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Immunohistochemical expression of HER2/neu in colorectal carcinoma in Erbil city, Kurdistan Region

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Abstract

Background and objective: Colorectal carcinoma is one of the commonest cancers and the second leading cause of cancer death in Europe and North America. Despite the improved surgeries and adjuvant therapies, the prognosis of colorectal cancer remains poor, and the survival rate is unsatisfactory. Overexpression of HER2 gene has been noticed in many human cancers, and it is associated with poor prognosis. This study aimed to detect the frequency of the HER2/neu immunoexpression in colorectal carcinoma and investigate its association with the clinicopathological parameters.

Methods: A retrospective study was carried out in Rizgary teaching hospital and private laboratories in Erbil city for the period from January 2014 - December 2017. A total of 103 formalin fixed, paraffin embedded, archival tissue blocks of colectomy samples of colorectal carcinoma cases were collected. Immunostaining was done in a private laboratory (Vin Lab) in Dohuk city. Scoring of the HER2/neu immunostaining was managed according to published criteria for breast carcinoma.

Results: Fifty-five cases (53.4%) were labeled as positive for HER2/neu immunoexpression. While 48 (46.6%) of the cases were labeled as negative. HER2/neu status was significantly associated with the tumor grade (P = 0.02), while no significant association was found between HER2/neu expression and other clinicopathological parameters.

Conclusion: HER2/neu immunoexpression was observed in 53.4% of the cases of colorectal carcinoma, and it was significantly associated with tumor grade.

Keywords: Colorectal carcinoma; HER2/neu; Immunoexpression.

Introduction

Colorectal carcinoma is one of the commonest cancers and the second leading cause of cancer death in Europe and North America. Annually there are about one million newly diagnosed cases and a half million cases die from the disease worldwide.[1-3] Its oncogenesisis characterized by complex interactions between genetic alterations, host immune system, and environmental carcinogenic factors.[4,5] Despite the improved surgeries and adjuvant therapies, the prognosis of colorectal cancer remains poor, and the survival rate is unsatisfactory. This is most probably because of two factors; recurrence and metastasis, which obstacle the prognosis.[3,6] Therefore, it is quite important to concern about the characteristic of the tumor itself and its environment. Consequently, pertaining to colorectal cancer subtypes will help to achieve target therapy and improve prognosis. Apart from understanding prognostic factors which are concerned in colorectal cancer; further understanding of mechanism and biology of the tumor is of great value for application of target therapy and consequently improving prognosis and increasing the life span of the patients.[6] HER2/neu is one of the members of the epidermal growth factor (EGF) family of

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receptor tyrosine kinases, which consists of EGFR (ErbB1), HER2/Neu (ErbB2), ErbB3, and ErbB4. Among the members of the ErbB family, HER2/neuhas been concerned to play an oncogenic role in human tumors along with EGFR.[7,8] HER2/ neu is positioned on chromosome 17q21 and translates a 185 kD transmembrane glycoprotein receptor that is stimulated by the phosphorylation of deposits. Activation of HER2/neu induces initiation of various signal cascades such as the MAPK, PI3K/AKT, and PKC pathways, which are essential for cell differentiation, proliferation, persistence and cell survival.[5,9,10] Overexpression of HER2/neu gene has been noticed in many other human cancers, and it is associated with poor prognosis. About 7 to 43% of breast cancer patients noticed to have overexpression of HER2/neu gene and subsequently influence the line of treatment and prognosis.[11] However, overexpression of this gene in colorectal cancer is not well-understood regarding the prognosis and target therapy.[6] Research on immunohistochemical expression of HER2/ neu in colorectal cancer is lacking in our region, and there is no available statistical data about the evaluation of HER2/neu status in colorectal cancer in Erbil city. Therefore, the present study aimed to assess the immunohistochemical expression of HER2/neu antigen in colorectal cancer and investigate its association with the clinicopathological parameters like age, gender, tumor site, histological type, tumor grade, nodal involvement, and tumor stage.

Methods

After obtaining approval of the study protocol form the research ethics committee at the College of Medicine at Hawler Medical University, this retrospective study was carried out. A total of 103 formalin fixed, paraffin embedded, archival tissue blocks of colectomy samples of colorectal carcinoma cases were recruited from January 2014 to December 2017. The samples were randomly selected from the histopathology laboratory of Rizgary Teaching Hospital and some private histopathology laboratories in Erbil city. The patients' clinicopathological parameters such as age, gender, tumor site, histological type, tumor grade, nodal involvement, and tumor stage were also collected and analyzed statistically. Two sections of four-micron thickness were prepared for each tumor case. They were taken from the most representative paraffin blocks of the tumors that contained more than 50% of tumor tissue, avoiding necrotic and hemorrhagic areas. Consequently, one of them was stained with Hematoxylin and eosin stain and histopathologically re-evaluated for the tumor type and grade. The other sectionwas dewaxed and processed for immunohistochemical staining. The pathological tumor staging was performed according to American Joint Committee on Cancer (AJCC) and the Union InternationaleContre Le Cancer (UICC).[12]

Immunohistochemical staining:

Dako Manufacturer's recommendations were followed for immunostaining. The tissue was stained by Labeled polymer and enhanced polymer systems (Autostainer Link 48) method. Dako recommendation was used to stain the tissue by Labelled polymer and enhanced polymer systems (DakoEnVision™ Flex) method. Thin sections (four µm) were cut, mounted on salinized slides, and dried at 60 °C for one hour. Tissue slides had been deparaffinized and rehydrated at room temperature (20-25 °C). The slides placed in a xylene bath and incubated for five minutes and had been put in absolute ethanol for 3 minutes. After that, slides placed in 95% ethanol for 3 minutes. Finally, slides were immersed in distilled or deionized water for a minimum of 30 seconds. A specific epitope retrieval method in 10 mmol/L citrate buffer was used using 1:10 ratio with distilled water. Two expert pathologists had examined the sections with the use of light microscopy

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independently. Any disagreements were reviewed, followed by definitive decisions. HER2/neu expression was evaluated semi quantitatively and was scored according to the percentage of labeled cells. Negative controls were prepared simultaneously for all 103 samples by replacing the primary antibody with distilled water. The positive control was a sample of invasive ductal carcinoma of the breast, with strong complete membranous staining for HER2/ neu immunoexpression in the tumor cells.

HER2/neu scoring system:

Scoring of the HER2/neu immunostaining was managed according to published criteria for breast carcinoma. Cytoplasmic staining was not included. The intensity of membranous staining was graded from zero to three. Score zero is defined as undetectable staining or membranous staining in <10% of the tumor cells. Score one+ is defined as faint membranous staining in >10% of the tumor cells. Score two+ is defined as weak to moderate complete membranous staining in >10% of the tumor cells; Score three+ is defined as strong complete membranous staining observed in >10% of the tumor cells. HER2/neu protein expression was defined as negative (scores 0 and 1+) or positive (scores 2+ and 3+).[13-16]

Statistical analysis

The collected data were analyzed using computerized software statistical package for the social sciences program (version 23). By using the Pearson Chi-square test and Fisher's exact test, assessment of the association between HER2/neu expression and clinicopathological parameters had been calculated. The level of significance was set at $P \leq 0.05$.

Results

In this study, 103 cases of colorectal cancer have been included. The patient's agewas ranged from 19-85 years, with a mean age of 54.48 years ± 14.818 years, and the median age was 56 years. There were two age groups; 65% were 50 years or above, and 35% were less than 50 years old. There were 45 males and 58 females with a male to female ratio of 0.77:1. The clinicopathological characteristics of the studied cases are described in Table 1.

Table 1: Numbers and percentages of different clinicopathological characteristics of the studied cases

Clinicopathological parameters		No.	%
Age	≥ 50 years	67	65.0
	< 50 years	36	35.0
Gender	Male	45	43.7
	Female	58	56.3
Tumor Site	Right colon	34	33.1
	Left colon and rectum	69	66.9
Tumor type	(conventional)	89	86.4
	Mucinous	14	13.6
Tumor grade	Well – moderately differentiated	85	82.5
	Poorly differentiated	18	17.5
Nodal involvement	Negative	47	45.6
	Positive	56	54.4
Tumor stage	Stage 1 & 2	47	45.6
	Stage 3 & 4	56	54.4
Total		103	100.0

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Only in 9 cases (8.7%), there were not any detectable membranous staining and thus were scored as zero. Thirty-nine cases (37.9%)were scored as 1+. Thirty-seven cases (35.9%) were scored as 2+. Only 18 cases (17.5%) were scored as 3+, as shown in Figure 1. Fifty-five cases were labeled as positive for HER2 immunoexpression, while 48 cases were labeled as negative. HER2/neu status was significantly associated with the tumor grade (*P* = 0.02), while no significant association was found between HER2/neu expression and other clinicopathological characteristics, as shown in Table 2.

Table 2: Association of HER2/neu expression with clinicopathological characteristics.

Clinicopathological characteristic		Total No.	HER2/neu negative Frequency (%)	HER2/neu positive Frequency (%)	*P* value
Age	≥50 years	67	30 (44.7)	37 (55.3)	
	<50 years	36	18 (50)	18 (50)	0.61
Gender	Male	45	21 (46.6)	24 (53.4)	
	female	58	27 (46.5)	31 (53.5)	0.99
Tumor site	Right colon	34	13 (38.3)	21 (61.7)	
	Left colon	69	35 (50.7)	34 (49.3)	0.23
Tumor type	Non-mucinous	89	40 (44.9)	49 (55.1)	
	mucinous	14	8 (57.2)	6 (42.8)	0.39
Tumor grade	Well-moderately differentiated	85	44 (51.8)	41 (48.2)	
	Poorly differentiated	18	4 (22.2)	14 (77.8)	**0.02**
Nodal involvement	Negative	47	20 (42.5)	27 (57.5)	
	Positive	56	28 (50)	28 (50)	0.45
Tumor stage	Stage I and II	47	20 (42.6)	27 (57.4)	
	Stage III and IV	56	28 (50)	28 (50)	0.45

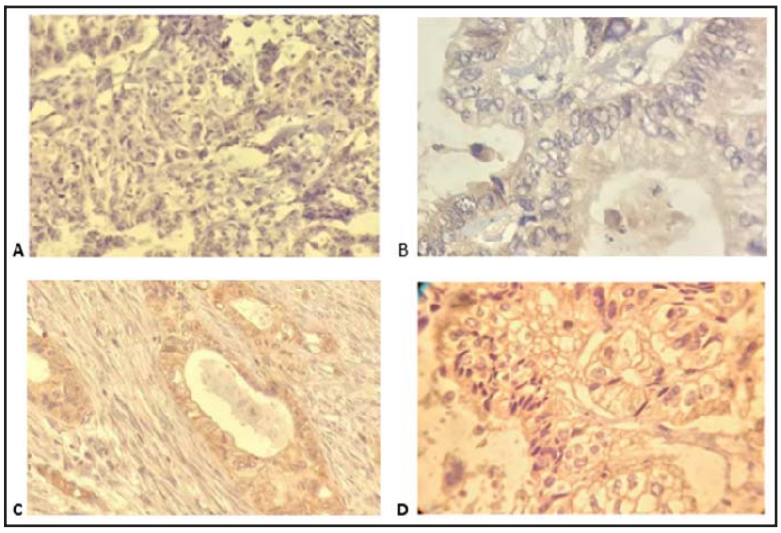

Figure 1: Showed four scores of HER2/neu immunoexpression: A- HER2/neu score zero (IHCx400). B- HER2/neu score 1+ (IHCx400). C- HER2/neu score 2+(IHCx400). D- HER2/neu score 3+(IHCx400).

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Discussion

Colorectal cancer develops because of the uncontrolled growth of cells in the large intestine.[17] It has been regarded as one of the most common cancers, and it is graded as the second cause of cancer mortality in the western world.[18] Its incidence increases annually by 2%.[19] In the colon cancer, HER2/neu expression is considered as a marker for poor prognosis. In colorectal cancer patients, it is utilized as a predictor for the patient's response to adjuvant chemotherapy.[20,21] The present study has shown that more than half of the cases (55 cases) (53.4%) were labeled as positive for HER2/neu, and 48 cases (46.6%) were labeled as negative. This result was a little bit higher than other studies, as shown in Table 3. The discrepancy in HER2/ neu expression in different studies might be due to the type and concentration of antibody that had been used, differences in the detection system, and differences in the method of HER2/neu scoring, or it may be due to genetic or environmental factors. In the present study, most of those who had positive HER2 expression tumors were older ones and aged more than 50 years. However, no statistically significant association was found. Many of the previous studies obtained the same results.[23,24,26,27] Comparable results were found in previous studies regarding gender,[2,28-30] that showed HER2/neu expression was more frequent among females than males (56.3% versus 43.7%), with no statistically significant association. The majority of the positive status HER2/ neu tumors of the present study were located in the left side colon and rectum (66.9%), while only (33.1%) located on the right side. However, no statistically significant association was found between them. our result is in close alignment with what has been observed by others.[22-24] In concordance with the present results that 86.4% were non-mucinous and 13.6% were mucinous, many studies found that non-mucinous tumor type shows highest

Table 3: HER2/neu immunoexpression results of previous research in comparison to the present study.

Study (year)	Location	No. of cases	HER2/neu status	
			Negative%	Positive%
Park et al. (2007)[22]	South Korea	137	52.6	47.4
Kavange et al. (2009)[23]	Ireland	106	90	10
Pappas et al. (2013)[24]	Greece	51	96.1	3.9
Seo et al. (2014)[14]	Republic of Korea	365	94	6
Tu et al. (2015)[25]	China	878	88.4	11.6
Meng et al. (2015)[26]	China	717	55	45
Torabizadeh et al. (2016)[27]	Iran	50	60	40
Suma et al. (2017)[19]	India	50	76	24
Current study(2018)	Erbil/Kurdistan	103	46.6	53.4

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HER2/neu positivity rate rather than the mucinous type, with no statistically significant association.[28,30,31] HER2/neu expression was positive in 55 cases, 82.5% of them were well to moderately differentiated tumors, while 17.5% of them were poorly differentiation ones. A significant association was found between positive HER2/neu expression and tumor grade, in which HER2/neu expression was more in well-moderately differentiated tumors than poorly differentiated ones (P = 0.02). Similar results were found by Madani and his colleagues, in which significant association was found between HER2/neu expression and tumor differentiation.[30] On another hand, in a study done by Lu and Hu, a significant association between HER2/neu expression and tumor grade had been detected, which was opposite to the present study. It showed that HER2/neu overexpression was more in poorly differentiated tumors than those well-differentiated ones.[32] The most likely reason for the differences in findings might be due to differences in HER2/neu positivity between the two studies, as in the current study, only 2+ and 3+ HER2/neu expressed tumors regarded as positive while in previous studies 1+, 2+ and 3+ HER2/neu expressions were regarded as positive. In addition, unlike to present study, other studies found no significant association between HER2/neu expression and tumor grade.[22,24,25] The current study indicated that the rate of HER2/neu overexpression in the patients who had presented with lymph node metastasis (54.4%) were slightly more than those who did not present with lymph node metastasis (45.6%). However, there was no statistically significant association with HER2/neu immunoexpression. Our results were in alignment with other studies.[1,2,22,25,26,30] Concerning the association of HER2/neu expression with the stage of the disease, although statistically non-significant, it was slightly more expressed in stages III and IV of the disease, that 45.6% were in stages I and II and 54.4% were in stages III and IV. This result was in agreement with other studies that found HER2/neu more expressed in the advanced stage of the disease without statistical significance.[14,28,31-33] Conversely, other studies showed that HER2/neu expressed more frequently in stage I and II of the disease rather than stage III and IV.[1,5,22,25,34] This wide range of HER2/neu expression and differences in association with the clinicopathological parameters among the different studies might be due to different scoring systems, variable technical issues in IHC methods, using different concentrations of antibody, and different IHC procedures that had been used to detect HER2/neu protein. In addition to discrepancies among the pathologists in the evaluation of HER2/neu expression scoring.

Conclusion

HER2/neu was frequently expressed in colorectal carcinoma, and it was expressed more in well-moderately differentiated tumors. It was significantly associated with tumor grade, while no significant association was observed with other clinicopathological parameters.

Competing interests

The authors declare no competing interests.

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