ORIGINAL RESEARCH PAPER

Pathology

KEY WORDS: EGFR (Epidermal Growth factor Receptor), IHC (Immunohistochemistry), H&E (Hematoxylin and Eosin stain), CRC (Colorectal carcinoma)

ROLE OF EPIDERMAL GROWTH FACTOR RECEPTOR IN COLORECTAL CARCINOMA

Dr Monika Aggarwal MD(Pathology), Assistant professor, Dept. of Pathology, Base Hospital, Delhi Cantt, New Delhi.

Dr VS Srinivas* Consultant pathology, Comdt, Military Hospital, Secundrabad. *Corresponding Author

Dr VS Nijhawan Prof and HOD. Dept. of Pathology, MMIMSR, Mullana, Ambala.

Dr Vibha Dutta MD (Path), PhD (AIIMS Delhi), MIAC Director & CEO, All India Institute of Medical Sciences, Nagpur

ABSTRACT

Colorectal carcinoma has etiopathogenesis related to both environmental and genetic factors. Epidermal growth factor receptor (EGFR) and its downstream signaling pathways regulate the key cellular events that drive the progression of many neoplasms. Thirty cases of colorectal carcinoma (CRC) were studied using H&E stain for morphology, grade and stage and further IHC was done for EGFR mutations. Our study found that the poorly differentiated adenocarcinoma and moderately differentiated adenocarcinoma showed stronger EGFR staining as compared to well differentiated adenocarcinoma. So, higher the grade of tumor, stronger is the EGFR positivity. EGFR expression detection by IHC is a sensitive and specific marker in CRC. The findings of positivity will help in guiding the therapy and better survival in patients of CRC.

INTRODUCTION

Colorectal carcinoma is one of the leading causes of death.[1] Adenocarcinoma of colon and rectum is most common (>90%). The etiopathogenesis of colorectal cancer is related to both environmental and genetic factors. Epidermal growth factor receptor (EGFR) and its downstream signaling pathways regulate the key cellular events that drive the progression of many neoplasms. EGFR belongs to a family of receptors known as ErbB family (tyrosine kinase receptors). It is a 170kDa transmembrane protein composed of an intracellular tyrosine kinase domain, a transmembrane lipophilic segment and an extracellular ligand binding domain.[2] In normal cells, the EGFR signalling cascade begins with ligand activation of EGFR. After binding its ligand, EGFR is known to homodimerize, then transphosphorylate several tyrosine kinase domains and thus to initiate intracellular EGFR signalling pathway. Two main intracellular pathways activated by EGFR are the mitogen activated protein kinase (MAPK) pathway and the phosphatidylinositol 3-kinase-(PI3K-) protein kinase B (AKT) pathway, inducing the transcription of genes involved in cellular processes.[3,4] These pathways lead to the activation of various transcription factors that then impact cellular responses such as proliferation, migration, differentiation, and apoptosis. Importantly, these two axes are closely related and have some overlap. When this tightly regulated system goes awry, it can contribute to malignant transformation and tumor progression through increased cell proliferation, prolonged survival, angiogenesis, anti-apoptosis, invasion, and metastasis. Mutations, gene amplification and overexpression of protein not only contributes to carcinogenesis but also impact prognosis and provide specific targets for therapeutic intervention. EGFR is expressed and is found to be associated with tumor progression and poor survival in various tumors such as gliomas, carcinomas of the lung, colon, head and neck, pancreas, breast, ovary, bladder, and kidney.

We studied the EGFR mutation using immunohistochemistry to detect EGFR expression in CRC. IHC is highly dependent on the antibody clone that is used, staining protocols, selection of scoring methods and cut-off values. However, in spite of these drawbacks, EGFR testing using IHC continues to be the ideal method of testing for these mutations. In the molecular testing of EGFR gene mutations in carcinoma, seven exons have been amplified using the polymerase chain reaction and mutations in these seven exons have been analysed. However, the method is cumbersome and there is always a chance that some mutations may be missed. Therefore, looking for the EGFR protein expression by IHC is a better, faster and more efficient method.

AIM of this study was to study about the expression of EGFR with the grade and stage of adenocarcinoma of colon on H&E stain.

MATERIALS AND METHODS.

This study selected both prospective and retrospective diagnosed cases of colorectal carcinoma in a tertiary care Hospital (Malignant disease treatment centre) after ethical clearance to assess the role of EGFR mutation in cases of CRC over a period of two years. Total of 30 cases were studied. Blocks and tissues of confirmed cases of CRC from patients who underwent colectomy with lymph node sampling have been included. However, Melanoma, mesenchymal tumors, carcinoids and other tumors of colon and rectum were excluded. Patients with metastatic CRC, who were already treated and presented with a second colorectal malignancy and metastatic tumors to colon from other viscera were also excluded.

METHODOLOGY.

Formalin fixed paraffin embedded (FFPE) blocks of colorectal carcinoma were selected for HPE evaluation and Immunohistochemistry (IHC) for EGFR. H&E stained slides were evaluated and graded as per TNM staging of the CRC. Lymph node sections were reassessed for the metastasis.

EGFR was studied by IHC protocol using Avidin-Biotin peroxidase method. Polyclonal rabbit anti-EGFR was used made by BioGenex laboratories, USA. This antibody is for specific localisation of EGFR in FFPE tissue sections.

STASTICAL ANALYSIS.

Results of conventional histologic prognostic markers (grade and stage) were correlated with proliferation markers (EGFR positivity). Relevant clinical data was collected for all cases included in the study. The data generated was noted and excel sheet was generated to analyze the data. The data for EGFR was combined into two grades based on staining intensity i.e., weak staining and strong staining. The data was analysed using stastical software SPSS for windows (Version 17). Chi-

square test was applied and p-value of ≤0.05 was considered significant.

RESULTS .

In our study, the age group of the study subjects was between 43 to 84 yrs with the mean age of 63 years. Among these 30 patients, 26 (86%) were males and 04 (14%) were females. Out of these cases, 10 (34%) cases presented with symptoms of intestinal obstruction and remaining 20 cases had symptoms of change in bowel habits, anemia and vague abdominal pain. Total of 20 cases (66%) had involvement of the left side of colon, 02 (06%) involved transverse colon and 08 patients (28%) had right-sided involvement. Grossly, 12 cases were of annular growth and 10 cases had ulceroproliferative growth on left side of colon while 08 cases on the right side were ulceroproliferative in nature.

Histopathological grade.

The grade was assessed on H&E stain and graded into well differentiated, moderately differentiated and poorly differentiated adenocarcinoma.

Figure 1 : Distribution of cases according to WHO grade of CRC(n=30)

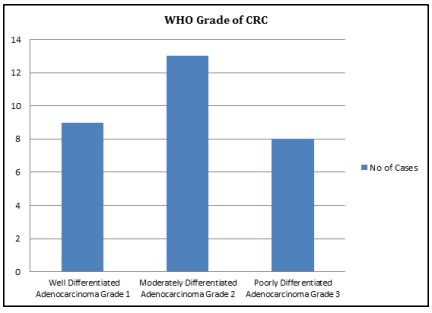
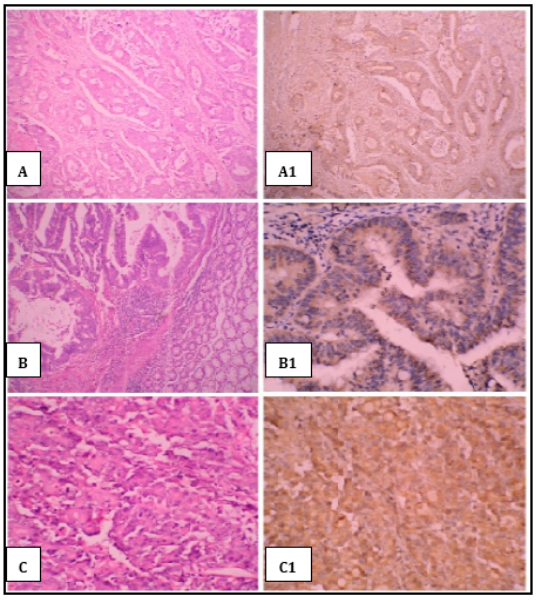


Fig 1 shows that distribution of cases as per grading of tumors (WHO Grade) based on glandular architecture and differentiation, predominantly into three grades : Well differentiated (Grade 1) 09 (30%), Moderately differentiated (Grade 2) 13 (43%) and Poorly differentiated (Grade 3) 08 (27%) cases.

Grade (H&E) and EGFR staining. Staining intensity of EGFR protein overexpression was studied with the grade of tumor on H&E stain.

Figure 2 : Photomicrograph showing Grade and EGFR in CRC cases



Legend :

(A) HPE showing Well Differentiated Adenocarcinoma,
(A1) EGFR 1+ positivity.
(B) HPE showing Moderately Differentiated Adenocarcinoma,
(B1) EGFR 2+ positivity.
(C) HPE showing poorly Differentiated Adenocarcinoma,
(C1) EGFR 3+ positivity.

Association of EGFR and Histopathological Grade. The grade of EGFR immunostaining was assessed and categorised into four grades based on membranous positivity of the malignant epithelial cells, arranged in glandular pattern as well as in singly scattered infiltrating tumor cells. The staining was assessed, scored as 0, 1+, 2+, 3+ and compared with the histopathological grade of the tumor.

Table No 1 : Grade and EGFR staining intensity

Grade	EGFR				Total
	0	1+	2+	3+	
Well Differentiated Adenocarcinoma - Grade 1	2(7%)	3(10%)	3(10%)	1(3%)	9(30%)
Moderately Differentiated Adenocarcinoma - Grade 2	1(3%)	0(0%)	5(17%)	6(20%)	12(40%)
Poorly Differentiated Adenocarcinoma - Grade 3	0(0%)	0(0%)	3(10%)	6(20%)	9(30%)
Total	**3(10%)**	**3(10%)**	**11(37%)**	**13(43%)**	**30(100%)**

Table No 1 showed an association among the EGFR staining intensity and grade of the tumor. For stastical analysis, EGFR staining was grouped into two categories - Weak staining(0&1+) and Strong staining (2+&3+). The stastical analysis was done using Chi-square test, it was found that the poorly differentiated adenocarcinoma and moderately differentiated adenocarcinoma showed stronger EGFR staining as compared to well differentiated adenocarcinoma. So, higher the grade of tumor, stronger is the EGFR positivity. The results were found to be stastically significant with a p value of 0.006 (<0.05).

Association of EGFR with Grade and Stage of CRC. The higher the grade of the tumor, more advanced is the stage and stronger is the EGFR staining intensity.

Table No 2 : Grade, Stage and EGFR

Stage	Grade	EGFR				Total
		0	1+	2+	3+	
pT1N1	Well Differentiated Adenocarcinoma				1	1
	Moderately Differentiated Adenocarcinoma				1	1
pT2N0	Moderately Differentiated Adenocarcinoma	1			1	2
pT2N2	Poorly Differentiated Adenocarcinoma				1	1
pT2N2a	Moderately Differentiated Adenocarcinoma				1	1
pT3N0	Well Differentiated Adenocarcinoma			1	0	1
	Moderately Differentiated Adenocarcinoma			5	0	5
	Poorly Differentiated Adenocarcinoma			1	2	3
pT3N1	Well Differentiated Adenocarcinoma			2		2

pT3N1b	Well Differentiated Adenocarcinoma		1			1
pT3N1c	Well Differentiated Adenocarcinoma	1			0	1
	Moderately Differentiated Adenocarcinoma	0			1	1
pT3N2	Well Differentiated Adenocarcinoma		1		0	1
	Moderately Differentiated Adenocarcinoma		0		2	2
	Poorly Differentiated Adenocarcinoma		0		2	2
pT3N2a	Poorly Differentiated Adenocarcinoma				1	1
pT3N2b	Well Differentiated Adenocarcinoma		1			1
pT4aN0	Poorly Differentiated Adenocarcinoma			1		1
pT4bN1	Well Differentiated Adenocarcinoma	1				1
pT4N1	Poorly Differentiated Adenocarcinoma			1		1
Total	**Well Differentiated Adenocarcinoma**	**2**	**3**	**3**	**1**	**9**
	Moderately Differentiated Adenocarcinoma	**1**	**0**	**5**	**6**	**12**
	Poorly Differentiated Adenocarcinoma	**0**	**0**	**3**	**6**	**9**
	Total	**3**	**3**	**11**	**13**	**30**

Table No 2 showed the stage, grade and EGFR positivity association. The higher the grade of the tumor, more advanced is the stage and stronger is the EGFR staining intensity.

DISCUSSION.

In our study, the age group of the study subjects was between 43 to 84 yrs with the mean age of 63 years. In our study, there was statistically significant association between age and incidence of CRC. Our findings are compatible with similar study done by Eddy DM et al [5], Cunningham D [6] as well as database on incidence of CRC is Surveillance, Epidemiology, and End Results (SEER) Program released in April 2010.[7] Out of 30 cases - 12 cases were of annular growth and 10 cases had ulceroproliferative growth on left side of colon while 08 cases on the right side were ulceroproliferative in nature. Our findings are stastically significant with other studies that most of the colorectal carcinomas are of ulceroproliferative type on the right side and that annular growths are more common on the left side.[8,9,10,11] Our study has shown that the patients of CRC have increased EGFR expression as the grade of tumor increases. There are various other studies on EGFR levels in CRC and show similar results as our study. A study done by Jinru Shia et al has shown that EGFR expression is present in approximately 60–80% of CRC and the receptor has emerged as a rational target for anticancer therapy in these tumors. [12] A study done by SiShi L et al has shown that EGFR and HER2/Neu levels are frequently elevated in colon cancer cells. [13] EGFR and/or HER2/Neu elevation play an important role in the development of the majority of colon cancers, and targeting EGFR and/or HER2/Neu could serve as an effective common strategy for therapeutic intervention or prevention of colon cancer. A review done by Santinia D et al about the study of monoclonal antibodies against EGFR represent one of the most important recent advancements in the treatment of colorectal cancer. [14] A review article by Hindwai et al on EGFR Signaling in CRC has shown that EGFR expression typically determined by IHC is associated with CRC and further with a faster progression and poorer prognosis of cancer. Our study and other studies showed that if we could perhaps block the EGFR pathway in CRC patients we could alter course of the disease favourably. The EGFR gene mutation, amplification and protein overexpression have been shown to contribute to colorectal carcinogenesis. [3] A number of biomarkers have been evaluated in their potential to predict the response to anti-EGFR-based therapies. These include marker related to EGFR amplification, activation and phosphorylation, EGFR polymorphisms, but also markers related to Ras/Raf/MAPK and the PI3K/Akt signalling pathways and angiogenesis. [15]

CONCLUSION.

We studied the role of EGFR protein overexpression in colorectal carcinoma and its usefulness in day to day practice. We found significant correlation between the grade of EGFR staining in the malignant epithelial cells showing higher positivity than non-neoplastic tissues. Also, there was significant correlation between the score/grade of EGFR staining in the malignant epithelial cells with the positivity percentage status.

We found that EGFR expression detection by IHC is a sensitive and specific marker in CRC. The findings of positivity will help in guiding the therapy and better survival in patients of CRC. EGFR has been shown to be overexpressed in patients of CRC. The development of a panel of EGFR inhibitors could reduce the proliferation of tumor cells when used alone or in combination with cytotoxic drugs or radiation. Regarding all these findings, the identification of inhibitors of the different EGFR signalling pathways in a new therapeutic strategies setting could contribute to reduced tumor growth by restoring the pro-apoptotic mechanisms and by reducing cell proliferation. Our study strengthens the results of previous studies highlighting the detection of EGFR as an important prognostic marker in CRC. As EGFR has proved useful in predicting the therapeutic outcomes as well as response to therapy, its further usefulness needs to be assessed based on multi-institutional studies with larger sample sizes. Further research needs to be carried out on this concept to reap net therapeutic benefits.

Limitation of the study.

Our study has limited number of cases and more cases with multicentric studies can be done to further strengthening the results.

Conflict of interest.

There is no conflict of interest.

REFERENCES

1. Ferlay J, Sin HR, Bray F, , Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer.2010; 127:2893-917.
2. Hynes NE and Lane AH. ERBB receptors and cancer: the complexity of targeted inhibitors. Nature Reviews Cancer. 2005;5;341-354.
3. Krasinskas AM. SAGE-Hindwai access to research. Pathology research international: Review article. Hindwai.com/journals/pri/2011/Article ID932932, 6pages doi : 10.4061/2011/932932.
4. Mitsudomi T and Yatabe Y. Epidermal growth factor receptor in relation to tumor development: EGFR gene and cancer. FEBS Journal. 2010;277;301-308
5. Eddy DM. Screening for colorectal cancer. Ann Intern Med. 1990; 113:373-384.
6. Cunningham D, Atkin W, Lenz HJ, Lynch HT, Minsky B, Nordlinger B, Starling N. Colorectal cancer. Lancet. 2010;375:1030–47.
7. SEER Stat Database: Incidence, Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov), National Can¬cer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2010, based on the November 2009 submission.
8. George SM, Makinen MJ, Jernwal P, Makela J,Vihko P, Kartunnnen TJ. Classification of advanced colorectal cancer by tumor edge morphology: evidence for different pathogenesisand significance of polypoidal and non-polypoidal tumors. Cancer. 2000;89:1901-1909.
9. Rasool A, Bari S, Rashid S, Wani A, Wani R, Peer G. Outcome of patients with acute intestinal obstruction due to colorectal carcinoma. The internal journal of surgery. 2008;20.
10. Raphael Rubin, David S. Strayer, Emauel. Rubin's pathology: Clinicopathological Foundations of Medicine. 2008;609-610.
11. Snaebjomsson P, Jonasson L, Jonsson T, Pall Helgi Moller, Asgeir Theodors, Jon G Jonsson. Colon cancer in Iceland – A nationwide comparative study on various pathology parameters with respect to right and left tumor location and patients age. International journal of cancer. 2010;127(11).
12. Shia J, Klimstra DS, Li AR et al. Epidermal growth factor receptor expression and gene amplification in colorectal carcinoma: An immunohistochemical and chromogenic in situ hybridization study. Modern Pathology. 2005; 18: 1350-1356.
13. Sishi L, Buchbinder E, Wu L, Bjorge JD, Fujita DJ, Zhu S. EGFR and HER2 levels are frequently elevated in colon cancer cells, Discoveries report 2014, May-

Aug; 1(1):e1. doI:10.15190/drep.2014.1.
14. Santinia D, Pantanoa F, Vincenzia B et al. Molecular predictive factors of response to anti-EGFR antibodies in colorectal cancer patients. EJCEJC Supplement 2008;86-90.
15. Spano J P, Fagard R, Soria J C, Rixe O, Khayat D and Milano G. epidermal growth factor receptor signaling in colorectal cancer: preclinical data and therapeutic perspectives. Annals of oncology. 2005; 16:189-194.