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Original Article

Evaluation of Expression of EGFR, HER-2 and COX-2 in Colorectal Cancer

Behrang Kazeminegad, Abbas Mirafsharieh, Freidoon Solhjoo

Dept. of Pathology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ABSTRACT

Background and Aims: **EGFR and HER-2 are two members of ERbB/HER family of Type I Transmembrane growth factor receptors. Cox2 is an enzyme responsible for the conversion of arachidonic acid to prostaglandins, which has a major role in angiogenesis and can modelate tumor growth. The aim of this study was to determine the level of expression of EGFR, HER-2 and Cox2 in colorectd cancer.**

Material and Methods: **IHC study was performed in paraffin-embedded blocks of 47 patients underwent colectomy due to colorectal cancer in Modarres Hospital, Tehran, Iran from 2008 to 2009. Three separated pathologists analyzed the slides after complete IHC staining for EGFR, HER-2 and COX-2.**

Results: **EGFR, HER-2 and Cox2 revealed over expression in colorectal cancer as 80.9%, 25.5% and 72.4% respectively, EGFR revealed no statistically significant association with clinicopathologic parameters, but Cox2 overexpression exhibited statistically significant association with higher stages tumors (III, IV) (*P* value: 0.037) and tumor with lymph node metastasis(*P*= 0.005). On the other hand, HER2 overexpression showed statistically significant association with lower grade (well and moderately differentiation) tumors (*P*= 0.042).**

Conclusion: **According to over expression of three markers, EGFR, HER-2, and COX-2 in colorectal cancers, using drugs that act against these receptors and investigation of survival improvement of patients with these drugs in other studies are recommended.**

Keywords: **EGF Receptor, HER-2 Proto-Oncogene Protein, Cox2 protein, Colorectal cancer**

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Address communications to: Dr Behrang Kazeminegad, Department of Pathology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: dkazeminezhad@gmail.com

Introduction

Colorectal cancer is one of the most common neoplasms (1) and affects men and women equally (1, 2). The mean age of incidence is 62 years (2). Despite the improved survival rates achieved with the use of newer antineoplastic agents, approximately 20% of stage 2 patients will die from recurrent disease, indicating the need to identify further targets and compounds as well as subsets of patients who can benefit from targeted therapy (1).

EGFR, HER-2, COX-2 participate in carcinogenesis of several tumors (3-5). The first two receptors (HER-2, EGFR) are the members of ERbB/HER family of type 1 transmembranous growth factor receptors and play a vital role in morphogenesis and epithelial organs' maintenance (3, 4). They also by affecting on differentiation, proliferation, migration, and apoptosis of tumor cells participate in carcinoma development and extension. COX-2 is the enzyme that converts arashidonic acid to prostaglandins and play an important role on angiogenesis and tumor growth (1, 5).

According to these molecules expression in colorectal cancer, some studies reported increased expression (1, 6-8), some unchanged expression (9) and some decreased expression (10) of them. In addition, there is contradictory information about expression of these molecules in different stages (2, 11) and grades (2) of colorectal carcinoma. Thus, some consider the difference of these markers with stage and grade changes to be significant (1, 8) and some consider it no significant (6, 7), which has reduced the importance of these markers in the prognoses of the diseases. In addition, some studies suggest that there are major molecular differences among different races tumors (12).

This information caused us to conduct this study to evaluate COX-2, HER-2 and EGFR expression and assessment of relation with clinicopathologic parameters affecting on prognosis.

Material and Methods

Patient tissue specimens

This study included patients who underwent colectomy due to colorectal cancer in Modarres Hospital, Tehran, Iran from 2008 to 2009. There were 47 patients that their demographic and clinicopathologic information using pathology report and studying the slides by three separated pathologists were collected and confirmed. Staging and grading of patients had been performed by means of pathology reports, review of the archived slides, and were based on American Joint Committee on cancer / international union against cancer. All demographic and clinicopathologic information can be seen in Table 1.

IHC were performed with standard envision method and by means of these antibodies, (all were DAKO manufactured): EGRR (RTT-EGFR-384), HER-2(code-Nr.AQ485), COX-2(clone CX-294). Positive control of EGFR using skin tissue was prepared according to manufacture recommendation. In addition, breast carcinoma slices with IHC +3 staining for HER-2 and COX-2 were used as positive controls (with cytoplasmic and membranous staining, respectively) and the slides, which their primary antibodies were deleted, were used as negative control for all three markers.

Immunohistochemical evaluation

Three separated pathologists analyzed the IHC slides by light microscopy without knowledge of patient results. For EGFR and HER-2, membranous and for COX-2, cytoplasmic staining was considered positive and staining intensity were classified according to literature and valid references (1, 2) as follows: for COX: 0: without staining, +1: poorly staining, +2: moderately staining, +3: strong staining (Table 1). For EGFR as positive (presence of staining) and negative (absence of staining), and for HER-2 classification were based on the method as used for breast carcinoma. Finally, for COX-2

and HER-2 cases of 0 and +1 were considered as negative and +2 and +3 as positive (1). If there was a contrast idea among pathologists (two pathologists positive and one pathologist negative or vice versa) idea of majority were considered.

Table 1- Grading of the immunohistochemical staining for HER-2/ neu overexpression

Staining pattern	Score	Her2/ neu protein overexpression assesment
No staining is observed or membrane staining is observed in less than 10% of the tumor cells	0	Negative
A faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane	+1	Negative
A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells	+2	Weakly positive
A strong complete membrane staining is observed in more than 10% of tumor cells	+3	Strongly positive

Statistical analysis

Statistical data analyses were conducted in two forms of descriptive and analytic statistics Chi square test, Exact Fisher test, and Kruskal Wallis.

Results

After completion of IHC, normal tissue was 100% negative for three makers. Results and their relation to different clinicopathological parameters are summarized in Table 2.

After completion of above statistical tests it was determined that COX-2 expression in high stages (III, IV) was significantly higher than low stages (I,II) (P value: 0.037) (Fig. 1). In addition, the expression of this marker had significant increase in tumors with lymph node metastases (P= 0.005) (Fig 2). Thus, it was found for HER-2 that the expression was significantly higher in low grades (I, II) than high grades (III) (P= 0.042) (Fig. 3).

There was no significant correlation between COX-2 expression with grade of tumor and HER-2 with stage of diseases and other clinicopathological parameters (age, sex, vascular invasion, tumor type, and tumor position).

EGFR expression had no significant correlation with any clinicopathological parameters.

Discussion

The expression and clinical importance of EGFR, HER-2 and COX-2 in colorectal cancers is controversial. Different causes have been mentioned for this dispersion in markers expression rate, which one of them is different evaluation techniques (IHC, PCR ...), even in IHC cases using monoclonal antibodies, use of different monoclonal antibodies can be the cause of differences in the results (13). In addition, mutations in different domains of extracellular structure, inter cellwall or intra cellular tyrosine kinase receptors (EGFR, HER-2) (14) are other causes mentioned. The other cause of these differences, described by Soliman *et al*, is race differences in colorectal cancers. They found that colon cancers in Egypt in com-

Table 2- EGFR, HER-2 and COX-2 expression in relation to some clincopathological parameters in patients underwent colectomy in Modarres Hospital between 2008 and 2009 (In case of HER-2 and COX-2 +2 and +3 were considered positive) (Total percentages is written in parenthesis)

Marker type		EGFR		HER2		COX2	
		-	+	-	+	-	+
Condition		Number of specimen(%)	Number of specimen(%)	Number of specimen(%)	Number of specimen(%)	Number of specimen(%)	Number of specimen(%)
SEX	Female	7(77.78)	18(47.37)	16(50.00)	9(60.00)	5(41.67)	20(57.14)
	Male	2(22.22)	20(52.63)	16(50.00)	6(40.00)	7(58.33)	15(42.86)
Stage	I	4(44.44)	15(39.47)	13(40.63)	6(40.00)	7(58.33)	12(34.29)
	II	2(22.22)	10(26.32)	8(25.00)	4(26.67)	4(33.33)	8(22.86)
	III	3(33.33)	11(28.95)	9(28.13)	5(33.33)	0(0.00)	14(40.00)
	IV	0(.00)	2(5.26)	2(6.25)	0(0.00)	1(8.33)	1(2.86)
Grade	Well	5(55.56)	27(71.05)	20(62.50)	12(80.00)	9(75.00)	23(65.71)
	Moderate	1(11.11)	6(15.79)	4(12.50)	3(20.00)	2(16.67)	5(14.29)
	Poor	3(33.33)	5(13.16)	8(25.00)	1(8.33)	7(20.00)	
Angioinvasive	-	5 (55.56)	29(76.32)	23(71.88)	11(73.33)	10(83.33)	24(68.57)
	+	4(44.44)	9(23.68)	9(28.13)	4(26.67)	2(16.67)	11(31.43)
Lymph node metastasis	-	6(66.67)	26(68.42)	22(68.75)	10(66.67)	12(100.00)	20(57.14)
	+	3(33.33)	12(31.58)	10(31.25)	5(33.33)	0(0.00)	15(42.86)
Type	Non-mucinous	8(88.89)	30(78.95)	24(75.00)	14(93.33)	11(91.67)	27(77.14)
	Mucinous	1(11.11)	8(21.05)	8(25.00)	1(6.67)	1(8.33)	8(22.86)
Location	Rectosigmoid	6(66.67)	23(60.53)	22(68.75)	7(46.67)	9(75.00)	20(57.14)
	Left Colon	2(22.22)	5(13.16)	3(9.38)	4(26.67)	2(16.67)	5(14.29)
	Transvers Colon	0(0.00)	4(10.53)	2(6.25)	2(13.33)	0(0.00)	4(11.43)
	Right Colon	1(11.11)	6(15.79)	5(15.63)	2(13.33)	1(8.33)	6(17.14)

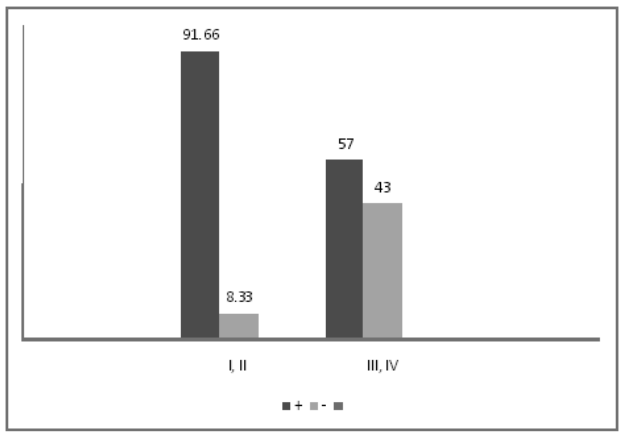

Fig. 1- Comparison of COX -2 abundance according to high stages (III, IV) and low stages (I, II) tumors

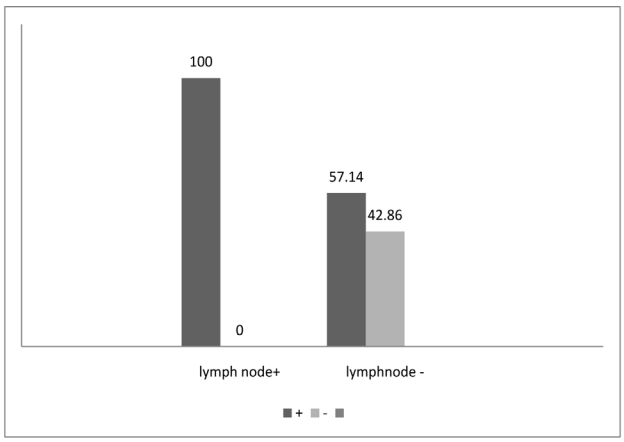

Fig. 2- Comparison of COX-2 abundance according to presence of absence of lymph node metastasis

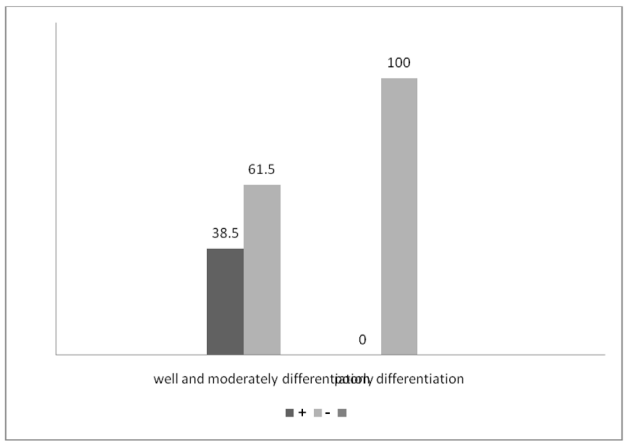

Fig. 3- Comparison of abundance percentage of HER2 according to well, moderately (grades I , II) and poorly differentiation (grade III)

parison to west patients were less differentiated and showed higher levels of microsatellite instability and K-ras mutation (12). According to mentioned information, it is not surprising that there is controversy about the relation between marker expression and clinicopathological characteristics.

In addition, in our study, all three markers of EGFR HER-2 and COX-2 had over expression (for tumor tissues by 80.9%, 31.9%, and 74.5% respectively). Yet, it was seen that there was significant relation between COX-2 expression with higher stages (III, IV) tumors and with tumors with lymph node metastasis. On the other hand, there was significant relation between HER-2 over expression and tumors with well differentiation (grades I, II) that is corresponded to the results of previous studies (1). In the case of HER-2 lesser expression in tumors with poorly differentiation (which is significantly different from differentiated tumors), one possible cause mentioned is over expression of histon dacetylases in poorly differentiated adenocarcinomas that decreases histon acetylation and then HER-2 expression (15). To confirm this, in one study with the use of histon deacetylase inhibitors in breast cancers can promotes differentiation in cell lines (16). Further investigations are need to further confirmation of this issue in colorectal cancers.

Conclusion

According to over expression of three markers, EGFR, HER-2, and COX-2 in colorectal cancers and according to need for new treatments in these patients, we can use drugs available that act against these receptors, enzymes and histon deacetylases. In addition, clearly, investigation of survival improvement of patients with these drugs need more studies in this regard.

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Tehran, Iran. The authors declare that there is no conflict of interests.

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