Japanese Journal of Gastroenterology and Hepatology

Research Article

ISSN: 2435-1210 | Volume 10

Evaluation of HER2 in Colorectal Cancer with Aggressiveness of the Tumor and Follow-Up Patients

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Received: 16 Sep 2023
Accepted: 19 Oct 2023
Published: 26 Oct 2023
J Short Name: JJGH

Citation:

Mohsenifar Z. Evaluation of HER2 in Colorectal Cancer with Aggressiveness of the Tumor and Follow-Up Patients. J Gastro Hepato. 2023; V10(4): 1-8

Keywords:

Her2/neu; CRC; Trastuzumab; Immunohistochemistry; IRAN

1. Abstract

1.1. Background & Objective: Colorectal cancer is a common cancer with a high incidence and mortality rate of worldwide. HER-2 is a proto-oncogene that its overexpression leading to malignant transformation. This study was performed for evaluation of HER2 protein in colorectal cancer.

1.2. Methods: In this cross-sectional study, 60 consecutive malignant colorectal cancer in Taleghani hospital of Tehran for 2 years were be enrolled and the IHC of HER2 were determined and compared to demographic and pathologic findings. Also, we followed the patients.

1.3. Results: The results of the present study showed that out of 60 patients, 32 (53.3%) were men and 28 (46.7%) were women, and most of them were in the age group of 50-59 years (33.3%). The most involved sites were sigmoid, rectum and transverse colon. Most tumors were in grade 2 (51.7%). Most tumors were deep in T3 (61.7%) and most tumors were in stage 3 (50%). Most tumors were less than 5 cm (60%). The main findings of this study were the positive correlation between HER2 intensity and tumor depth (T). Our results also showed that increasing the depth of the tumor (T) can be 3.36 times positive for HER2. There was also a positive correlation between tumor depth and HER2 using Pearson test (r = 0.343, P = 0.007).

1.4. Conclusion: Our results showed that increasing tumor depth (T) could be positive for HER2. Differences in HER2 expression in various studies are probably due to techniques used in the IHC and demographic and genetic differences in the study environments.

2. Introduction

Cancers are one of the deadliest factors in the world after cardiovascular diseases [1]. CRC is a common cancer with a high incidence and mortality worldwide [54]. Colorectal cancer has two types, hereditary (Familial) and sporadic, about 80% of which are sporadic and the remaining 20% are hereditary [2]. Colorectal cancer is the third most common malignancy in men and the second most common malignancy in women. Worldwide, it accounts for 8% of cancer deaths [3]. CRC is the second most common cause of cancer death in the United States. [55] It is also the most growing cancer in terms of prevalence in Asian and Eastern European countries [3, 4]. CRC is the third most common cancer in Iran [17].

Recent cancer statistics show that the incidence of colorectal malignancies is declining in the United States due to early detection and resection of precancerous lesions by colonoscopy [5]. Apart from skin malignancies, colorectal malignancies are the third most common

cancer among Iranians and occur at a younger age and its incidence is increasing [6]. Among the diseases of the gastrointestinal tract, on the shores of the Caspian Sea in Iran, the upper gastrointestinal malignancies have been widely addressed, while little attention has been paid to colorectal malignancies. One reason for this could be the low incidence of this type of malignancy in reports submitted before the 1970s [7]. The study between the incidence and prevalence of cancers in Iran during the recent years showed an increase in colorectal cancer. As it has climbed from the ninth to the fifth rank [18].

Currently, one of the most common gastrointestinal cancers in Iran is colorectal cancer, which is the third most common cancer in Iranian men and the fourth in Iranian women [19]. Colorectal cancer was the cause of 1.2 million new cases and 608,700 deaths in 2008 worldwide [20]. Areas with high incidence of colorectal cancer include Australia, New Zealand, North America, Canada and parts of Europe, and areas with low incidence include Africa, South and Central Asia, China, India and South America [20, 21].

Colorectal malignancies are influenced by environmental and genetic factors and occur in all classes of society. Today, living standards have risen dramatically across the country, and over the past three decades, rapid socioeconomic advances have led to changes in lifestyle, a tendency to inactivity, and a high-fat, high-protein diet, low fiber [8, 9]. Other risk factors consist of men gender, smoking and alcohol consumption. People with a family history of polyps, colorectal cancer, and intestinal polyposis have a higher risk of progressing to colorectal cancer [22]. They play a role and sometimes the continuation of both factors will cause cancer. Genetic susceptibility is one of the most important risk factors for the development of colorectal cancer [23]. Colon carcinomas often originate from adenomatous polyps and take an average of ten years for precancerous lesions to turn into cancer [10, 11].

Histological studies are an essential step in determining the heterogeneity of any tumor. Immunohistochemistry (IHC) also plays an important role in differentiating different types of tumors and assessing their degree of malignancy [12, 13, 24]. One of the genetic factors involved in tumorigenesis is the human epidermal growth factor receptor 2 (HER2), HER2 is a transmembrane protein with tyrosine kinase activity which is a proto-oncogene located on chromosome 17q and is responsible for the production of intramembrane tyrosine kinase growth factor receptor and can be produced by IHC Assess [14, 15, 25].

Overexpression of HER2 significantly leads to malignancy by increasing cell survival, proliferation and decreasing the apoptotic potential of cells [25]. HER2 gene amplification and protein expression in several human epithelial malignancies, including breast, stomach, prostate and thyroid are seen. In these tumors, increased HER2 expression is generally associated with less differentiated phenotypes and poor prognosis [14, 15, 26-28].

HER2 overexpression occurs in about 20 to 30% of breast tumors, which is associated with an invasive disease, with a higher recurrence rate and lower survival. Trastuzumab is a monoclonal antibody against HER2 that is effective in treating HER2 positive breast cancer in combination with chemotherapy in early and metastatic cases of the disease [29, 30].

In gastric cancer, the increase in the incidence of HER2 in various articles has suffered. Various studies have presented conflicting findings on its prognostic relevance. However, with the recent introduction of trastuzumab for the treatment of patients with advanced gastric cancer, the clinical demand for HER2 evaluation is increasing rapidly [31, 32].

HER2 has also been studied in thyroid cancer. Interestingly, in these studies, overexpression of HER2 has a wide range, with a positive rate of 0% to 70%. Despite the clinical significance of this marker for breast cancer, the role of HER2 in prostate cancer is still controversial. Nishio Y et al. Have suggested that overexpression of HER2 may be a useful indicator of an unfavorable prognosis. Excessive incidence of HER2 in colorectal cancer shows a wide range in various studies in different parts of the world [33]. Therefore, conducting molecular studies in order to find markers predicting the outcome of patients has research priorities [16].

In a cohort study by Seo et al The incidence of HER2 protein and its clinical implications for primary colorectal cancer (CRC) were investigated. HER2 status was evaluated in two retrospective groups including 365 patients without metastasis (group 1) and 174 advanced patients with concomitant or delayed distant metastasis (group 2). HER2 status was assessed by silver in-situ hybridization (SISH) IHC and mRNA in-situ hybridization (ISH). The incidence of HER2 protein (IHC +2 + 3 +) in groups 1 and 2 was approximately 6% and was not associated with aggressive cancer behavior and patient prognosis in both groups [34].

Pappas et al Investigated the immunohistochemical incidence of HER2 and its relationship with clinical-pathological parameters and prognosis of CRC patients. This study showed that there is a moderate incidence of this protein (+2) in a small proportion (3.9%) of colorectal cancer patients and there is no clear association between HER2 expression and age, sex, tumor location, tumor grade, stage and there is no patient survival [35].

A meta-analysis by Li et al Examined 11 studies, all of which used IHC to assess HER2 expression. A wide range of HER2 expression was reported in 11 different studies ranging from 8.3 to 81.9%, but ultimately its occurrence was negatively correlated with CRC survival [36].

In another meta-analysis performed by Wu et al A total of 18 studies including 2867 CRC patients were included to evaluate the association between HER2 immunohistochemical expression and clinicopathological features and survival. The overall analysis showed that

there was no detectable relationship between HER2 expression and prognosis in CRC patients [37].

In contrast, in another meta-analysis conducted by Sun et al A total of 30 studies, including 4942 patients with CRC and 521 healthy individuals with inclusion criteria, showed that HER2 expression levels in CRC patients were significantly higher than those Healthy and also in patients with lymph node metastasis is higher than patients without lymph node metastasis [38].

Ingold Heppner et al retrospectively assessed the prevalence of HER2 in a large complex of colorectal carcinomas using IHC and chromogenic local hybridization (CISH). Overall, in 1645 cases of primary colorectal cancer, 1.6% of cases were HER2 positive, which was significantly associated with higher stages of UICC and lymph node metastasis. They believe that although the prevalence of HER2 positive cases in CRC is low, it seems reasonable to evaluate it in advanced cases of colorectal cancer with lymph node involvement and may offer new therapeutic goals in advanced cases of the disease [39].

Moussa et al in 2020 published HER2 results in benign and malignant colorectal lesions, that positive HER2 was detected in 15% of CRC patients and was not associated with nodal involvement or metastasis [57].

Due to different results and different prevalence of this proto-oncogene in colorectal cancer in different studies and little information about it is available in the Iranian population, and also due to the growing trend of colorectal cancer and the need to use new treatments such as target therapy to improve survival. In this study, we investigated the incidence of HER2 in colorectal cancer and its relationship with the severity of tumor invasion and other factors.

This study was performed for determination of immunohistochemical expression of HER2 protein in CRC and its correlation with aggressiveness of tumor and compared according to other variables such as age and gender of patient and pathologic findings.

3. Materials and Methods

In this cross-sectional descriptive study, 60 patients with CRC who referred to Taleghani Teaching Hospital of Shahid Beheshti University of Medical Sciences, Tehran, IRAN from March 2016 to March 2018 and their tumors were surgically removed. Exclusion Criteria consisted of: Presence of other malignancies, Presence of related background disease such as FAP, IBD or any other conditional diseases and Neoadjuvant chemoradiotherapy

The information required by patients was prepared using a checklist that contains demographic variables including: age and sex of patients and pathological information including: tumor size, tumor location, tumor penetration depth, perineural invasion, lymphovascular invasion, histologic differentiation, pathological staging of the tumor, Lymphatic metastasis and the presence of distant metastases were extracted from pathologic reports and slides.

Patients' pathological slides were reviewed and a suitable slide and its paraffin block with the lowest amount of necrosis were selected for IHC staining. Immunohistochemical staining of HER2 was performed on deparaffined blocks using IgG monoclonal antibody and according to the manufacturer's instructions (Master diagnostic). For positive internal control, an invasive ductal breast cancer sample with a strong membrane expression for this antibody was used, and for negative internal control, no antibody was shed on the tissue.

The process was supervised by a pathologist and the result of immunohistochemical staining of tissues were evaluated by two pathologists separately. The severity of HER2 was determined based on HER2 in the CAP protocol and Valtorta et al [56].

After 4-5 years (depending on the case) we called and asked for treatment results consists of complete response, recurrence, metastasis or death. Then we compare these findings with IHC results.

Statistical analysis: Statistical methods for data analysis

Data analysis will be performed by SPSS (version 24.0) software [Statistical Procedures for Social Sciences; Chicago, Illinois, USA]. Chi-Square, Fisher, and Independent-Sample-T tests will be used and considered statistically significant at P values less than 0.05.

4. Results

In this study, histopathological examination and its associated factors in paraffin blocks of CRC in Taleghani Hospital from march 2016 to march 2018 have been investigated. A total of 60 people were studied, of which 32 (53.3%) were men and 28 (46.7%) were women, most of whom were in the age group of 50-59 years (33.3%). Table 1 provides more detailed information on the distribution of patients' age groups by sex in the study population.

Due to Table1: the highest frequency is in the age group of 50-59 years (40.6%) and in women in the age groups of 40-49 years, 50-59 years and 69-60 years (each with 25%). Using Chi Square test, it was found that there is no statistically significant difference between gender and age groups (P = 0.351)

As Table 2: As can be seen in the table above, the most involved sites were in the sigmoid (no: 22, 36.7%), rectum (no:13, 21.7%) and transverse colon (no: 8, 13.3%), respectively.

Table 3 shows the immunohistochemical results in terms of HER2 expression intensity in the study population. As can be seen, in 19 cases (31.7%) the marker was positive, of which 13 were +2 and 6 were +3. According to the interpretation of the positive method of considering the samples in the implementation method, we interpret cases 0 and +1 as negative finding of HER2.

Table 4 shows the pathological findings in the studied samples. As can be seen in the Table 4, most tumors were in grade 2 (51.7%). Most tumors were T3 deep (61.7%) and most tumors were in stage 3 (50%). Also, in terms of size, most tumors were less than 5 cm (60%).

The results showed that there was only a significant relationship between the expression intensity of HER2 marker and tumor depth (P = 0.023). Other correlations between the other variables and the intensity of the marker expression are shown in Table 5.

As can be seen in the Table 5, among the evaluated variables, only there was a significant correlation between tumor depth (T) and HER2 marker expression, which was positive. P: 0.23.

There was no significant relationship between HER2 expression and PNI (P = 0.353). Also, there was no significant relationship between HER2 expression and metastasis (P = 0.613) and between HER2 expression and tumor grade (P = 0.544).

Binary logistic test showed that among the studied variables, only tumor depth (T) could significantly predict HER2 positivity. Table 6 shows the effect of this factor on predicting HER2 positivity.

As can be seen, increasing the depth of the tumor (T) can result in a 3.36-fold positive. There was also a positive correlation between tumor depth and HER2 using Pearson test (r = 0.343, P = 0.007).

Finally, we followed the CRC cases for up to 4 to 5 years. We were unable to follow up 10 patients because their phone number had changed. 37 had no recurrence or metastasis and the rest had recurrence or distant metastasis. Unfortunately, 11 patients died during this follow-up period. We compared the IHC results with successful treatment in Table 7. There was no Significant value as shown in the table. We also calculated the correlation ratio for successful treatment and IHC results that described in Table 8.

Above table shows no correlation between IHC results and successful treatment. But in different types of IHC results, positive slides (3+) are more correlated with successful treatment.

As a summary, the results of the present study showed that out of 60 patients, 32 (53.3%) were men and 28 (46.7%) were women, and most of them were in the age group of 50-59 years (33.3%). The most involved sites were sigmoid (22, 36.7%), rectum (13, 21.7%) and transverse colon (8, 13.3%), respectively. Most tumors were in grade 2 (51.7%). Most tumors were deep in T3 (61.7%) and most tumors were in stage 3 (50%). Also, in terms of size, most tumors were less than 5 cm (60%). The main findings of this study were the positive correlation between HER2 expression intensity and tumor depth (T). Our results also showed that increasing the depth of the tumor (T) can be 3.36 times positive for HER2. There was also a positive correlation between tumor depth and HER2 using Pearson test (r = 0.343, P = 0.007).

Table 1: Distribution of age groups by gender

Age groups/ gender	Less than 40	40-49	50-59	60-69	70-79	80-90	Sum
Male	3, 9.4	3, 9.4	13, 40.6	6, 18.8	6, 18.8	1, 3.1	32, 53.3
Female	3, 10.7	7, 25	7, 25	7, 25	2, 7.1	2, 7.1	28, 46.7
Sum	6, 10	10, 16.6	20, 33.3	13, 21.6	8, 13.3	3, 5	60, 100

Table 2: Distribution of cancer involvement site in the subjects.

Cancer site	Number	Percentage
Sigmoid	22	36.70%
Rectum	13	21.70%
Transverse colon	8	13.30%
Ascending colon	6	10%
Cecum	6	10%
Descending colon	5	8.30%

Table 3: Distribution of HER2 marker expression intensity in the studied patients

IHC results	Number	Frequency
0	28	46.60%
1	13	21.70%
2	13	21.70%
3	6	10%

Table 4: Distribution of positive pathological findings in the studied patients

Number and percentage Pathologic findings	Number	Percentage
Metastases	2	3.3
Perineural invasion	17	28.3
Lymphovascular invasion	38	63.3
Grade 1	21	35
Grade 2	31	51.7
Grade 3	8	13.3
Lymphatic metastasis	29	48.3
T1	3	5
T2	11	18.3
T3	37	61.7
T4a	8	13.3
T4b	1	1.7
Pathologic stage I	11	18.3
Pathologic stage II	17	28.3
Pathologic stage III	30	50
Pathologic stage IV	2	3.3
Less than 5 cm	36	60
5-10 cm	20	33.3
More than 10 cm	4	6.7

Table 5: Distribution of HER2u marker expression intensity in the studied patients according to clinical and pathological features

HER2 Clinical and Pathological Data	Correlation coefficient	P value
Tumor grade	0.581	o.544
T (depth of tumor)	0.259	0.023
Stage	0.15	0.164
Metastases	0.061	0.613
Perineural invasion	0.599	0.353
Lymph node metastases	0.125	0.166
Lymphovascular Invasion	0.164	0.436
Tumor Size	0.127	0.407
Gender	0.133	0.492
Age	0.176	0.183
Site of tumor	0.155	0.146

Table 6: Predictive effect of tumor depth on HER2 positivity in patients studied

Variable	B	S.E.	O.R.	P.
T	1.215	0.499	3.36	0.015

Table 7: The comparison of IHC and treatment successful

IHC results	IHC interpretation	P value
0 or 1+	Negative	0.45
2+	Equivocal	2.78
3+	Positive	0.11

Table 8: The correlation ratio between successful treatment and IHC results

IHC results	IHC interpretation	Correlation ratio
0 or 1+	Negative	0.047
2+	Equivocal	-0.03
3+	Positive	0.03

5. Discussion

HER2 is an antigenic marker in immunological studies in intestinal carcinoma that can be used in prognosis [42]. HER2 protein overexpression has been shown to be significant in the treatment of breast cancer [43]. The biological role of this marker has been evaluated in many solid tumors [42,44].

In 2003, Pellegrini et al reported a correlation of about 93% between 83 real-time PCR and FISH methods on 83 samples. They also reported PCR sensitivity of about 96% [41].

In 2014, a study looked at the expression of HER family members in tumor samples from 86 patients with colorectal cancer. A total of 43%, 77%, 52% and 92% were positive for EGFR, HER2, HER3 and HER4, respectively. They announced that They stated that the simultaneous expression of all members of the HER family in colorectal cancer confirms the need to further investigate the predictive value of responding to treatment with anti-EGFR mABs [40].

In contrast of most of our results in a study by Pappas et al., In 51 CRC samples, 3.9% reported HER2 expression. However, no correlation was observed between overexpression of this protein and any clinical or prognostic variables [35].

Such as most of our results, in another retrospective study, Schuell et al. Examined 77 colorectal cancer specimens; Using Hercep kit, they showed that intensities of +2 and +3 IHC staining were observed in only 1 and 3% of cases of HER2 expression and were not related to clinicopathological data and their survival [59]. Also, in the study of Nathanson et al. In 139 patients, high expression of HER2 was reported in only 5 cases and was not associated with clinical-pathological features and patient survival [45]. In the study of Kavanagh et al. In 132 patients with colorectal cancer, moderate expression was present in 8% of cases and severe expression was present in only 2% of cases, with gender, age, grade, TNM and 5-year survival of patients was not found to be relevant [46].

In our study, the expression of HER2 was positive in 31.7% of cases. But no association was observed with any clinical, pathological or demographic features except tumor depth (T). Almost similar to our study, in a study by Park et al., Out of 137 patients with colorectal cancer, 65 (47.4%) showed HER2 expression, but there was a significant relationship between the expression of this protein and its clinical and pathological features. Seo in 2014 showed the incidence of HER2 protein overexpression (IHC 2+/3+) was approximately 6% in two cohorts studies and was not significantly associated with aggressive CRC behavior or patients' prognosis in both the cohorts (34). Also, Pappa wrote similar findings in 2013 [35]. Li did a me-

ta-analysis of 11 studies and expressed no relationship between HER2 and survival [36]. Wu had another meta-analysis and showed no detectable relation between HER2 and prognosis in CRC [37].

Conversely Sun in a meta-analysis showed HER2 is significantly higher in CRC. In addition is higher than positive lymph node metastasis [38]. The same results for lymph node involvement and prognosis were shown by Ingold Heppner in a retrospective investigation [39].

Abdul Razzaq et al found 47% positive IHC results of HER2(2,3 +) in North African patients. They showed relation between HER2 and tumor grading and surveillance too [58].

For Prognostic value of HER2 in CRC researchers showed different results. Osako et al. Showed that in 146 colorectal cancer samples, they were positive for HER2 in 100 cases (68.5%). High expression of HER2 was correlated with tumor stage in this study and an independent prognostic factor was introduced [47]. In a study by Kay et al. In 164 patients, 33.5% were positive and correlated with patient survival independently [48]. A study by Zhou et al. In 173 colorectal carcinomas showed that HER2 was positive in 52% of cases and was an independent factor in the prognosis of patient survival [49].

The role of HER2 protein in CRC is still in question. Perhaps the best explanation for this is that differences in different studies are probably due to the techniques used in implementing the IHC. The diagnosis of HER2 protein is highly dependent on tissue fixation and the choice of primary antibody [50].

In addition, the length of the antigen retroviral period, antigen dilution, and duration of the peroxidase reaction are critical stages in HER2 staining [51]. Prolonged storage of specimens can be a major problem, especially when specimens are stored in colorless slides [52].

Another point in interpreting HER2 intensity is that there is still no agreement on whether cytoplasmic staining or membrane staining can be considered in staining. Because cytoplasmic localization of HER2 is more common, but its diagnostic value is not yet clear [48,49]. In a study by Half et al., It was shown that only membrane HER2 was associated with high levels of gene amplification and mRNA overexpression, indicating its oncogenic role [53].

Differences in HER2 expression in various studies are probably due to techniques used in the IHC and demographic and genetic differences in the study environments.

Our study focused on the high expression of HER2 by IHC technique and its gene amplification was not performed. Given that in breast cancer, FISH is used to find HER2 expression, it is suggested that in future studies, this method be used for HER2 expression in CRC.

6. Conclusion

Expression of HER2 in an Iranian population was reported to be high in this study (31.7%). There was only a significant correlation between the expression of this protein and tumor depth (T), but there was no significant correlation between it and other variables.

Our results showed that increasing tumor depth (T) could be positive for HER2. However, this issue needs further investigation and studies with a larger sample size. If this finding is confirmed in future studies in the Iranian population, target therapy can be used as adjunctive therapy in advanced cases of the tumor that is directly related to the depth of its penetration. What is being done today in cases of advanced gastric cancer.

We have some suggestion for further searches: Evaluation of the CRC cases by FISH test and evaluation of clinicopathological picture and accompanying factors in CRC with larger sample size and evaluation of the effect of clinical pathology and associated factors in paraffin blocks of colon polyps in relation to HER2 prospectively

Conducting multi-center studies to cover the error of demographic diversity

7. Financial Support and Sponsorship

This study was funded by Taleghani Hospital Research Developmental Committee of Shahid Beheshti University of Medical Science.

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