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ORIGINAL: HER2 Amplification in Non-Mutant RAS Status in Patients with Metastatic Colorectal Cancer and the 2-Year Survival Rate

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ABSTRACT

Introduction: HER2 amplification is a therapeutic target in breast and stomach cancer, but the relationship between HER2 and response to treatment in metastatic colorectal cancer has not been fully determined. This study investigated the incidence of HER2 overexpression in non-mutant RAS patients with metastatic colorectal cancer to determine the two-year survival rate in patients referred to Imam Khomeini Hospital in Sari between 2014-2018.

Material and Methods: This cohort study was conducted between 2014 and 2018 among patients with metastatic colorectal cancer who were identified as non-mutant (wild) NRAS, KRAS, and BRAF genes. Patients received cetuximab. The clinical course of the patients was evaluated simultaneously during this evaluation period, and the two-year survival rate of the patients and the response to cetuximab were evaluated. Data were entered into SPSS software version 24 and analyzed.

Results: Twenty-five patients were examined, 60% were men. The most common symptom in patients was abdominal pain (32%). The majority (72%) of the study patients did not undergo surgery, 24% underwent hemicolectomy and 4% underwent total colectomy. The incidence of HER2 positive in women was higher than in men (10% vs. 6%) and in grade 1 patients was higher than grade 2 (8.3% vs. 1.1%). Forty-four percents of patients had positive two-year survival. Among patients with positive two-year survival, 9.1% were HER2 positive and 90.9% were HER2 negative.

Conclusion: The results of the study showed that infectious causes (influenza) and underlying CLD including asthma were major causes of ARF in pregnant women. Thus, more careful attention is needed to control the underlying disease, and planning for easier access to the influenza vaccine can play an effective role in reducing the incidence of pulmonary infections.

Introduction

Colorectal cancer (CRC) is the third most common cancer in the world (1). In Iran, this cancer includes 12.8% of them (2). More than 90% of patients are diagnosed

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after the age of 50 (3). Since this cancer is one of the most treatable, early detection is critical (4). The most common sites of metastases are the liver, lungs, bones, brain, and spine (5). In benign and non-metastatic stages, surgical treatment is chosen as the first step (6). Targeted chemotherapy blocks the function of some specific proteins in colorectal cancer (7, 8). In addition, radiation therapy is also typical in the treatment because it can reduce the tumor size (9).

Recent advances in the biology of metastatic colorectal cancers (mCRC) helped identify target receptors, vascular endothelial growth factor receptor (VEGFR), and epithelial growth factor receptor (EGFR). Detection of these receptors led to producing biological drugs with inhibitory action (10-12).

RAS oncogene is one of the principal oncogenes in CRC, which has three variants: HRAS, KRAS, and NRAS, which KRAS is the most common mutation in CRC (13, 14). Cetuximab is a humanized monoclonal IgG1 antibody that targets the extracellular domain of EGFR, which causes clinical improvement in mCRC with two non-mutant (Wild Type (WT)) K-RAS exons, either alone or in combination with classical chemotherapy drugs (15, 16). Anti-EGFR drugs such as Cetuximab and Panitumumab are effective only in RAS non-mutant mCRC (17, 18). RAS oncogenes act in a cascade manner. If a mutation occurs in KRAS or NRAS genes, the EGFR family drugs will be ineffective. BRAF V600E mutation in EGFR down-stream signaling pathway was identified as a prognostic factor in treating mCRC patients with anti-EGFR drugs (19).

Various biomarkers, such as oncogenes or tumor suppressor agents in angiogenesis and cell proliferation, are used to detect and predict cancer prognosis. Human epidermal growth factor receptor 2 (HER2) is a tyrosine kinase receptor of the EGFR family, and its amplification is known as a possible mechanism of resistance to Cetuximab (20, 21). A possible mechanism of the resistance is the activity of the HER2 signaling pathway or Heregulin Upregulation, and continuous ERK1/2 signaling cause is cetuximab resistance (22).

Recent guidelines recommend checking the RAS signal before starting anti-EGFR treatment. However, some patients with non-mutant RAS (WT) also do not respond to Cetuximab treatment, and the investigations show the B-RAF mutation as the cause of the resistance (23, 24). After that, Cetuximab and Panitumumab were prescribed for patients with non-mutant (WT) BRAF, KRAS, and NRAS. However, the responses to this treatment in mCRC patients were different, which suggested the necessity of knowing other causes of drug resistance (25). HER2 overexpression is one of the newly identified elements in the drug resistance pathway. HER2 amplification is seen in 1-4% of mCRC (26). Several studies show that HER2 overexpression in mCRC causes a decrease in 2 compared to patients without HER2 amplification (27, 28). The overall survival in patients with HER2 amplification is unclear because there was a decrease in some patients; in others, it had no effect (29).

HER2 expression in normal adult cells is low, while it is overexpressed in 20 to 30% of breast and ovarian cancers; on the surface of breast tumor cells, more than 2 million molecules of this receptor are observed (30, 31).

HER2 amplification is a critical predicting and prognostic factor in response to anti-HER2 therapy (such as Herceptin) in treating metastatic breast cancer and gastroeso-phageal adenocarcinoma (32). Sawada et al. showed that the HER2 is a prognostic factor and an appropriate predictor for response to anti-EGFR therapy in mCRC patients (26). For this reason, HER2 amplification is a therapeutic target in breast and stomach cancer. Science, HER2 amplification is used as a therapeutic target in breast and stomach cancer. However, the relationship between HER2 and Cetuximab in mCRC has yet to be entirely determined (33, 34).

In Bertotti et al.'s study, HER2 amplification was identified as a principal mechanism of cetuximab resistance in the WT population of KRAS, NRAS, BRAF, and PIK3CA3. The authors observed HER2 amplification in a

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small percentage (2-3%) of CRC patients (35). This ratio is increased by considering KRAS WT patients resistant to Cetuximab. Today, HER2 is the first therapeutic target in mCRC, a good predictor of response to targeted treatment (36). Current therapies for CRC include endoscopic resection, local ablation, local surgery, targeted chemotherapy, palliative chemotherapy, and immunotherapy. Chemotherapy includes monotherapy, mainly based on fluoropyrimidine, and multiple drug therapy with oxaliplatin, irinotecan, and capecitabin. Several targeted drugs have also been tested as treatments for mCRC, including the monoclonal antibodies cetuximab and panitumumab against the EGFR, bevacizumab against VEGF-A, the fusion protein aflibercept, and the small molecule multikinase inhibitor regorafen, all of which target various angiogenesis factors (37). Although these therapies have doubled the overall survival (up to 3 years) of patients with advanced disease, CRC is still associated with a poor prognosis and survival (38). On the other hand, it should be noted that HER2 is a part of the RAS pathway, the block of which causes the RAS cascade to stop.

Therefore, this study investigated the incidence of HER2 in non-mutant RAS and BRAF patients with mCRC treated with Cetuximab.

Methods

Study Design and Cohort Selection

This study is a retrospective cohort to investigate the HER2 amplification in mCRC patients with WT RAS and BRAF and their 2-year survival rate. The study population included patients diagnosed with CRC from March 2015 to March 2020. First, patients with mCRC were selected from 2015 to 2020 through the files of Touba Clinic (Sari, Iran), and those with 2 WT exons of NRAS, KRAS, and BRAF were isolated. The patients in our study were all receiving cetuximab. The pathology blocks of the patients were collected from the hospital and checked for HER2.

Patients' age, gender, symptoms, family history, time of diagnosis, the primary location of the tumor (right or left colon and rectum), histology, disease grade (1, 2, and 3), disease stage (local, locally advanced, metastasis), Metastasis location (lung, liver, etc.), lymph node involvement, lympho-vascular invasion, surgery status (hemicolectomy, total colectomy), HER2 status, and RAS mutation (wild type, mutant) were investigated. The 2-year survival rate and their response to the cetuximab were studied. PFS was assessed every two months with CT and MRI and evaluating it with RECIST version 1.1 criteria. Examination of the pathology blocks was done through IHC (by two pathologists, one of whom was also one of the authors of this article (F.N.). Simultaneously, the clinical course of the patients was evaluated during this period.

HER2 Status Characterizations

Scoring was based on the Hercep-Test evaluation system: zero score means no immunoreactivity or is present in less than 10% of tumor cells (negative). One plus (1+) means weak immunoreactivity in more than 10% of tumor cells but only in part of the membrane. (Imperfect and also negative). Two plus (2+) means weak to moderate immunoreactivity of all membranes, more than 10% of tumor cells (weak positive). Three plus (3+) means complete to strong immunoreactivity in more than 10% of tumor cells (Strong positive).

Study Objectives and Endpoints

The primary study aim was to asses HER2 amplification in KRAS 2 exon wild type in mCRC and its two-year surveillance. The secondary objective was to compare the clinicopathological features in positive and negative HER2 groups.

Statistical analysis

Data description was presented with frequency, mean, standard deviation, median and interquartile range. The Shapiro-Wilk Test was used to check the normal distribution. Qualitative variables were

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compared with the Chi-Square or Fisher Exact Test. Quantitative variables were compared between two groups (HER2 +/-) by Independent Samples T-test or Mann-Whitney test. The survival analysis was done with the life expectancy table and Kaplan-Meier test. A comparison of the survival rate between the two groups (HER2 +/-) was made with Log Rank Test. Also, the hazard ratio of two-year survival was determined by univariate and multivariate methods with Cox-Proportional Hazard Model Test. The data was entered and analyzed in SPSS version 24 software. A significance level of 0.05% was defined, and a 95% confidence interval was selected.

Results

Three thousand cases of CRC were examined from Touba Clinic's reviewed cases, of which 300 cases were metastatic cancer. Among them, 35 patients had non-mutant (WT) NRAS, KRAS, and BRAF. Among these, we discovered complete information and pathology block of twenty-five cases. Ten were female (40%), and 15 were male (60%). The average age of the patients was 63±7.8 years, with a range of 44-79. The most common symptom in patients was abdominal pain (32%), manifested with other symptoms in 44% of cases. Primary symptoms were abdominal pain (8 patients (32%)), weight loss (5 patients (23%)), constipation (1 patient (4%)) and 11 patients (41%) had more than one symptom (***Table 1***).

The primary tumor location was 6 cases (24%) in the right colon, 11 cases (44%) in the left colon, 3 cases (12%) in the rectum, and 5 cases (20%) with more than one side involvement. HER2 was positive in 2 (8%) out of 25 patients. Histologically, the mass was adenocarcinoma in 24 cases (96%). HER2 was positive in 2 cases (8%) and negative in 23 cases (92%) (***Table 2***).

Eighteen patients (72%) had no surgery. Six patients (24%) had hemicolectomy, and one (4%) had a total colectomy. Lymph nodes were involved in 14 patients at the time of diagnosis. The average number of involved lymph nodes in these patients was 18 ± 17 (mean ± SD). Fifteen patients (60%) had metastases to the liver. Metastases were in two patients (8%) to other organs and eight (32%) in more than one location (***Table 3***).

Table 1. Clinicopathological features of both HER2+ patients

Case	1	2
Age	72	79
Sex	Female	Male
Symptoms	Abdominal pain	Abdominal pain
Family history	Negative	Negative
Primary tumor location	Right	Right
Histology	Adeno-carcinoma	Adeno-carcinoma
Grading (at the onset)	2	1
Staging	Metastatic	Metastatic
Location of Metastasis	Liver and lung	Liver
Lymph node involvement (N)	30	35
Lymphovascular invasion	Unknown	Positive
Surgery	Negative	Right hemicolectomy
HER2	3+	3+
RAS	WT	WT
Surveillance	Less than 2 years	More than 2 years

Table 2. Tumor indicators

Variable		N (%)
Grade	1	12 (48)
	2	9 (36)
	Unknown	4 (16)
Stage	Locally Advanced	2 (8)
	Metastasis	23 (92)
Lymphovascular invasion	Positive	11 (44)
	Negative	3 (12)
	Unknown	11 (44)

The HER2 over-expression in women was one out of ten (10%) and one out of 15 (6.6%) in men. Four patients had a positive family history (all 1st-degree relatives), none of which were HER2 positive. HER2 amplification was present in about two out of 19 cases (10%) in primary tumors, and none were HER2-positive in secondary ones. HER2 amplification was found in two of six

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cases (33.3%) in the right colon, while negative in the left colon, rectum, and multifocal mass (***Figure 1***).

HER2 was positive in two (8.3%) of 24 patients with adenocarcinoma. It was positive in one of 12 patients (8.3%) of grade 1 tumors at the time of diagnosis and one of nine (11%) of grade 2 tumors. In the locally advanced stage, no patients were HER2 positive, and two were HER2 negative (100%). In the metastatic stage, only two of the 23 patients were HER2 positive (8.7%) (one with only liver metastasis, none in other organ involvements). In cases of more than two metastasis sites, one patient was HER2 positive (12.5%) (metastases to liver and lung), and seven were HER2 negative. HER2 was positive in one of ten patients with lymphovascular involvement at the time of diagnosis (10%), and no positive cases were observed in non-lymphovascular involvement.

Table 3. A comparison of tumors features between HER2 +/- status

Variable		HER2 +	HER2 -
Sex	Female	1	9
	Male	1	14
Source of tumor	Primary	2	17
	Secondary	0	2
Tumor location	Right	2	4
	Left	0	11
	Rectum	0	3
	More than one	0	5
Histology	Adenocarcinoma	2	22
	Non-adenocarcinoma	0	1
Grading	1	1	11
	2	1	8
Staging	Locally advanced	0	2
	Metastases	2	21
Location of metastases	Liver	1	14
	Other organs	0	2
	More than 2 organs	1	7
Lympho-vascular involvement	Positive	1	10
	Negative	0	3
	Unknown	1	10
Surgery	No surgery	1	17
	Hemicolectomy	0	5
	Total hemicolectomy	0	1

Among patients with two-year survival, 9% were HER2 positive, and 91% were HER2 negative. Among the patients with less than two years of survival, 7% were HER2 positive, and 93% were HER2 negative (P-value>0.05) (***Table 4*** and ***Table 5***).

Table 4. Two-year survival in HER2 +/-

	Two-year survival		P-value
	Yes	No	
HER2 +	1 (9%)	1 (7%)	> 0.05
HER2 -	10 (91%)	13 (93%)	> 0.05

Table 5. PFS (progression free survival) in HER2 +/-

	PFS		
	Less than 6 months	more than 6 months	P-value
HER2 +	1 (6.5%)	1 (11%)	> 0.05
HER2 -	15 (93.5%)	8 (89%)	> 0.05

Discussion

This study investigated HER2 over-expression in non-mutant RAS patients with mCRC and determined the two-year survival rate. In previous articles, HER2 amplification among all cases of mCRC was 2-3%, and in cases of mCRC with two exons wild type KRAS, it was 4 to 5% (26, 35). In our study, 19 cases (76%) were primary, 2 cases (8%) were secondary tumors, and four cases (16%) were unclear. The two-year survival rate in our study was lower in patients with left colon involvement than in other primary tumor sites (Although this rate was not statistically significant p-value=0.69). In Salem et al.'s study, the probability of less than two-year survival was higher in patients with right colon involvement (16.7 months versus 36 (p-values <0.001)) (40). Therefore, the findings of subsequent studies can determine the significance of the initial location of the tumor during the treatment. In our study, the most manifested symptom in patients was abdominal pain (32%), accompanied by other symptoms in 44%. In Safai's study,

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abdominal pain was also the most common (57.1%) symptom (42). Therefore, in both studies, a manifestation of abdominal pain as the initial presentation can indicate a slow growth rate and exert a compressive effect on the nerves due to increasing the tumor size

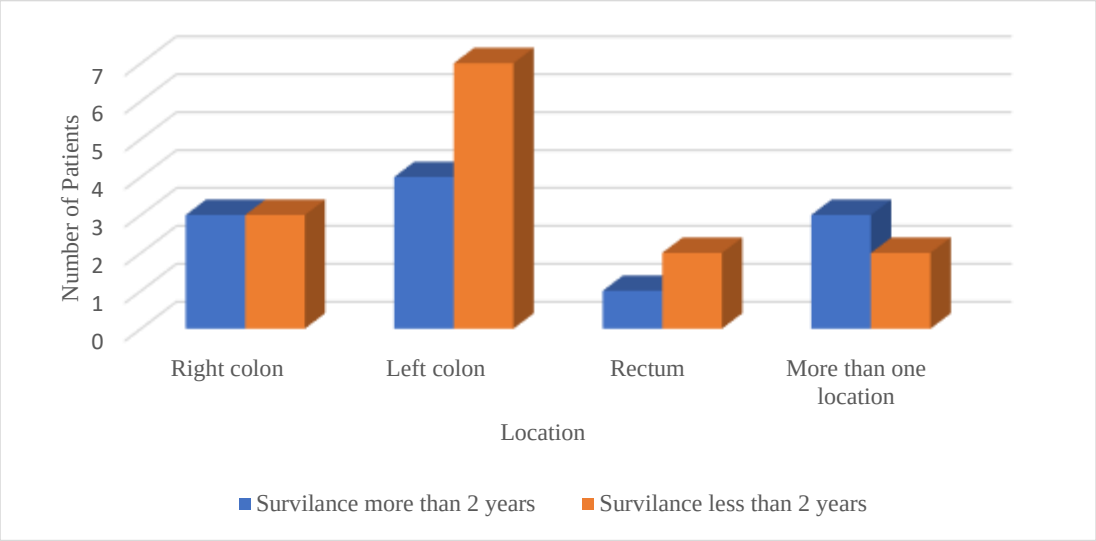

Figure 1. Two-year surveillance of the tumors and their primary sight

over time. It means that the mass is diagnosed at a higher stage and may have a worse prognosis.

Adenocarcinoma (96%) was the most common histology in our study. In Safai and Takahashi's study, also the most common histological type of tumor was adeno-carcinoma (85.2% and 89.6%, respectively) (19, 42). Therefore, it can be concluded that most colorectal tumors are adenocarcinoma, and this finding can substantially affect the treatment plan.

In our study, the liver was the site of most metastasis (60%). In Bianchi's study, most metastases were in the lung, contrary to ours (36). Since the venous circulation of the intestines enters the hepatic portal system first, it is evident that the hepatic system is the most common site of metastasis. Since Blood flows from the heart to the lungs, the lung is also one of the important sites of metastasis in CRC (36).

In our study, the HER2 amplification in women was 10% (1 person out of 10) and 6.6% in men (1 person out of 15). In contrast with us, in Liu's study, HER2 amplification in males was 14.8% and in females was 11.1% (39). Also, in Bianchi's study, males were more (75%) than females (25%) (36). Therefore, and the HER2 amplification in mCRC is probably independent of gender.

In our study, the HER2 amplification in patients with a primary mass in the right semicolon was 33.3% (2 out of 6). No positive case was observed in patients with a left semicolon tumor, rectal, and multifocal mass. In Bianchi's study, HER2 over-expression was associated with left semicolon mass in 89.5%, contrary to ours (36). Therefore, HER2 occurrence is likely related to factors other than the location of the colorectal mass. On the other hand, the differences in findings of various locations of the colon can be due to their embryonic origin, which leads to genetic and biological differences as well as clinicopathological behaviors. In our study, the HER2 amplification in grade 2 was higher than in grade 1 (11% vs. 8.3%) at the time of diagnosis. In Liu's study, HER2 positivity was higher in grade 1 patients than in grade 2 (16.7% vs. 15.8%), contrary to ours (39). Conversely, similar to us, HER2 was more positive in grade 2 patients than in grade 1 (65.8% vs. 2.5%) in Bianchi's study.

Both patients were HER2 negative (100%) in the locally advanced stage. In the metastasis group, two patients were HER2 positive

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(8.7%), and 21 others were HER2 negative. One case was just related to liver metastasis, and no positive case was observed in other organs on their own metastasis without the liver. In more than two metastases group, one patient was HER2 positive (12.5%), and seven were HER2 negative (87.5%). In Bianchi's study, HER2-positive patients showed more lung metastases and higher tumor burden (36). Also, 48.6% of subjects had two or more metastases, of which 53% were HER2 positive, and 47% were HER2 negative; that was more than our study. It may be HER2 over-expression independent of the location of metastasis.

HER2 was positive in 1 of 11 cases (11%) in patients with lymphovascular involvement. Furthermore, no positive cases were observed in non-lymphovascular involvement.

In our study, 44% of patients had more than two-year survival. 9.1% of them were HER2 positive, and 90.9% were HER2 negative. Moreover, 7% were HER2 positive, and 93% were HER2 negative (p-value>0.05) in the patients with less than 2-year surveillance. On the other hand, in our study, PFS was not significantly different between the two groups. Also, in Liu et al.'s study, PFS was not significantly different in both HER2 positive and negative groups (39). In contrast to our study, Jeong and Bianchi showed that PFS in HER2-positive patients was significantly lower than in HER2-negative (In Jong's study, 3.1 months in people with HER-2 overexpression versus 5.6 months in people without HER-2 (p-value=0.019)) (36, 41).

The retrospective nature of our study is one of the limitations. The limited number of participants can influence the findings; hence conducting a study with more participants would be more reliable. The impossibility of complete access to the information of Touba Clinic files caused the reduction of our statistical population. The small number of HER2-positive patients in our study limited the statistical comparison of HER2 subgroups.

Our study was conducted in a population of almost the same race, which can be a confounding factor in genetic studies.

Prospective cohort studies can give us more reliable results. Future studies should be carried out in a wider geographical area to obtain more reliable results. It is necessary to conduct future studies with a larger sample size.

Conclusion

There was no significant correlation between HER2 amplification and the two-year survival of patients in our study.

Ethical standards statement

All the investigation procedures used in the current study were reviewed and approved by the Research Ethics Committee of the Mazandaran University of Medical Sciences (code: IR.MAZUMS.IMAMHOSPITAL.REC.1400.002).

Conflicts of interest

The authors declare no conflict of interest.

Authors' contributions

Each author has made an important scientific contribution to the study and has assisted with the drafting or revising of the manuscript.

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