RESEARCH ARTICLE

Editorial Process: Submission:12/12/2023 Acceptance:06/14/2024

HER2-neu Expression and Survival in Colorectal Cancer in the South of Egypt; Immunohistochemistry and Genetic Study

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Abstract

Background: Colorectal cancer (CRC) is a major public health problem and one of leading cancer related death all over the world. One of the prognostic parameters that play a role in different types of cancer is *HER2*. However, the role of *HER2* in CRC and its relation with clinicopathological features and survival is conflicting. We hypothesize that *HER2* has different patterns of expression in CRC which may affect the prognosis of patients. **Material & Methods:** We studied sixty specimens of colorectal carcinoma for *HER2* immunohistochemistry and gene amplification and correlate it with clinicopathological features and patients` survival. **Results:** Our data showed that negative *HER2* expression was statistically associated with female gender (P = 0.010) and low & intermediate tumor budding (P = 0.030). There was a statistically significant relation between *HER2* IHC and *HER2* FISH amplification (P=0.000). Although neither *HER2* immunoexpression and FISH amplification showed significant relation with overall survival nor disease free survival, *HER2* amplified CRCs tended to have a worse survival compared with negative CRCs (40 months versus 50 months). The presence of male gender, lymphovascular invasion, nodal metastasis and distant metastasis (P = 0.013, 0.006, 0.006 and 0.000 respectively) were significantly statistically associated with poor overall survival. The presence of tumor grade III and high tumor budding (P = 0.035 and 0.007 respectively) were significantly statistically associated with shorter disease free survival. **Conclusions:** Our results showed that *HER2* IHC 3+ staining is highly predictive of *HER2* gene amplification in colorectal carcinomas. There is a tendency towards poorer prognosis in amplified *HER2* CRC cases.

Keywords: Colorectal carcinoma- *HER2/neu*- IHC- FISH- survival

Asian Pac J Cancer Prev, **25 (6)**, 2023-2032

Introduction

Colorectal cancer (CRC) is a major public health problem and one of leading cancer related death all over the world [1]. It is the third most commonly diagnosed form of cancer globally, comprising 11% of all cancer diagnoses and the second most deadly cancer worldwide [1]. In spite of great advance in the diagnosis and developing therapeutic options, the prognosis of some patients is still grim. Many prognostic factors have been identified to date, with TNM (Tumor, Node, Metastasis) staging system one of the most important factors. However, patients with same stage may exhibit different outcome [2]. Therefor it is important to establish other prognostic & predictive parameters other than conventional ones. One of the prognostic parameters that play a role in different types of cancer is *HER2* (human epidermal growth factor receptor) [3].

HER2 is a transmembrane glycoprotein located at the long arm of human chromosome 17 which belongs to the epidermal growth factor receptor (EGFR) epithelial tyrosine kinase protein family [4]. This family plays a central role in a variety of cellular responses including cell growth, survival, and differentiation via multiple signal transduction pathways and participate in cellular proliferation and differentiation [3].

It is well established that *HER2* has a prognostic role in some types of cancer like breast and gastric carcinoma [5, 6]. It`s considered a therapeutic target, with targeted therapy against *HER2* using the monoclonal antibody Trastuzumab (Herceptin) having become the standard for patients whose breast carcinomas exhibit *HER2* gene amplification [5] and selected cases of gastric carcinoma [6]. However, *HER2* overexpression and its relation with clinicopathological features and survival in CRC is conflicting [7]. In CRC, Diverse rates & patterns of *HER2* overexpression have been reported [8], with cytoplasmic staining pattern showed an average around 30% [9] and only 2% - 11% showed membranous expression pattern [10].

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To date, our knowledge about the concordance between *HER2* expression by immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) in CRC is unclear. In addition, the relation between *HER2* expression and patient prognosis is still limited. We hypothesize that *HER2* has different patterns of expression in CRC and this may affect the prognosis of patients. To assess the credibility of our hypothesis, we investigated *HER2* expression both by IHC & FISH techniques in CRC patients and correlate this expression with different clinicopathological parameters and patient's survival.

Materials and Methods

Case selection

This is a retrospective study included sixty specimens of colorectal carcinoma. These were retrieved from biopsy specimens received at laboratory of pathology in south Egypt cancer institute at the period (2013-2016).

The available clinicopathological data of the cases were obtained from the hospital medical records. These data included: the age of patient at time of diagnosis, sex, tumor site, operation type, clinical follow up information as occurrence of distant metastasis or local recurrence and survival data including overall survival, disease free survival and progressive free survival. Follow up was performed for 2 years.

Representative Hematoxylin and eosin-stained slides were examined for each specimen for detailed histopathological features including tumor histological type & grade (according to WHO (World Health Organization) classification of colon and rectal tumors, 5th Edition, 2019) [11], perineural and lymphovascular invasion (LVI) , depth of tumor invasion, lymph node metastasis, distant metastasis (according to the TNM classification of the American Joint Committee on Cancer (AJCC) 8th Edition, 2017) [12]. In addition, we evaluate tumor budding and presence of poorly differentiated clusters (according to The International Tumor Budding Consensus Conference (ITBCC) 2016 group) [13].

Immunohistochemical staining of HER2

Immunohistochemical staining was performed using avid-biotin immunoperioxidase method. Four-micrometer-thick formalin-fixed, paraffin embedded tissue sections were mounted over coated slides. Sections were dewaxed and rehydrated through graded alcohols to distilled water. The hydrogen peroxide block was applied and incubated for 15 minutes then for antigen retrieval; sections were treated in microwave of (600 watt) by immersion of the slides in citrate puffer solution (PH 7) for 20 minutes. Sections were incubated with primary antibody (c-erbB-2/HER-2/neu Ab-17) for 1 hour at room temperature in the humid chamber. Antibody used was Clone e 2-4001 + 3B5, Catalog #MS-730-P0 (0.1ml) supplied by Thermo Scientific Company of 1/400 concentration. Then, the slides were washed 2 to 3 times using phosphate buffer saline solution. After washing, immunostaining was performed using a universal staining kit (Thermo Scientific Company) according to the manufacturer`s instructions. Counter staining was done using Mayer's hematoxylin.

Positive control

It was examined first to ascertain that all reagents are functioning properly. Sections from breast (known as *HER2* score 3) was used as positive control for *HER2*

Negative control

Tumor tissue section was processed in the above-mentioned sequence but the primary antibody was not added and instead phosphate buffered saline (PBS) was used in this step.

Evaluation of HER2 protein expression

HER2 staining was known by brownish staining of the cytoplasm. The scoring method for *HER2* expression in colorectal cancer according to HERACLES (*HER2* Amplification for Colorectal Cancer Enhanced Stratification) criteria [14] is as following: IHC score 0: no staining, IHC score 1+: faint staining (segmental or granular); moderate staining < 50% of cells; intense staining < 10% cells, IHC score 2+: moderate staining in > 50% of cells and IHC score 3+: intense staining in > 50% of cells. Score 0 and 1 are considered as negative, score 2 is equivocal and score 3 is positive.

Fluorescent in situ hybridization (FISH) HER2 amplification probe

All cases of our study were evaluated by FISH probe. FISH was performed using the Cytocell HER-2 (ERBB2) Amplification probe. The *HER2* amplification probe consists of a red probe spanning the *HER2* (ERBB2) gene and neighboring regions and a green probe for the chromosome 17 centromere. The FISH procedure was performed according to the manufacturer` instructions. In brief, 4µm sections were dewaxed and rehydrated through xylene and ethanol and heated in pretreatment reagent at 98 - 100°C for 20 - 30 min. 5µl of probe mixture (as the probes were provided premixed in hybridization solution (Formamide; Dextran Sulphate; SSC)) was applied to the slide and overlaid with a coverslip then underwent denaturation at 80°C for 5 minutes and hybridization at 37°C for 12 – 16 hours. After hybridization, slides were rinsed in 0.4× SSC/0.3% NP-40 at 72°C for 2 minutes, and counterstained with 4,6-diamidino-2-phenylindole (DAPI).

Evaluation of HER2 amplification probe

HER2 FISH positive: an average of >6 *HER2* gene copies/nucleus or *HER2*/CEP (centromere enumeration probe) 17 ratio of more than 2.2 in more than 10% of cells, where CEP17 is a centromeric probe for chromosome 17 on which the *HER2* gene resides. The equivocal range for *HER2* FISH assays: HER/CEP ratios from 1.8 to 2.2 or average gene copy number between 4.0 and 6.0. Negative *HER2* FISH amplification: *HER2*/CEP17 ratio of less than 1.8 or an average of fewer than four copies of *HER2* gene per nucleus [15].

Statistical analysis

Results were statistically analyzed using statistical

package for Social Sciences (SPSS version 22). Data were presented as frequency and percentage for qualitative variables, and as mean and standard deviation for quantitative variables. Chi-square test was used to compare qualitative variables. Kaplan Meyer (using Log-rank test) was done for overall survival, disease free survival and progression free survival. Multi-variate analysis was done using the Cox regression model. Significance was defined as P-value < 0.05.

Results

Clinicopathologic features of CRC Cases of the Study

The clinicopathologic characters of 60 patients with CRC involved in the present study were summarized in Table 1. Briefly, the age of patients ranges from 23 to 86 with the mean age was 50 years. 47 cases were adenocarcinoma, NOS and 13 cases were mucinous adenocarcinoma. 20 cases of CRC were in right colon and 40 cases were in left colon & rectum. 25 cases were grade I, 26 cases were grade II and 9 cases were grade III (Table 1).

Immunohistochemical expression of HER2

HER2/neu was expressed in colorectal carcinoma cells with mainly cytoplasmic staining pattern. Cytoplasmic staining was detected in 33/60 (55%) cases, while membranous staining was detected in only 1/60 case (1.7%). Positive *HER2* staining (score 3) was noticed in 14 cases (23.3%), negative staining (score 0&1) in 28 cases (46.7%) and equivocal staining (score 2) in 18 cases (30%) (Figure 1)

Relationship between HER2/neu protein expression and clinicopathological features

Statistically significant relation was detected between *HER2* expression and sex of the patients as 67.9% of cases with negative *HER2* expression were female gender (P = 0.010) . Also statistical significant relation was detected between HER2 expression and tumor budding as tumors with low & intermediate budding showed negative *HER2* expression (P = 0.030) (Table 2).

No significant relationship was detected between *HER2* expression and other parameters as; age, site of the tumor, histologic type and grade, presence of necrosis, lymphovascular emboli, poorly differentiated clusters, tumor invasion, lymph node metastasis and distant metastasis (P=0.191, P=0.651, P=0.060, P=0.334, P=0.651, P=0.245, P=0.160, P=0.194, P=0.095, P=0.338, P=0.110 and P=0.671 respectively) (Table 2).

Cytogenetic finding

Amplification of *HER2*/neu FISH probe were noticed in 32 cases of CRC (53.3%) while 28 cases (46.7%) showed negative expression (Figure 2).

Relationship of HER2/neu FISH probe amplification and clinicopathological features

Statistically significant relation was detected between *HER2*/neu FISH probe amplification and male gender (P=0.010) and presence of grade 1 poorly differentiated

Table 1. Clinicopathological Features of the Studied Cases

Clinicopathological features	No. (60)	%
Sex		
Male	30	50.00
Female	30	50.00
Age: (years)		
< 50	26	43.30
≥ 50	34	56.70
Mean ± SD (Range)	50.20 ± 13.70 (23.0-86.0)	
Tumor type		
Adenocarcinoma	47	78.30
Mucinous adenocarcinoma	13	21.70
Site of tumor:		
Right colon	20	33.30
Left colon & rectum	40	66.70
Grade		
Grade I	25	41.70
Grade II	26	43.30
Grade III	9	15.00
Necrosis:		
Positive	16	26.70
Negative	36	60.00
NA	8	13.30
LVI		
Positive	17	28.30
Negative	35	58.30
NA	8	13.30
Tumor budding:		
1	16	26.70
2	14	23.30
3	18	30.00
NA	12	20.00
Poorly differentiated clusters:		
1	23	38.30
2	11	18.30
3	14	23.30
NA	12	20.00
Tumor invasion:		
T2	2	3.30
T3	49	81.70
T4	1	1.70
TX	8	13.30
Lymph node metastasis		
N0	23	38.30
N1	16	26.70
N2	10	16.70
NX	11	18.30
Distant metastasis:		
M0	39	65.00
M1	21	35.00

NA, not assessed

Table 2. Relationship between *HER2* Expression and Clinicopathological Features

Clinicopathological features	HER2 IHC						P-value
	Positive		Negative		Equivocal		
	No.	%	No.	%	No.	%	
Sex							
Male	7	50.00	9	32.10	14	77.80	0.010*
Female	7	50.00	19	67.90	4	22.20	
Age: (years)							
< 50	5	35.70	10	35.70	11	61.10	0.191
≥ 50	9	64.30	18	64.30	7	38.90	
Tumor type							
Adenocarcinoma	9	64.30	23	82.10	15	83.30	0.344
Mucinous adenocarcinoma	5	35.70	5	17.90	3	16.70	
Site of tumor							
Right colon	6	42.90	8	28.60	6	33.30	0.651
Left colon & rectum	8	57.10	20	71.40	12	66.70	
Grade							
Grade I	5	35.70	13	46.40	7	38.90	
Grade II	9	64.30	9	32.10	8	44.40	0.245
Grade III	0	0.00	6	21.40	3	16.70	
Necrosis							
Positive	4	28.60	10	35.70	2	11.10	0.16
Negative	10	71.40	13	46.40	13	72.20	
NA	0	0.00	5	17.90	3	16.70	
LVI							
Positive	2	14.30	9	32.10	6	33.30	0.194
Negative	12	85.70	14	50.00	9	50.00	
NA	0	0.00	5	17.90	3	16.70	
Tumor budding							
1	5	35.70	9	32.10	2	11.10	
2	5	35.70	6	21.40	3	16.70	0.030*
3	2	14.30	5	17.90	11	61.10	
NA	2	14.30	8	28.60	2	11.10	
Poorly differentiated clusters:							
1	8	57.10	7	25.00	8	44.40	
2	2	14.30	8	28.60	1	5.60	0.095
3	2	14.30	5	17.90	7	38.90	
NA	2	14.30	8	28.60	2	11.10	
Tumor invasion:							
T2	0	0.00	2	7.10	0	0.00	
T3	14	100.00	20	71.40	15	83.30	0.338
T4	0	0.00	1	3.60	0	0.00	
TX	0	0.00	5	17.90	3	16.70	
Lymph node metastasis							
N0	10	71.40	12	42.90	1	5.60	
N1	3	21.40	7	25.00	6	33.30	0.11
N2	0	0.00	4	14.30	6	33.30	
NX	1	7.10	5	17.90	5	27.80	
Distant metastasis							
M0	8	57.10	18	64.30	13	72.20	0.671
M1	6	42.90	10	35.70	5	27.80	

*Statistical significant difference (P < 0.05); NA, not assessed

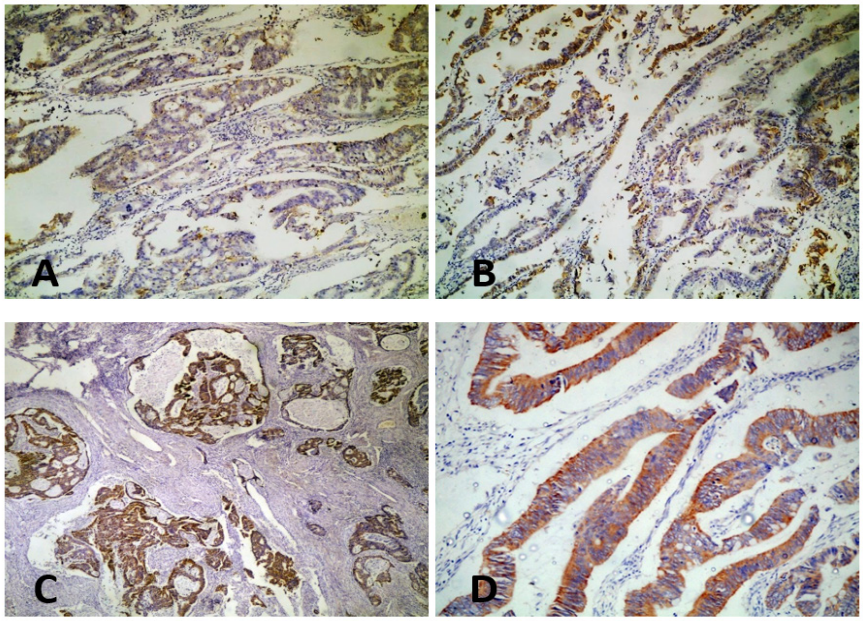

Figure 1. Expression of *HER2* in Colorectal Carcinoma. A, Negative expression of *HER2* in colorectal carcinoma (x10); B, Equivocal expression of *HER2* in colorectal carcinoma (x10); C, Positive cytoplasmic expression of *HER2* in colorectal carcinoma (x10); D, Positive cytoplasmic expression of *HER2* in colorectal carcinoma (x40).

clusters (P=0.045) (Table 3).

No significant relation was detected between *HER2*/neu FISH probe amplification and other parameters as; age, site of the tumor, histologic type and grade, presence of necrosis, lymphovascular emboli, tumor budding, tumor invasion, lymph node metastasis and distant metastasis (P=0.265, P=0.464, P=0.396, P=0.197 P=0.133, P=0.427, P=0.194, P=0.159, P=0.179, P=0.914 and P=0.592 respectively) (Table 3).

Relation between HER2/neu immunoexpression and HER2/neu FISH probe amplification

According to our study, all 28 cases with negative *HER2* expression showed negative Fish probe. All positive (14) and equivocal (18) cases showed amplified Fish probe. There was a statistically significant correlation between *HER2*/neu immunoexpression and *HER2*/neu FISH amplification (P=0.000) (Table 4).

Survival Analysis

Univariate Kaplan-Meier-survival analysis demonstrated that neither *HER2* immunoexpression and FISH amplification showed significant relation with overall survival (OS), disease-free survival (DFS) and progression-free survival (PFS) (*HER2* expression; OS, P = 0.113, DFS; P = 0.130 and PFS; P = 0.143) and

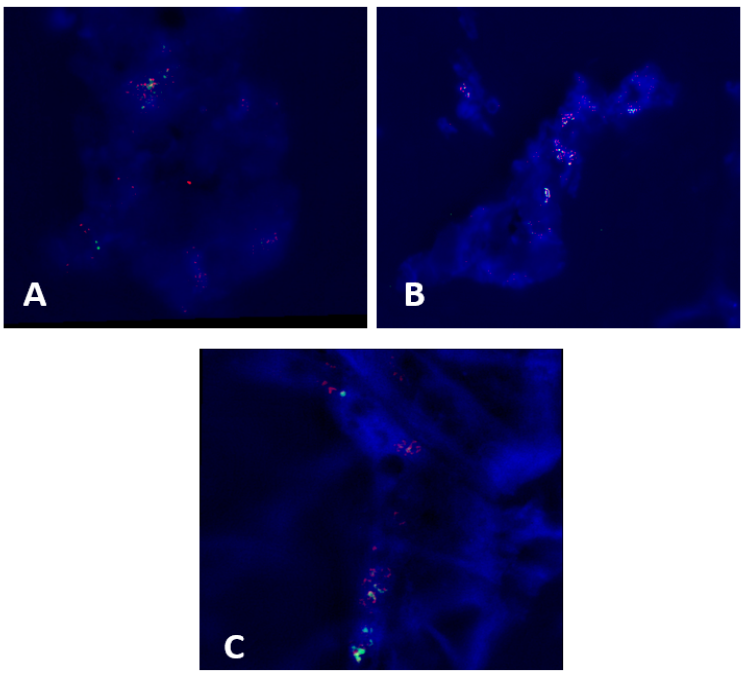

Figure 2. A, B & C; *HER2* Amplification in Colorectal Carcinoma

Table 3. Relation between *Her2/neu* FISH Probe Amplification and Clinicopathological Features

Clinicopathological features	HER2 FISH probe				P-value
	Amplified		Negative		
	No.	%	No.	%	
Sex					
Male	21	65.60	9	32.10	0.010*
Female	11	34.40	19	67.90	
Age: (years)					
< 50	16	50.00	10	35.70	0.265
≥ 50	16	50.00	18	64.30	
Tumor type					
Adenocarcinoma	24	75.00	23	82.10	0.503
Mucinous adenocarcinoma	8	25.00	5	17.90	
Site of tumor					
Right colon	12	37.50	8	28.60	0.464
Left colon & rectum	20	62.50	20	71.40	
Grade					
Grade I	12	37.50	13	46.40	
Grade II	17	53.10	9	32.10	0.197
Grade III	3	9.40	6	21.40	
Necrosis					
Positive	6	18.80	10	35.70	
Negative	23	71.90	13	46.40	0.133
NA	3	9.40	5	17.90	
LVI					
Positive	8	25.00	9	32.10	
Negative	21	65.60	14	50.00	0.427
NA	3	9.40	5	17.90	
Tumor budding					
1	7	21.90	9	32.10	
2	8	25.00	6	21.40	0.159
3	13	40.60	5	17.90	
NA	4	12.50	8	28.60	
Poorly differentiated clusters:					
1	16	50.00	7	25.00	
2	3	9.40	8	28.60	0.045*
3	9	28.10	5	17.90	
NA	4	12.50	8	28.60	
Tumor invasion:					
T2	0	0.00	2	7.10	
T3	29	90.60	20	71.40	0.179
T4	0	0.00	1	3.60	
TX	3	9.40	5	17.90	
Lymph node metastasis:					
N0	11	34.40	12	42.90	
N1	9	28.10	7	25.00	0.914
N2	6	18.80	4	14.30	
NX	6	18.80	5	17.90	
Distant metastasis					
M0	21	65.60	18	64.30	0.914
M1	11	34.40	10	35.70	

*Statistical significant difference (P < 0.05); NA, not assessed

(*HER2* FISH probe; OS, P = 0.102, DFS, P = 0.121 and PFS; P = 0.438). However, *HER2* amplified CRCs tended to have a worse survival compared with negative CRCs (Figure 3, A). The median survival time of patients with amplified *HER2* was 40 months versus 50 months in patients with negative *HER2* expression.

The univariate analysis of the other parameters examined showed that there was a significant progressive decline in OS with male gender (P = 0.013; Figure 3, B), with presence of LVI (P = 0.006; Figure 3, C), with lymph node metastasis (P = 0.006; Figure 3, D) and also with distant metastasis (P = 0.000; Figure 3, E). The remaining clinicopathological parameters examined, namely: age, tumor site, histologic type, grade, presence of necrosis, tumor budding, poorly differentiated clusters and depth of tumor invasion were not found to be associated significantly with OS (P > 0.05).

There was also a significant progressive decline in DFS with tumor grade III (P = 0.035; Figure 3, F) and high tumor budding (P = 0.007; Figure 3, G). The remaining clinicopathological parameters examined, namely: age, sex tumor site, histologic type, presence of necrosis, lymphovascular emboli, poorly differentiated clusters, depth of tumor invasion, lymph node metastasis and distant metastasis were not found to be associated significantly with OS (P > 0.05).

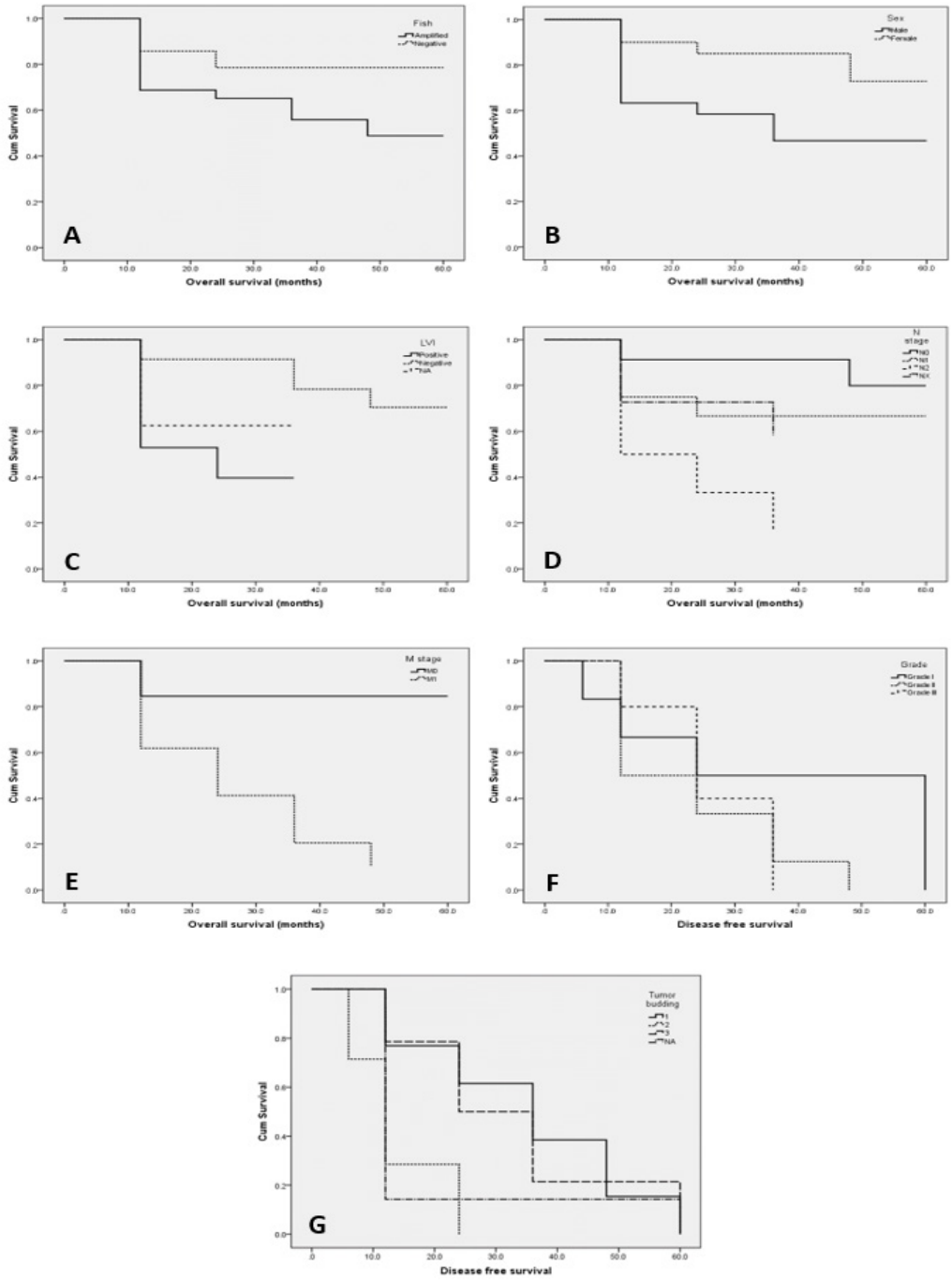

Figure 3. A, Relation between HER2 FISH probe amplification and OS; B, Relation between sex and OS; C, Relation between lymphovascular emboli and OS; D, Relation between lymph node metastasis and OS; E, Relation between distant metastasis and OS; F, Relation between tumor grade and DFS; G, Relation between tumor budding and DFS.

Table 4. Relation between *HER2* Immunoexpression and *HER2* FISH Probe Amplification

HER2 IHC	HER2 FISH probe				P-value
	Amplified		Negative		
	No.	%	No.	%	
Positive	14	43.80	0	0.00	0.000*
Negative	0	0.00	28	100.00	
Equifocal	18	56.30	0	0.00	

*Statistical significant difference (P < 0.05)

PFS was not found to be affected by any clinicopathological parameters. After multivariate analysis using Cox proportional hazard model, distant metastasis (P = 0.002; HR = 6.988; 95% CI, 2.065-23.652) proved to be the only significant independent factor for OS.

Discussion

The *HER2* signaling network is known to influence a wide range of cellular processes, including proliferation, motility, and survival [16]. Overexpression of the *HER2* receptor is variable in different tumors; as it is detected in 13–20% of human breast cancer [17] but the level and incidence of *HER2* overexpression in primary colon tumors appears to be different than those observed in breast cancer. Conflicting data exist about the prevalence of *HER2* overexpression in colorectal cancer which ranges from 2.7% to 47.4% [18].

In this work, we studied 60 cases of colorectal carcinoma in South Egypt Cancer Institute in period from 2013 to 2016, *HER2* protein expression by immunohistochemistry and gene amplification by fluorescence in situ hybridization, its predictive and prognostic values and relation to DFS, PFS and OS.

Positive *HER2* staining (score 2&3) was noticed in 18 and 14 cases respectively (53.3%) and negative staining (score 0&1) in 28 cases (46.7%). Our findings were similar to Shabbir et al. [19] and Kamaland Jalal [20] who detected positive immunostaining in 55% and 53.4% of colorectal cancer cases respectively. However, these results were very different from other researches done by Sawada et al. [21] and Wang et al. [18] who found *HER2* positivity in 4.1% and 11.7% of cases respectively.

Possible explanation for this dramatic range in *HER2* overexpression positivity across studies may be that antibodies used for staining varied among research groups and also variation in the definition of the positivity pattern either membranous or cytoplasmic. The *HER2* antibody used in the study with the highest positive *HER2* staining (47.4%) was a polyclonal rabbit antibody. The positive rates of *HER2* protein staining in studies using the DAKO *HER2* antibody were 2.7% to 15.5%, while it were 8% to 11.4% in the studies using Ventana pathway antibody [18].

HER2 gene amplification was identified in all (18) cases with *HER2* IHC scores of 2+ and (14) cases with *HER2* IHC scores of 3+, which totally represent 53.7% of all colorectal cancer patients. No tumors with *HER2* IHC scores of 1+ showed evidence of *HER2* gene amplification by FISH. This indicates that there is a high concordance between *HER2* IHC 3+ staining and *HER2* gene amplification in colorectal adenocarcinomas. This is similar to Marx et al. [22] who found that 100% of tumors with 3+ *HER2* staining showed *HER2* gene amplifications and nearly similar to Wang et al. [18] who detected 83% of tumors with 3+ *HER2* staining exhibited *HER2* gene amplification.

Variations in the antibodies used likely led to differential staining and scoring, contributing to the disagreement in *HER2* gene amplification in tumors with 3+ *HER2* scores. All these studies, including ours, have shown that *HER2* IHC 3+ staining is highly predictive of *HER2* gene amplification in colorectal carcinomas.

In contrast, the percentage of tumors with *HER2* IHC 2+ staining showing evidence of *HER2* gene amplification was highly variable. In our study, *HER2* amplification was evident in 100% of tumors, nearly similar to Marx et al. [22], who observed *HER2* gene amplification in 75% of tumors with *HER2* scores of 2+. In contrast, in the study by Sawada et al. [21] and Wang et al. [18], such amplification was only evident in 36% and 20% of tumors respectively.

In this study, we found a statistically significant association between negative *HER2* expression and female patients (P=0.010), low and intermediate tumor budding (P=0.030). No other similar studies in association between *HER2* expression and tumor budding were found in the literature. However, tumor budding is an independent adverse prognostic factor in colorectal cancer as it is associated with a higher TNM stage, high tumor grade, the presence of lymphovascular invasion and consequently with lymph node and distant metastases [23, 24]. Therefore, there may be a relation between high tumor budding and positive *HER2* expression.

We didn`t find significant relation with site of the tumor, but Seo et al. [25] detected a statistically significant association with tumor location in the rectum (P=0.033). It has been clearly demonstrated that *HER2* gene amplification differs significantly between right/left-sided and rectal carcinomas [26, 27].

We also didn`t find significant relation with histologic grade or lymph node metastasis. However, Heppner et al. [28] found statistically significant association of *HER2* positivity with nodal status (P =0.033). Conradi et al. [29] reported that *HER2* positivity was associated with high grade tumors and positive nodal status. We didn`t find significant relation with depth of tumor invasion or distant metastasis. On the other hand, Wang et al. [18] found a statistically significant association between *HER2* gene amplification and tumor depth of invasion and distant metastasis (P = 0.001 and 0.028 respectively).

On the other hand, Marx et al. [22] failed to

find an association of positive *HER2* status with clinicopathological parameters. All these differences between many studies are probably due to differences in groups analyzed, *HER2* testing methods, and tumor biological characteristics. In our study, *HER2* positivity had no statistically significant impact on patients' overall survival, but *HER2* positive tumors displayed a tendency to poorer courses, which is in line with Heppner et al. [28]. However, Seo et al. [25] and Wang et al. [18] didn`t detect an association between *HER2* expression and survival rates.

In our study, OS showed significant pro¬gressive decline in association with male gender (P = 0.013), presence of LVI (P = 0.006), lymph node metastasis (P = 0.006) and distant metastasis (P = 0.000). This is similar to Chao-Hsien et al. [30] who reported significant decline in OS with lymph node metastasis (P < 0.001) and distant metastasis (P < 0.001). Also, Joachim et al. [31] detected significantly worse median OS in men (P = 0.0394). Finally, Pei et al. [32] reported that presence of lymphovascular emboli was statistically associated with worse OS (P < 0.001).

Unfortunately, we have no detailed information about the different applied therapy regimen, which may be a limitation of the presented study. However, the tendency of poorer overall survival of *HER2* positive CRC probably reflects the association with advanced CRC, independent of treatment. In conclusion, *HER2* protein overexpression is evident in 53.7% of CRC. Both *HER2* IHC scores 2 and 3 are highly correlated with *HER2* gene amplification. There is a tendency towards poorer prognosis in amplified *HER2* CRC cases.

Recommendation; future studies are recommended for the possibility of benefit of *HER2* targeted therapy in certain group of colorectal carcinoma patients.

Author Contribution Statement

Conceptualization: HEB; Data curation: HEB; Formal analysis: HEB, FAT; Investigation: HEB, FAT, THA; Methodology: HEB; Project administration: HEB, HAN. EMA; Resources: HEB, FAT; Supervision: HEB.THA; Validation: HEB; Visualization: HEB; Writing – original draft: HEB, MOM; Writing – review & editing: HEB; Approval of final manuscript: all authors.

Abbreviations

CRC: colorectal cancer
TNM: Tumor Node Metastasis
HER2: human epidermal growth factor receptor 2
EGFR: epidermal growth factor receptor
IHC: immunohistochemistry
FISH: fluorescent in situ hybridization
WHO: World Health Organization
AJCC: American Joint Committee on Cancer
ITBCC: International Tumor Budding Consensus Conference (ITBCC)
PBS: phosphate buffered saline
HERACLES: *HER2* Amplification for Colorectal Cancer Enhanced Stratification
DAPI: 4,6-diamidino-2-phenylindole
CEP: centromere enumeration probe
SPSS: Statistical Package for Social Sciences
LVI: lymphovascular invasion
OS: overall survival
DFS: disease free survival
PFS: progression free survival

Acknowledgements

We are grateful for the Research Grant office, Quality Assurance Unite at Faculty of Medicine, Assiut University as the work funded by them; Grant number (2017107/12-003-R1). We also grateful for Surgical Pathology Laboratories, Assuit University Hospital, Faculty of Medicine and South Egypt Cancer Institute, Assuit University as we take from them our speciemens.

Funding sources

This work was funded by the Research Grant office, Quality Assurance Unite at Faculty of Medicine, Assiut University. Grant number (2017107/12-003-R1).

Ethical issue

This research received ethics approval from the Committee of Medical Ethics of Faculty of Medicine, Assuit University, number (17200089).

Data availability

All data generated or analyzed during this study are included in this published article.

Funding

This work was funded by the Research Grant office, Quality Assurance Unite at Faculty of Medicine, Assiut University. Grant number (2017107/12-003-R1).

Conflict of interest

The authors declare that they have no conflict of interest exist.

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