Original Article

Immunohistochemical Expression of Epidermal Growth Factor Receptor in Human colorectal Adenocarcinoma (A clinicopathological study)

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Abstract:

Background: Colorectal Adenocarcinoma contributes one of the most common malignancies and the second leading cause of death from cancer in the western world. Epidermal growth factor receptor (EGFR) belongs to a family (ErbB-tyrosine receptors), EGFR plays an important role in the pathogenesis of colorectal cancer.
Objectives: to evaluate the immunohistochemical expression of epidermal growth factor receptors (EGFR) in colorectal adenocarcinoma and to correlate this expression with different clinicopathological parameters.
Pateints and method: In this study clinicopathological parameters of twenty five colorectal adenocarcinoma cases diagnosed in private pathology laboratories in Baghdad /Iraq from November 2012 to September 2013 were respectively evaluated in terms of age, gender, pathological diagnosis including; tumor location, lymph node status. EGFR expression was investigated immunohistochemically.
Results: twenty five colorectal cancer patients were included in this study with median age 54.5, range from (28-81)years, 15 cases (60%) were female and 10 (40%) cases were male. Tumor size range from 3-10 cm with mean 6.5 cm, 10 (40%) cases were from rectum, 7 (28%) from the right side colon & 8 (32%) cases were from from left side colon. Twenty three (92%) cases were moderately differentiated, and two (8%) cases were poorly differentiated, five (20%) cases were T1, 10 (40%) cases were T2, 5 (20%) cases were each T3 & T4 respectively. 3 (12%) cases were N1, 7 (28%) cases were N2, and fifteen (60%) cases have no lymph node involvement. Three (12%) cases with distant metastasis. Eighteen (72%) cases of colorectal adenocarcinoma demonstrate EGFR reactivity in > 1% of the tumor cells. No significant statistical correlation was noticed between EGFR expression and each of age, gender, site of the tumor and grade of tumor (P value > 0.05). A significant statistical correlation was noted between EGFR expression and local tumor invasion (T) and lymph node involvement (p value <0.05).
Conclusion: Epidermal growth factor receptor plays an important role in colorectal adenocarcinoma oncogenesis. EGFR expression appears to have a value as a prognostic biomarker, since it's expression by the tumor cells is significantly correlated to lymph node involvement and tumor local invasion.
Keywords: Colorectal Adenocarcinoma, EGFR

Introduction:

Colorectal cancer is one of the most common malignancies and the second leading cause of death from cancer in Europe and North America. It is responsible for approximately one million new cases and half million death per year wold wide (1). The ErbB family of receptors tyrosine kinases comprises ErbB1,2,3 and 4. Erb B1 also known as EGFR, is a typical member of the Erb Bfamily stimulated upon ligand binding (2,3). EGFR catalyse the transfer of phosphate molecules from ATP to an active site of tyrosine kinase to mediate signals, triggering a cascade of well- identified molecular events that will protect cells from apoptosis, facilitate invasion and promote angiogenesis reaction (4+5). Enhanced activity or overexpression of EGFR has been found to be associated with tumor progression and poor survival in various malignancies such as head and neck (6), lung(7) , breast (8) , gastrointestinal tract (9) , and bladder cancer(10). There is a clear evidence that EGFR plays an important role in pathogenesis of colorectal carcinoma. EGFR is widly present in advanced colorectal carcinoma, its expression ranges from 72% to 80%, in recent published series (11,12). Moreover, EGFR expression appears to be associated with poor survival and increased risk of invasion and metastasis (13). This study aims to evaluate the immunohistochemical expression of EGFR in colorectal carcinoma and to correlate this expression with different clinicopathological parameters.

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Pateints and methods:

This study is retrospectively designed, a total of 25 cases of colorectal adenocarcinoma diagnosed in private pathology laboratories in Baghdad/ Iraq, in the period from November 2012 to September 2013, all cases were evaluated in terms of age, gender, pathological diagnosis, including tumor size, location, grade, lymph nodes status and TNM staging system. Immunostaing for EGFR was performed using the immunohistochemical system kit EGFR pharmaDx (Dak0 cytomation, Carpinteria, Ca, USA), on freshly cut, formalin fixed, paraffin embedded tissue from patients with colorectal carcinoma and positive control cases were used squamous epithelium from skin (stained membraneous and cytoplasmaic)

EGFR status evaluation: Sections were analysed using light microscopy by two observers, positivety for EGFR expression was defined as any membranous staing above background level. The tumor was considered positive when ≥ 1% of the tumor cells had memberanous staining. Cytoplasmic staining with out associated membrane staining was reported as negative.

The intensity of EGFR reactivity in the adenocarcinoma cells was scored as in the following system:-

1+ (weak intensity): faint brown membraneous staining.

2+ (moderate intensity):brown membraneeous staining of intermediate darkness producing complete or incomplete circular outline of the neoplastic cells.

3+ strong intensity: dark brown or black memberanous staining producing a thick outline, complete or in complete on the outline of the neoplastic cells.

The level of EGFR expression was expressed according to the percentage of the cells stained with weak, moderate, intense as follow:1-20%, 20-50%, and > 50%(16).

Results:

Twenty five colorectal cancer pateints comprised the study population. Pateint's characteristics are described in table 1. The median age is 54.5 years, with a range between (28-81). Ten (40%) cases were male and 15 (60%) cases were female. Tumor size ranges from 3-10 cm, with a mean 6.5 cm. Ten (40%) cases were from the rectum, 7 (28%)cases were from right-side colon, 8 (32%) cases were from left-side colon. Twenty three cases (92%) cases were moderately differentiated, two (8%) cases were poorly differentiated. Regarding the pathological staging, TNM staging system of American Joint cancer comity (AJCC) 2010 was applied to all tumors:

T1 5(20%) cases, T2 10(40%) cases, each T3 & T4 5 (20%) cases.

Fifteen cases (60%) have no lymph node involvement, 3 (12%) cases were N1(up to three lymph nodes involved) & 7 (28%) cases were N2 (more than 3 lymph nodes involved).

Regarding distant metastasis: 3(12%) cases had liver metastasis.

Table 1: Patients characteristics:

Characteristics		Number Percentage %
Age at diagnosis	Years Median 54.5 Range 28-81	
Sex	Male	10 (40%)
	Female	15 (60%)
Tumor size (cm)	Median 6.5 cm 3-10 cm	
Tumor location	Rectum	10 (40%)
	Right side colon	7 (28%)
	Left side colon	8 (32%)
Differentiation of tumor	Moderately differentiated	23 (92%)
	Poorly differentiated	2 (8%)
TNM	T1	5 (20%)
	T2	10 (40%)
	T3	5 (20%)
	T4	5 (20%)
	N0	15 (60%)
	N1	3 (12%)
	N2	7 (28%)
	M0	22 (88%)
	M1	3 (12%)

EFGR reactivity in colorectal carcinoma:

Overall 18 (72%) cases of colorectal carcinoma demonitrate EGFR reactivity in >1% of the tumor cells (table 2). Nine (36%) cases had expression in 1-20% of the cells (figure No 1), 5 (20%) cases had expression in 20-50% of the cells , 4(16%) had expression in > 50% of the cells. Six cases had +3 intensity reactive cells (figure No 2)

Table 2: Reactivity of EGFR:

EGFR reactivity	No of cases	Percentage %
< 1%	7	28%
1-20%	9	36%
20-50	5	20%
>50%	4	16%
total	25	100%

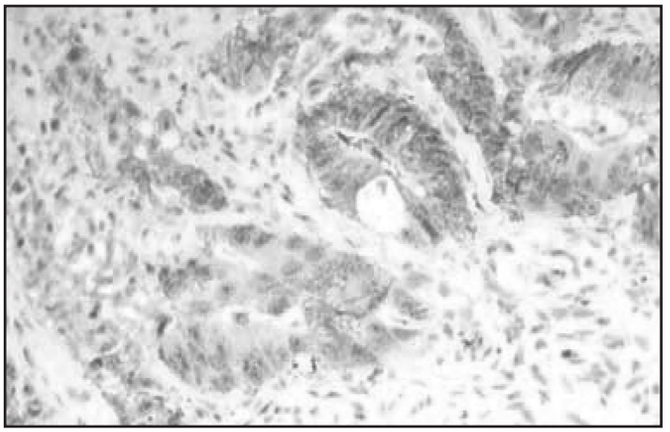

Figure no 1: Colorectal adenocarcinoma, membrane staining, 1+ staining intensity (20X)

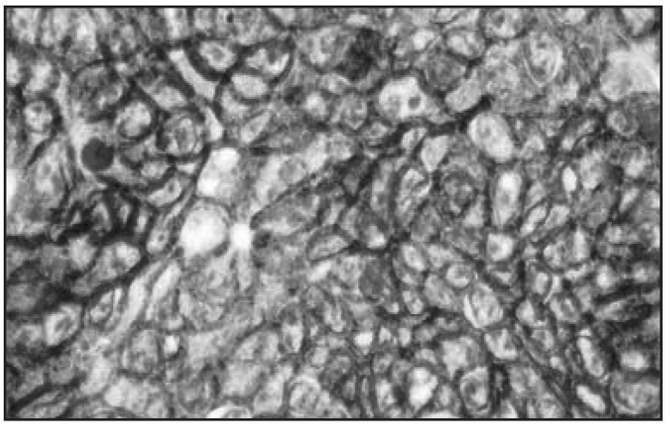

Figure no 2: Colorectal adenocarcinoma, membrane staining, 3+ staining intensity (40X)

Table (3) shows expression of EGFR in relation to clinicopathological parameters. Eight (32%) male cases and 10 (40%) female cases are positive for EGFR. No statistical correlation was observed between EGFR expression and the gender, P value > 0.05. Eight (32%) cases expressed EGFR located in rectum, & 5 (20%) cases located in the right and left colon respectively,No significant relation was noticed between location of the tumor and expression of EGFR, P value > 0.05 (NS). Sixteen (64%) cases expressed EGFR were moderately differentiated and 2(8%) cases were poorly differentiated. No significant correlation between EGFR expression and grade of tumor , P value > 0.05 (NS). Eight (32%) cases expressed EGFR have no lymph node involvement (N0), 3 (12%) cases expressed EGFR were N1, 7 (28%) cases were N2. There was a significant statistical relation between lymph node involvement by malignancy and EGFR expression, P value < 0.05 (S). None of the 5 (20%) cases of T1 expressed EGFR, 8 (32%) cases were T2 expressed EGFR, 5 (20%) cases including all T3 and T4 cases (each) expressed EGFR. A significant statistical relation was noticed between tumor extend (TNM staging system) and EGFR expression, P value < 0.05 (S).

Table(3)Expression of EGFR in relation to clinicopathological parameters.

EGFR expression	Parameter		P value
	Male	Female	
Positive	8 (32%)	10 (40%)	P > 0.05 NS
Negative	2 (8%)	5 (20%)	
total	10 (40%)	15 (60%)	

EGFR rexpression	Site			P value
	Rectum	Right colon	Left colon	
Positive	8 (32%)	5 (20%)	5 (20%)	P > 0.05 NS
Negative	2 (8%)	2 (8%)	3 (12%)	
total	10 (40%)	7 (28%)	8 (32%)	

EGFR rexpression	Nodal Involvement			P value
	N0	N1	N2	
Positive	8 (32%)	3 (12%)	7 (28%)	P < 0.05 S
Negative	7 (28%)	0	0	
total	15 (60%)	3 (12%)	7 (28%)	

EGFR expression	Grade		P value
	Moderatelly differentiated	Poorly Differentiated	
Positive	16 (64%)	2 (8%)	P > 0.05 NS
Negative	7 (28%)	0	
total	23 (92%)	2 (8%)	

EGFR rexpression	Tumor invasion (T)				P value
	T1	T2	T3	T4	
Positive	0	8 (32%)	5 (20%0	5 (20%)	P < 0.05 S
Negative	5 (20%)	2 (8%)	0	0	
total	5 (20%)	10 (40%)	5 (20%)	5 (20%)	

Disscussion:

Colorectal cancer is one of the most frequent occurring cancers in human(1). Activation of specific proto-oncogens such as KRAS by point mutation and inactivation of tumor suppressor genes like P53, DCC (deleted in colorectal cancer) and MCC (mutated in colorectal cancer)occurring during the progression of normal colonic epithelium to invasive carcinoma(14). Epidermal growth factor receptor belongs to a family of receptors known as Erb B tyrosine Kinase receptors which comprises four proteins encoded by the c-erbB proto-oncogens (2+3). EGFR has been shown to be overexpressed in colorectal cancer patient population but its positive value remains unclear (15). Currently by quantitative immunohistochemical detection scoring system has approved to evaluate EGFR expression in colorectal cancer patients (16). Possible relation of association between expression & overexpression of EGFR and other clinicopathological parameters in colorectal cancer remains unclear (15). In our study the overall expression of EGFR was in 18 (72%) cases, other studies showed its expression ranging from 72% to 82% (11,12). In this study the expression of EGFR has been studied in relation to the patient age, gender and results showed no significant correlation, these results was comparable to other published studies (15,16,17).In this study no siognificant correlation was demonstrated between EGFR expression and the site of the tumor, this finding was consistent with many published studies (16,18), only one study showed highr EGFR expression in cancers of the distal colon than rectal cancer (19). Some authers suggested that the higher levels of EGFR in the left side colon as opposed to right side could be related to different molecular mechanism between the two sites (20). Regarding our results, no significant statistical relation was observed between EGFR and degree of tumor differentiation (grade), in comparing with others, the results apper to be controversial with some studies showing a link (21) and other showing no relationship (16+17). A significant relation was noticed in this study between EGFR expression & tumor invasion (T) and lymph nodes involvements (N), these results were comparable with other studies that reported expression was correlated with more aggressive disease (22) and increase risk of metastasis (23), and advanced tumor stage (24) with higher rate of lymph node involvement (25).

Goldstein et al (16) reported that EGFR reactivity was higher in both lymph node and metastasis than in primary tumor.

Conclusiuon:

EGFR plays an important role in colorectal cancer oncogenesis, EGFR expression appears to have value as a prognostic biomarker, since its expression by tumor cells is significant correlated with lymph node involvement and tumor invasion

Auther's contributions:

Kifah hamdan : cases collections and study of acses.
Khitam razzak: IHC staing and interpretation of the results.
Sazan Abdulwahab: analysis of the data and writing

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