ilişkisi izlenmemiştir.

Anahtar Kelimeler: ERBB-2 proteini, kolorektal neoplazmlar, prognoz

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Submitted/Başvuru: 28.09.2023 • **Revision Requested/Revizyon Talebi:** 30.10.2023 • **Last Revision Received/Son Revizyon:** 25.11.2023 • **Accepted/Kabul:** 21.12.2023 • **Published Online/Online Yayın:** 05.03.2024

INTRODUCTION

Colorectal carcinoma (CRC) is one of the major worldwide health problems. It is the fourth most common cause of malignancies in the world and the second most common cause of cancer related deaths in the United States (US) and the western world (1).

The development of new cytotoxic agents and surgical techniques has improved the survival of CRC patients. But it is still not capable of preventing recurrence when patients become refractory to these modern chemotherapeutic regimens. Therefore, it is of great interest to identify molecular biomarkers for predicting outcome, therapeutic response, and potential therapeutic targets in CRC patients.

One of these biomarkers is the human epidermal growth factor 2 (c-erb B2) receptor. It has an oncogene located on the chromosome 17q21. Its increased expression plays a key role in cell proliferation, cell differentiation, inhibition of apoptosis and tumor progression. The overexpression of c-erb B2 has been reported in many epithelial tumors including breast, ovarian, gastric, colon and lung cancers. It is associated with more aggressive disease, poorer prognosis and high risk of recurrence (2, 3). Therefore, there are numerous ongoing researches for directed therapy alternatives for this target. Trastuzumab (Genentech, Inc. Roche Group, San Francisco, CA, USA), a humanized monoclonal antibody, targets the extracellular domain of the human epidermal growth factor 2 receptor. Its therapeutic benefit has been demonstrated in breast cancer and gastric cancer (4, 5).

Several studies evaluating c-erb B2 in CRC resulted in a large debate because overexpression rates varied between 0 and 84% (6). To optimize the wide range of these results, technical approaches should be standardized, and large-scale studies should be done.

This study aims to investigate the correlation of c-erb B2 expression with known prognostic parameters and to determine the availability in practical use.

MATERIALS and METHODS

This study was carried out at the Department of Pathology of the Mustafa Kemal University University Medical Faculty. A total of 71 cases diagnosed as CRC by processing their colectomy specimens at the Mustafa Kemal University Medical Faculty Department of Pathology between January 2007 and December 2012 were included in the study.

The prognostic parameters such as age, gender, and tumor localization, are determined for each patient respectively. The hematoxylin-eosin stained sections in the archive obtained from the formalin-fixed, paraffin-embedded blocks of colectomy specimens were evaluated for determining other prognostic predictors such as the microscopic tumor type, the histopathologic grade, the presence of lymphovascular invasion and lymph node metastasis, depth of invasion, and pathological Tumor-lymph nodes-metastasis (TNM) stage at diagnose duration. For microscopic type and histopathologic grade, the World Health Organization (WHO) 2010 classification was used.

For the immunohistochemical (IHC) process, new sections obtained from paraffin-embedded blocks were incubated with 1/800 diluted Polyclonal Rabbit Anti-Human c-erb B2 oncoprotein, for 30 minutes in an autostainer (DAKO Autostainer Link 48).

All sections were evaluated by two independent pathologists. Regarding c-erb B2 IHC staining, the scoring system for gastric cancer is used. Scores from 0 to 3 were used to determine the density of staining. No staining at all or incomplete membrane staining in <10% of the tumor cells was given the score 0, faint incomplete membrane staining in >10% of the tumor cells was given the score 1, weak to moderate staining of the entire membrane in >10% of the tumor cells was given the score 2, and strong staining of the entire membrane in >10% of the tumor cells was given the score 3. Scores of 0 and 1 were labeled as negative staining. Scores of 2 and 3 were labeled as positive staining (5, 7). The data was statistically analyzed by the Statistical Package for Social Sciences (SPSS) version 21 by using the Pearson chi-square test.

Approval was received for this study from the ethics committee of the Mustafa Kemal University Faculty of Medicine (Date: 04.10.2012, No: 24-2012/59). This study was exempted from informed consent because it is a retrospective study using previous data and materials from the archive.

RESULTS

The ages of the cases were between 24 and 88 (mean 58). There were 32 (45.1%) males and 39 (54.9%) females with a male-to-female ratio of 1:1.2. According to IHC c-erb B2 staining results, no expression or membrane staining in <10% of the tumor cells was observed in 34 (47.9%) cases, were scored 0 and labeled as negative. Faint incomplete membrane staining in >10% of the tumor cells was observed in 22 (31%) cases, were scored 1 and labeled as negative (Figure 1). Weak to moderate staining of the entire membrane in >10% of the tumor cells was observed in 10 (14.1%) cases, were scored 2 and labeled as positive (Figure 2). Strong staining of the entire membrane in >10% of the tumor cells was observed in 5 (7%) cases, were scored 3 and labeled as positive (Figure 3). Overall, 15 (21.1%) cases were stained positive with c-erb B2.

Regarding the morphologic types of colorectal carcinoma, among 64 adenocarcinoma (not otherwise specified) cases, 14 (21.9%) of them were observed positive for c-erb B2 overexpression. Out of 6 mucinous adenocarcinoma cases, 1 (16.7%) of them was positive for c-erb B2 overexpression and the only signet ring carcinoma case was c-erb B2 negative (Table 1).

The tumor was localized at the proximal colon in 31 cases, 7 (22.6%) of them were positive with c-erb B2. Among 40 distal colon sited cases, 8 (20%) of them were positive with c-erb B2.

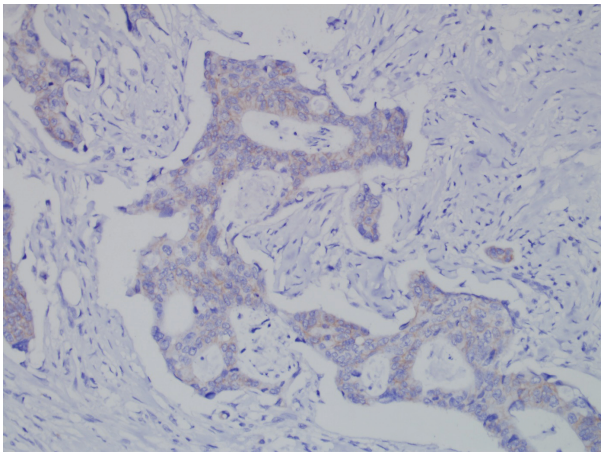

Figure 1: Score 1 c-erb B2 expression (x100)

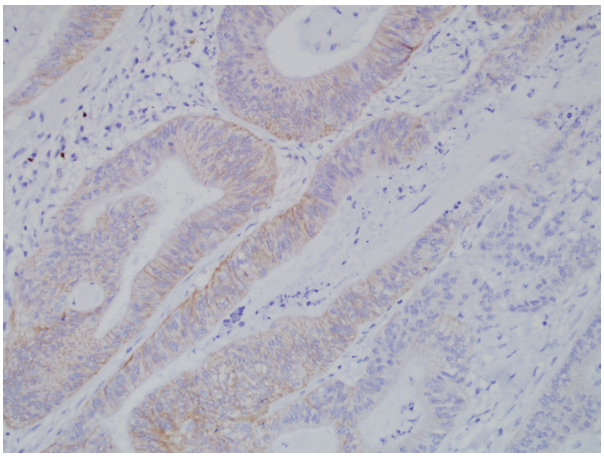

Figure 2: Score 2 c-erb B2 expression (x100)

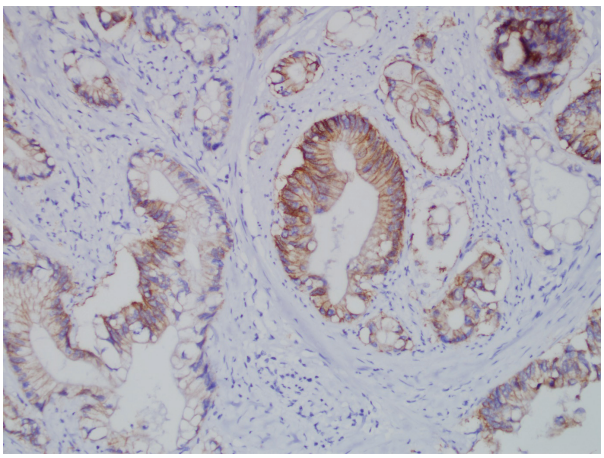

Figure 3: Score 3 c-erb B2 expression (x100)

The c-erb B2 overexpression were statistically similar between tumor localization sites (Pearson correlation (P))=0.792).

According to histopathologic grade evaluation, 64 cases were considered as low grade, 15 (23.4%) of them were positive with c-erb B2. Seven cases were considered high-grade tumors, but none of them were stained positive with c-erb B2. Statistically c-erb B2 staining features were similar between tumor grades (p=0.332).

According to pathological TNM stage procedure, 4 (5.6%) cases were considered as pT1, 8 (11.3%) cases were pT2, 9 (12.7%) cases were pT3 and 50 (70.4%) cases were pT4. In addition, 38 (53.5%) cases were considered as pN0, 20 (28.2%) of them were pN1, 13 (18.3%) of them were pN2.

The tumors have invaded to submucosa in four cases, 2 (50%) of them were positive with c-erb B2. In eight cases, the tumors have invaded to muscularis propria and 1 (12.5%) of them was positive with c-erb B2. None of 9 cases that have invaded to pericolorectal tissue were positive with c-erb B2. In addition, among 50 cases with serosal invasion, 12 (24%) of them were positive with c-erb B2. According to invasion depth, c-erb B2 overexpression was statistically similar (p=0.953).

Lymphovascular invasion was detected in 37 cases, 8 (21.6%) of them were positive with c-erb B2. Among the other 34 cases without lymphovascular invasion, 7 (20.6%) of them were positive with c-erb B2. The overexpression of c-erb B2 was statistically similar according to the presence of lymphovascular invasion (p=0.915).

Among 33 cases with lymph node metastasize, 7 (21.2%) of them were c-erb B2 positive and 26 (78.8%) of them were negative. Out of 38 cases without lymph node metastasis, 8 (21.1%) of them were c-erb B2 positive and 30 (78.9%) of them were negative. The overexpression of c-erb B2 was statistically similar according to the presence of lymph node metastasis (p=0.987).

DISCUSSION

Colorectal carcinoma affects a substantial patient population with an estimated 132,700 incident cases in the US in 2015 (8). Even with improvements in surgical treatment and its association with adjuvant chemotherapy, CRC still causes high mortality today. Thus, it justifies the search for new therapeutic targets and markers that can be used to define prognosis beside the standard criteria. The current study was undertaken with a view of establishing the positivity status and the potential role of c-erb B2 overexpression by IHC.

To date, several studies have reported that the frequency of c-erb B2 protein overexpression varies widely from 0 to 84% in CRC, also its prognostic significance is controversial (6). This debate might be attributed to several causes such as differences in scoring systems for c-erb B2 protein overexpression, technical approach, and heterogeneity of the study population. In our study, c-erb B2 protein overexpression was found to be positive in 15 (21.1%) patients among 71 CRC cases.

In a study conducted in Vienna, the ratio of c-erb B2 protein overexpression in CRC cases was 30%, more similar to our result. They also found no significant association with tumor grade, gender, localization, or survival (9). In our study, no significant relationship was found between c-erb B2 overexpression and standard prognostic predictors such as tumor grade, presence of lymph node metastasis and lymphovascular invasion,

Table 1: Clinicopathologic features of the CRC individuals and number of cases presenting c-erb B2 overexpression

Variable	Total no:71	C-erb B2 overexpression	p
Gender (M:F)	32:39 (1:1.2)		
Mean age (range in years)	58 (24-88)		
Tumor site			0.792
Proximal colon	31 (43.7%)	7 (22.6%)	
Distal colon	40 (56.3%)	8 (20%)	
Tumor type			
Adenocarcinoma (not otherwise specified)	64 (90.1%)	14 (21.9%)	
Mucinous adenocarcinoma	6 (8.5%)	1 (16.7%)	
Signet ring cell adenocarcinoma	1 (1.4%)	0	
Histopathologic grade			0.332
Low grade	64 (90.1%)	15 (23.4%)	
High grade	7 (9.9%)	0	
Tumor invasion depth			0.953
Submucosa	4 (5.6%)	2 (50%)	
Muscularis propria	8 (11.3%)	1 (12.5%)	
Pericolorectal tissue	9 (12.7%)	0	
Serosa	50 (70.4%)	12 (24%)	
Lymphovascular invasion			0.915
Positive	37 (52.1%)	8 (21.6%)	
Negative	34 (47.9%)	7 (20.6%)	
Lymph node metastasize			0.987
Positive	33 (46.5%)	7 (21.2%)	
Negative	38 (53.5%)	8 (21.1%)	

M: Male, F: Female

tumor localization site and invasion depth. In this aspect, the present study agreed with most other reports.

In a study conducted in Egypt, the ratio of c-erb B2 protein overexpression in CRC was 9.5% and no relationship between c-erb B2 overexpression and prognostic parameters of CRC was found (10). Kavanagh et al., found this ratio in rectal and colon cancer individuals at 7% and 11% respectively. They observed no correlation between c-erb B2 overexpression and age, gender, lymphovascular invasion, stage, perineural invasion or tumor size (11). In a study by Jesus et al., c-erb B2 protein overexpression was correlated with stage, prognosis and mortality of CRC; this ratio was 48.1% and no correlation was investigated (12).

Contrary to these results, in a study conducted in the US, Half et al. found a significant correlation between c-erb B2 overexpression (cytoplasmic staining) and tumor differentiation in colon cancer. They documented cytoplasmic staining in 63.5% of primary tumors, with strong membranous staining in 5% of primary CRC. Membranous but not cytoplasmic c-erb B2 staining has been strongly associated with gene amplification (13).

In a study performed in Pakistan in 2014, aimed to evaluate the frequency and staining pattern of c-erb B2 protein overexpression by using IHC in adenocarcinoma of the gastrointestinal tract, c-erb B2 positivity was found in 66% of CRC individuals. They found a highly significant association between tumor grade and c-erb B2 status but none with lymph node involvement (14). Seo et al. have observed that c-erb B2 protein overexpression and gene amplification were found in about 6% of CRC individuals. Human epidermal growth factor 2 gene amplification was more frequently found in the rectum than in the right or left colon, and a high concordance rate between IHC and dual color Silver in situ Hybridization methods has been demonstrated (15).

Elezoğlu et al. conducted a study with CRC individuals to investigate the relationship between c-erb B2 IHC staining and prognostic factors. They found a significant relationship between c-erb B2 staining and both stage and grade, whereas none were found with other prognostic factors or survival (16). Also, in a study conducted in Sweden in 2009, the ratio of c-erb B2 positively stained CRC cases was 54% (17).

In a study conducted in Iran, the ratio of c-erb B2 protein overexpression in CRC was 40 % and no significant relationship between c-erb B2 overexpression and prognostic parameters of CRC was found (18). Sayadnejad et al. performed a study with 50 invasive CRC patients, positive immunostaining was detected in 24% of the cases. They found no significant relationship between the expression of c-erb B2 and clinicopathological features (19).

In a study conducted in Istanbul, a total of 123 colorectal resection cases were studied, c-erb B2 immunohistochemical staining was observed in 61 cases (50%). Of these 61 cases, 19 (31%) had poor, 26 (43%) had moderate and 16 (26%) had strong intense membranous staining. They found a significant

relationship between c-erb B2 and distant metastasis, but no relation between c-erb B2 score and other parameters such as lymphocytes infiltrating the tumor, Crohn-like response, tumor nodules, presence of a mucinous component and poorly differentiated area (20).

Siena et al. conducted an open-label, phase 2 study recruited 78 patients with unresectable, recurrent, or metastatic colorectal adenocarcinoma who had required to have RAS and BRAF-V600E wild-type tumors, received and progressed on at least two previous treatment regimens from 25 clinics and hospitals in Italy, Japan, Spain, the UK, and the USA. Based on c-erb B2 expression, patients were allocated into one of three cohorts: 53 (68%) of them were c-erb B2 IHC 3+ or IHC 2+ and in-situ hybridization (ISH) -positive, 7 (9%) of them were IHC 2+ and ISH-negative, and 18 (2%) of them were IHC 1+ (21).

CONCLUSION

It would appear that we have not found a significant relationship between c-erb B2 overexpression and prognostic parameters such as tumor grade, lymph node metastasis, lymphovascular invasion, tumor localization site and invasion depth despite its crucial role in the occurrence of some tumors and its predicting value of worse prognosis for them. Furthermore, c-erb B2 protein overexpression may have a predictive role for targeted therapies like trastuzumab in CRC cases when standard therapeutic regimens are not efficient.

Although the prognostic significance of c-erb B2 has been demonstrated in gastric and breast cancer long since, it is still controversial in CRC. Nevertheless, because it is a target for anti c-erb B2 therapies and to evaluate these alternative managements in CRC, extensive studies using standardized techniques on large series are needed.

Ethics Committee Approval: This study was approved by Mustafa Kemal University Faculty of Medicine (Date: 04.10.2012, No: 24-2012/59).

Informed Consent: This study was exempted from informed consent because it is a retrospective study using previous data and materials from the archive

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- N.Ş.Ş., M.Y.; Data Acquisition- N.Ş.Ş., M.Y.; Data Analysis/Interpretation- N.Ş.Ş., M.Y.; Drafting Manuscript- N.Ş.Ş., M.Y.; Critical Revision of Manuscript- N.Ş.Ş., M.Y.; Final Approval and Accountability- N.Ş.Ş., M.Y.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: Grants and non-financial support were provided from Mustafa Kemal University Scientific Research Projects Coordination Unit (Project number: 11421), during the conduct of the study.

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