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# Expression of Membranous Epidermal Growth Factor Receptor in Colorectal Adenocarcinoma and It's Correlation with Clinicopathological Features

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**Abstract:** The aim of this study was to investigate the expression of membranous epidermal growth factor receptor in colorectal adenocarcinoma and it's correlation with clinicopathological features. Fifty formalin-fixed, paraffin embedded archival specimens of colorectal cancer were included randomly as cases. Immunohistochemical staining was performed to assess EGFR expression. The results were correlated with the clinicopathological features of colorectal tumor tissues. More than 1% of membranous EGFR expression was found in 24 (48%) of cancer specimens. The immunoreactions intensity was classified as weak, moderate and strong representing 2, 22 and 24%, respectively. According to multivariate analysis, EGFR expression was not significantly associated with age, sex, tumor site, stage, grade and type of tumor in cases. These results suggest that the assessment of EGFR expression in colorectal cancer by conventional immunohistochemistry has not proven its predictive value and can not be useful to predict about outcome of patients.

**Key words:** Clinicopathologic features, Colorectal cancer, Epidermal Growth Factor Receptor, Immunohistochemistry, Proliferation, TNM stage

## INTRODUCTION

Colon cancer is the fourth most leading cause of cancer-related mortality in the world, accounting for approximately 15% of all human cancers (Gursoy and Kinik, 2006; Zavarhei *et al*., 2007). The incidence of colorectal cancer (CRC) is almost similar in men and women (11%) (Sunkara *et al*., 2008). CRC is a common lethal disease with 5000 new cases reported each year in Iran but the genetic of this cancer and its pathophysiologic implications in this region has remained to be clarified (Esna-Ashari *et al*., 2008).

It is accepted that early diagnosis of colorectal cancer, successful surgical treatment, better knowledge of it's clinicopathological prognostic factors and response to adjuvant therapy have contributed to the improve outcome of affected patients. Therefore, identification of molecular markers associated with carcinogenesis, tumor growth, invasion and metastasis has been critical to developing potential therapeutic interventions (Doger *et al*., 2006; Tawfiek *et al*., 2010).

Epidermal Growth Factor Receptor (EGFR) is one of the most important genes along the colorectal carcinogenesis pathways. This 170-kDa transmembrane tyrosine kinase receptor belongs to a family of cell membrane receptors. This family comprises four proteins: EGFR (ErbBl-HER1), ErbB2 (HER2/neu), ErbB3 (HER3) and ErbB4 (HER4). EGFR composed of an extracellular ligand-binding region, a single membrane-spanning region and a cytoplasmic tyrosin-kinase-containing domain (Krasinskas, 2011). Activation of EGFR triggers intracellular signaling which regulates proliferation, differentiation, apoptosis and metastasis (Chen *et al*., 1987). Mutations or gene amplifications induce EGFR overexpression and structural rearrangements of this protein (De Castro-Carpeno *et al*., 2008). Aberrant EGFR activation appears to be an important factor in tumorigenesis, as well as an essential driving force for the aggressive growth behavior of cancer cells (El-Meghawry El-Kenawy *et al*., 2006). Overexpression of EGFR has been suggested as a factor of poor prognosis, decreased survival and/or increased metastasis in various malignant tumors including colorectal (Nicholson *et al*., 2001), lung (Hirsch *et al*., 2003), breast (Sainsbury *et al*., 1987), gastric (Yasui *et al*., 1988), bladder (Neal *et al*., 1985), esophageal (Ozawa *et al*., 1989), gallbladder (Kaufman *et al*., 2008), ovarian and cervical cancers (Bauknecht *et al*., 1989).

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EGFR expression has been reported in 80% of colorectal cancer and is one of the most promising targeted therapies in CRC treatment (Milano *et al*., 2008). To the best of authors knowledge, there is no published study about correlation between EGFR expression and clinicopathological features in Iranian patients. Therefore, the present study designed to evaluate the immunohistochemical expression of EGFR in colorectal adenocarcinoma and it's correlation with some of the clinicopathological features in this population.

## MATERIALS AND METHODS

**Sample collection:** This study was carried out in Moayyed Laboratory in Mashhad, Iran during the time period of Agust, 2009 to November, 2010. Fifty formalin-fixed and paraffin embedded colorectal cancer specimens were randomly selected. In patients the diagnosis was established by surgical resection and biopsy. A section from each specimen block was stained with hematoxylin and eosin for histological evaluation and representative blocks were chosen for immunostaining. Normal esophagus sections were used as positive control and for negative control, the primary antibody was omitted when the serial sections from each tissue were stained.

**Immunohistochemical staining:** Formalin-fixed, paraffin embedded tissue blocks were cut to 3-μm-thick sections for immunostaining. Immunohistochemical staining was performed using a streptavidin-biotin-peroxidase complex technique. Sections were dewaxed, rehydrated and incubated for 15 min with 3% hydrogen peroxide to block endogenous peroxidase activity. Antigen retrieval was performed by immersing the sections in EDTA Tris buffer (pH 7.0) for 20 min at 99°C in a water bath and washed with Tris buffer. Subsequently, sections were incubated for 30 min with primary mouse monoclonal antibody anti-EGFR (EGFR/113, Novacastra, Novacastra Laboratories Ltd, Newcastle, UK) at room temperature. The sections were again washed in Tris buffer and incubated with biotinylated link antimouse and antirabbit immunoglobulin and streptavidin-coupled horseradish peroxidase (Novacastra, United Kingdom) for 20 min. The sections were rinsed once again in Tris buffer and Staining was visualized with a 3, 3′- diaminobenzidine tetrachloride, supplemented with hydrogen peroxide. The slides were then washed in distillated water and counterstained with Mayer's hematoxylin and dehydrated before mounting.

**Interpretation of immunostaining:** Staining was scored independently by two observers and a high level of concordance (90%) was achieved. In case of disagreement, the slides were reviewed and consensus view achieved. The degree of membranous EGFR immunostaining based on intensity and relative abundance of the tumor cells was assessed using a semi-quantitative scoring system which was performed according to Chung *et al*. (2005): no membranous staining (0);<30% of the tumor cells stained positive (1); 30-50% of the tumor cells stained positive (2);>50% of the tumor cells stained positive (3). Although cytoplasmic staining of tumor cells was observed, but only membranous staining was considered to be specific.

**Statistical analysis:** Statistical analysis was performed using SPSS software. The correlation between clinicopathologic variables and EGFR expression was evaluated using Pearson's Chi-square, Fisher's exact, T-student and Mann-Whitney tests. Statistical significance was defined as $p<0.05$.

## RESULTS

The clinicopathological characteristics of colorectal adenocarcinoma patients were shown in Table 1. Fifty cases of colon carcinoma were evaluated in this study. Study population was consisted of 31 male and 19 female patients (median age: 59.14±14.3, ranged from 26 to 85).

Table 1: Clinicopathological characteristics of 50 colorectal cancer patients

| Characteristics | No. of patients | % |
|---|---|---|
| **Age (years)** | | |
| <59 | 26 | 52 |
| ≥59 | 24 | 48 |
| **Gender** | | |
| Male | 31 | 62 |
| Female | 19 | 38 |
| **TNM (Stage)** | | |
| StageI | 6 | 12 |
| StageIIA | 18 | 36 |
| StageIIB | 3 | 6 |
| StageIIIA | 4 | 8 |
| StageIIIB | 12 | 24 |
| StageIIIC | 6 | 12 |
| StageIV | 1 | 2 |
| **Site** | | |
| Proximal colon | 9 | 18 |
| Distal colon | 12 | 24 |
| Rectum | 24 | 48 |
| Other | 5 | 10 |
| **Histological type** | | |
| Adenocarcinoma | 47 | 94 |
| Mucinous | 3 | 6 |
| **Histological grade** | | |
| I | 33 | 66 |
| II | 16 | 32 |
| III | 1 | 2 |

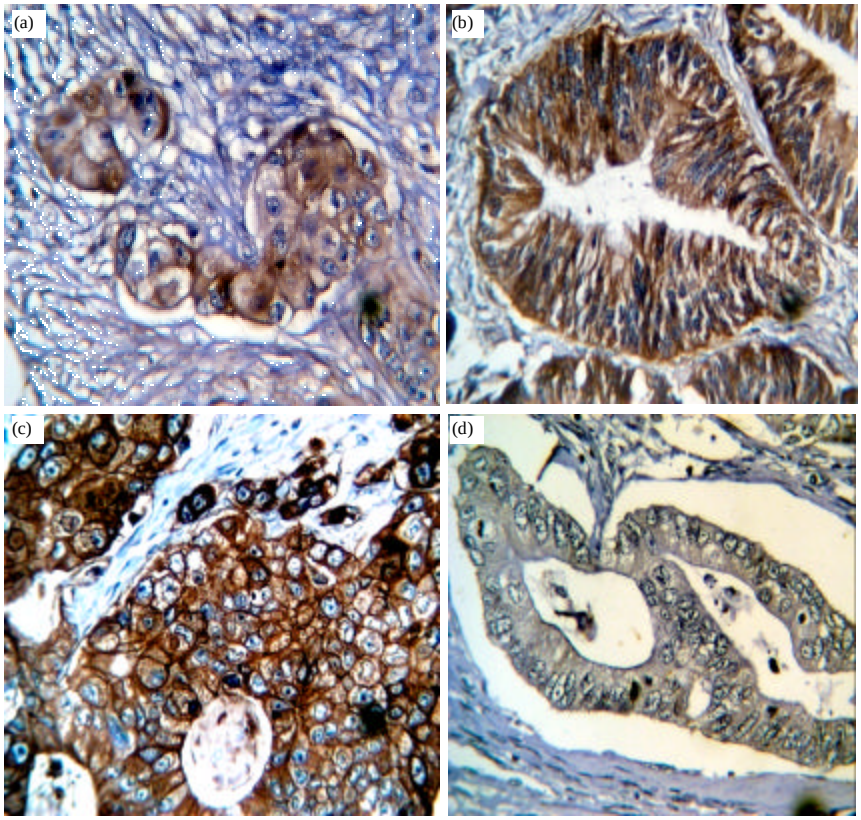


Fig. 1 (a-d): Epidermal Growth Factor Receptor (EGFR) expression in colorectal cancer. (a) <30% of membranous EGFR expression in adenocarcinoma type of colorectal cancer, stage IIB and grade I (1+), (b) 30-50% of membranous EGFR expression in adenocarcinoma type of colorectal cancer, stage IIIB and grade II (2+), (c) >50% of membranous EGFR expression in mucinous adenocarcinoma type of colorectal cancer, stage IIIC and grade II (3+) and (d) No membranous expression of EGFR in colorectal carcinoma (Negative control)

Table 2: Membranous EGFR expression: result of the scoring system in patients with colorectal cancer (n = 50)

| Score | No. | % |
|---|---|---|
| 0 | 26 | 52 |
| 1+ | 1 | 2 |
| 2+ | 11 | 22 |
| 3+ | 12 | 24 |

EGFR: epidermal growth factor receptor

A majority, 22 patients (44%) had stage III (IIIA, IIIB and IIIC) disease, 21 patients (42%) had stage II (IIA and IIB) tumors, 6 patients (12%) had stage I disease and 1 patient (2%) had stage IV tumors. Histological type of 47 patients (94%) was colon adenocarcinoma and 3 (6%) was mucinous adenocarcinoma.

Results of scoring system have been used to interpret the EGFR Immunohistochemical staining as summarized in Table 2. According to the EGFR positivity grade, 26 (52%) adenocarcinomas had no membranous reactivity and membranous EGFR expression was found in 24 (48%) of cases: 1 (2%) had<30% positive cells, 11 (22%) had 30-50% reactive cells and 12 (24%) had>50% labeled cells. In all tumor cells with positive expression, staining was encountered in more than 1% of the neoplastic cells. Figure 1(a-d) demonstrates different patterns of membranous EGFR staining.

The percentage of patients with EGFR immunoexpression was higher in stage IIIB (33.3%) and grade I (62.5%). Most of the EGFR expression (50%) was found in the Rectum of patients with colorectal carcinoma. In this study no statistically significant correlation was observed between membranous EGFR expression and clinicopathological features evaluated: sex, age, tumor site, histological type, grade and stage ($p>0.05$). Table 3 shows clinicopathological features of patients and their correlation to EGFR expression.

Table 3: Relationship between patients with Positive versus Negative membranous EGFR expression (expr.) in their colorectal adenocarcinomas and various clinicopathological parameters (n = 50 patients)

| | EGFR (Neg expr.) | | EGFR (Pos expr.) | | |
|---|---|---|---|---|---|
| Characteristics | n | % | n | % | p-value |
| **Age (years)** | | | | | |
| <59 | 14 | 53.8 | 12 | 50.0 | 0.563 |
| ≥59 | 12 | 46.2 | 12 | 50.0 | |
| **Gender** | | | | | |
| Male | 14 | 53.8 | 17 | 70.8 | 0.255 |
| Female | 12 | 46.2 | 7 | 29.2 | |
| **Site** | | | | | |
| Proximal colon | 5 | 19.2 | 4 | 16.7 | 1.000 |
| Distal colon | 6 | 23.1 | 6 | 25.0 | |
| Rectum | 12 | 46.2 | 12 | 50.0 | |
| Other | 3 | 11.5 | 2 | 8.3 | |
| **Histological type** | | | | | |
| Adenocarcinoma | 24 | 92.3 | 23 | 95.8 | 1.000 |
| Mucinous | 2 | 7.7 | 1 | 4.2 | |
| **Histological grade** | | | | | |
| I | 18 | 69.2 | 15 | 62.5 | 0.556 |
| II | 8 | 30.8 | 8 | 33.3 | |
| III | 0 | 0.0 | 1 | 4.2 | |
| **TNM (Stage)** | | | | | |
| Stage I | 4 | 15.4 | 2 | 8.3 | 0.261 |
| Stage IIA | 11 | 42.3 | 7 | 29.2 | |
| Stage IIB | 1 | 3.8 | 2 | 8.3 | |
| Stage IIIA | 2 | 7.7 | 2 | 8.3 | |
| Stage IIIB | 4 | 15.4 | 8 | 33.3 | |
| Stage IIIC | 3 | 11.5 | 3 | 12.5 | |
| Stage IV | 1 | 3.8 | 0 | 0.0 | |

EGFR: Epidermal growth factor receptor

## DISCUSSION

The EGFR gene, encoded on chromosome 7p12, is believed to be an important regulator of proliferation and apoptosis. However, it seems that the mutation or amplification of this gene induce overexpression of EGFR protein and leads to carcinogenesis. Previous studies have shown variable expression of EGFR in colorectal cancer ranging from 25% up to 77% of tumors (Messa *et al*., 1998; Radinsky *et al*., 1995). This differentiation in expression is attributed to the technical variables, the use of non validated antibodies and different scoring systems. On the other hand, several studies have examined the relation between EGFR protein expression and clinicopathological significances in colorectal cancer but EGFR remains a controversial prognostic factor yet.

The purpose of this study was to clarify the relationship between clinicopathological variables and membranous EGFR expression by immunohistochemistry in 50 colorectal carcinoma patients that may have an impact for patients' therapeutic strategies. The results of this study showed membranous EGFR expression in 48% of cases, consistent with those reported by Abd El All *et al*. (2008) who explained that 46.8% of patients showed positive membranous expression of EGFR and the intensity of tumor cells was classified as mild (8.9%), moderate (20.3%) and strong (17.7%). They also demonstrated that there is no significant association between TNM stage and EGFR expression. Supporting of these findings, results of present study did not show a correlation between TNM stage and EGFR expression ($p = 0.261$). In contrast, Spano *et al*. (2005) reported the overexpression of EGFR in CRC patient population and its significant association with TNM stage. In this study they examined the prognostic significance of EGFR expression by immunohistochemistry and assessed it's correlation with clinicopathologic variables in human colorectal cancer. They concluded that this expression-stage association may play a crucial role in a decision to initiate an adjuvant treatment in these patients. Karameris *et al*. (1993) also found a significant correlation between TNM stage and EGFR expression.

Kountourakis *et al*. (2006) studied the prognostic impact of Epidermal Growth Factor Receptor (EGFR) and Her-2/neu protein expression in colorectal cancer. They demonstrated a statistically significant expression of membranous EGFR in the older age group, but did not find a significant association between expression and sex of patients. In this regard, results of current study showed no significant correlation between EGFR expression and age ($p = 0.563$) or sex ($p = 0.255$) of patients.

Koenders *et al*. (1992) demonstrated no significant association between EGFR expression and tumor site while, Koretz *et al*. (1990) reported higher EGFR expression in cancers of the distal colon compared with rectal ones. Also they studied the expression of epidermal growth factor receptor in normal colorectal mucosa, adenoma and carcinoma and no correlation was observed between EGFR expression and tumor type. Findings of present study did not show a relationship between EGFR protein expression and different sites ($p = 1.00$) and types ($p = 1.00$) of tumor.

Few studies have shown a correlation between histological grade and EGFR overexpression (McKay *et al*., 2002; Steele *et al*., 1990), but according to many investigations (Goldstein and Armin, 2001; Baselga, 2002; Waksal, 1999), reseachers of this study found no significant association between histological grade and expression of EGFR protein ($p = 0.556$).

The results of this study have shown that the clinicopathological features in colon adenocarcinoma were not influenced by EGFR expression. In conclusion, findings of present study that are in agreement with the main previous reports, demonstrated that evaluation of EGFR expression by immunohistochemistry in colorectal cancer patients has still not proven its predictive value. However other assays such as EGFR fluorescence in situ hybridization may reveal the prognostic role of EGFR.

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