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Pathology Section

Clinicopathological Parameters and Immunohistochemical Expression of EGFR in Colorectal Carcinoma: A Cross-sectional Study

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ABSTRACT

Introduction: Colorectal Carcinoma (CRC) is the third most common type of cancer worldwide and is one of the leading causes of cancer-related death. Adenocarcinoma is the most common type. Histopathological examination is necessary to determine the type and extent of the tumour, which is essential for patient management and prognosis. Molecular markers play a major role in CRC, among which Epidermal Growth Factor Receptor (EGFR) has prognostic significance.

Aim: The aim of this study was to evaluate the immunohistochemical expression of EGFR in cases of colonic carcinoma and analyse its relationship with various histological and clinical parameters.

Materials and Methods: An observational (cross-sectional) study was conducted in the Department of Pathology, Sree Mookambika Institute of Medical Sciences, Kulasekharam, Kanyakumari district, Tamil Nadu, India. Data from colonic carcinoma patients confirmed by Haematoxylin and Eosin (H&E) staining were collected over a three-year period from January 2019 to December 2021. A total of 58 cases were included. The characteristics studied included age, sex, Carcinoembryonic Antigen (CEA) levels, tumour site, size, degree of histological differentiation (well, moderate, poor), TNM staging, and lymph node status. The EGFR score was obtained by multiplying the grade (% positive cells) by the intensity, and a composite score ranging from 0 to 9 was obtained. This score was used to define low EGFR expression (<6) or high EGFR expression (>6). Analysis was performed using the Chi-square test in Statistical Package for the Social Sciences (SPSS) version 20.0.

Results: Among the 58 patients included in the study, the most common age group affected by CRC was 61 to 70 years (28 cases; 48.3%), with a mean age of 63.32±14.36 years. The most common histological variant was low-grade adenocarcinoma (47 cases; 81.03%). EGFR reactivity was positive in 50 patients (86.20%). EGFR overexpression was significantly associated with histopathological type (low-grade adenocarcinoma) and tumour stage, with p-values of 0.03 and 0.001, respectively.

Conclusion: Immunohistochemical overexpression of EGFR in CRC is associated with histopathological type and tumour staging in this study. This overexpression is associated with poor prognosis and can be used as a predictive marker for patients who yield positive results with chemotherapy.

Keywords: Colonic carcinoma, Epidermal growth factor receptor, Immunohistochemistry

INTRODUCTION

Colorectal cancer accounts for approximately 9% of all cancers worldwide. Over the last three decades, the burden of CRC has increased in India, with an incidence of 4.3 and 3.4 per 100,000 populations for males and females, respectively [1]. A diet poor in fiber and rich in meat contributes to a higher risk of CRC, along with genetic predisposition as a non-dietary cause [2]. Adenocarcinomas are the most common malignancies arising in the colorectal region and are divided into three grades based on cell arrangement and tubule formation: well-differentiated (Grade-I), moderately differentiated (Grade-II), and poorly differentiated (Grade-III) [3]. The staging system for CRC is based on the depth of tumour invasion, involvement of regional lymph nodes, and the presence or absence of distant metastasis. Lymph node involvement is not seen in Stages I and II, while Stage III involves regional lymph nodes and Stage IV involves distant metastasis with or without lymph node involvement. Surgery, chemotherapy, and radiotherapy are the main treatment approaches for CRC [4]. However, while histopathological diagnosis and staging are important for treatment decisions, they are not sufficient as many patients have varied outcomes, necessitating the need for additional prognostic biomarkers [5].

Recently, the specific biomarker EGFR has gained attention due to its relevance in analysing its relationship with various histological and clinical parameters. This has helped substantiate the therapeutic benefit of anti-EGFR for CRC patients in the future, highlighting its role as a theranostic and prognostic marker for targeted therapy [6].

With this background, the present study focuses on evaluating EGFR expression and analysing its relationship with clinicopathological factors to assess its impact on patient prognosis, survival, and the potential for achieving targeted therapy in CRC patients.

MATERIALS AND METHODS

An observational (cross-sectional) study was carried out in the Department of Pathology at Sree Mookambika Institute of Medical Sciences, Tamil Nadu, India. The study was conducted over a period of three years, from January 2019 to December 2021, after obtaining Institutional Ethics Committee (IEC) Approval with IEC approval number: CSP MED/12AUG/03/18.

Inclusion criteria: All resected CRC samples received in the pathology department were included in the study.

Exclusion criteria: Colonoscopic biopsies and post-chemotherapy samples were excluded from the study.

Sample size: The sample size was calculated using the following formula:

$$Z1\text{-}\alpha/2^2\ pq/d^2$$

where

p=47.05%

q=52.95%

d=15% absolute error

Z1-α/2=1.96 for α=5%

=42.53=43 cases.

A total of 58 samples that met the inclusion criteria during the study period were included in the study.

Informed consent was obtained from all patients before surgery. Clinical details were recorded from the case sheets. All resected CRCs were received in 10% formalin, grossed, and underwent a detailed specimen description. Sampling was performed, and the samples were processed and embedded in paraffin. Tissue sections of 4 µm thickness were cut and stained with H&E for histopathological study. Slides were viewed, and clinical parameters such as age, sex, CEA levels, tumour site, tumour size, degree of histological differentiation (well/moderate/poor), and lymph node involvement were documented and staged according to the TNM/AJCC staging system [4]. The individual optimal cutoff value of CEA was considered according to TNM staging. A rise in CEA level >8 ng/mL indicates a high degree of certainty of relapse or disease progression. CEA is not a reliable indicator of clinical response to chemotherapy, but its increase is associated with prognostic value and survival [7].

Sections were taken on glass slides coated with adhesive (Poly-L-Lysine) for IHC using EGFR. IHC was performed using the avidin-biotin complex technique (Dako System, Peroxidase K675). The primary mouse monoclonal antibody (EGFR/113) was added to the tissue sections, followed by the secondary antibody horseradish peroxidase (HRP-conjugated antibody). Brown colour with Diaminobenzidine (DAB) was used to visualise positive cells, and the slides were counterstained with Harris hematoxylin, dehydrated, and mounted. Positive and negative controls were run with each batch of slides. A histological section of normal epidermis was used as a positive control in each staining batch, and for the negative control, 1% non-immune serum was used in place of the primary antibody.

Evaluation of EGFR:

The percentage of stained cells were graded as follows [8]:

- Grade-0: no positive cells
- Grade-1: 1-25% positive tumour cells
- Grade-2: 25-50% positive tumour cells
- Grade-3: >50% positive tumour cells.

The intensity of peroxidase deposits was assessed visually as light beige to dark brown staining in tumour cell membrane, cytoplasm, or both and was scored as follows:

Score: 0 (negative)

Score: 1 (weak)

Score: 2 (moderate)

Score: 3 (strong)

A composite score of 0 to 9 was obtained by multiplying the grade by the intensity. EGFR expression was considered low when <6 and high when ≥6 [9].

STATISTICAL ANALYSIS

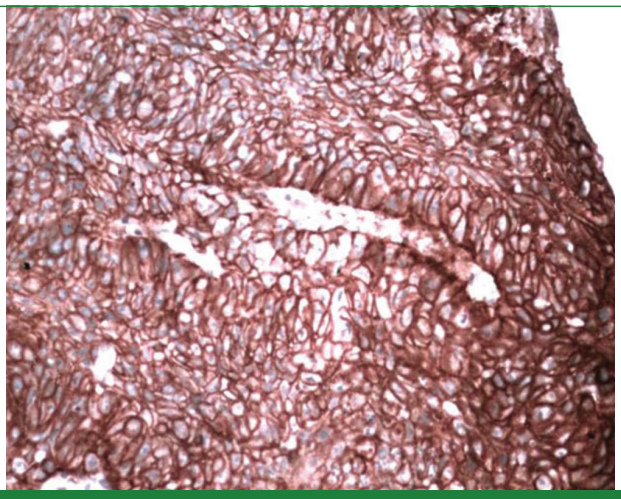
The data was entered into an Excel sheet, and analysis was conducted using SPSS 20.0 software. The frequency of CRC was presented as a percentage with a 95% confidence interval. The association between clinicopathological data and EGFR was tested for statistical significance using the Chi-square test. A p-value of <0.05 was considered statistically significant.

RESULTS

The present study comprised 58 histopathologically proven cases of adenocarcinoma of the colon. The most commonly affected age group was between 61 and 70 years, with 28 (48.3%) cases. Males were more commonly affected, with 43 (74%) cases compared to females, with 15 (26%) cases. The most common site of involvement was the proximal colon, with 25 (43.1%) cases, followed by the distal colon with 24 (41.3%) cases, and the rectum, with 9 (15.5%) cases. Left-sided colon tumours (20.3%) outnumbered right-sided tumours (7.54%).

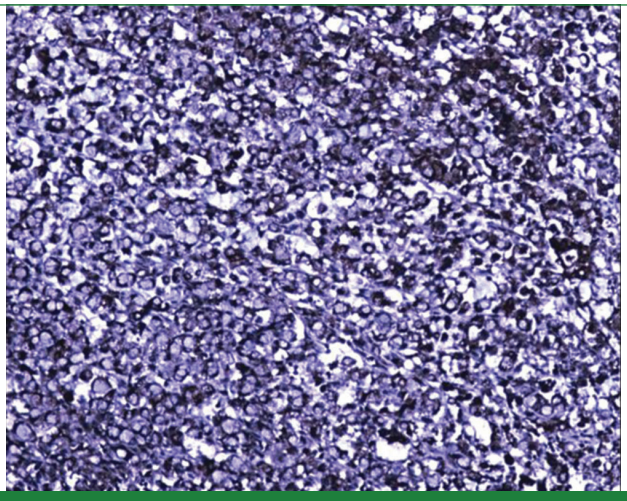
Lesions in 35 (60.3%) patients had a tumour size <5 cm in diameter, while 23 patients (39.7%) presented with a tumour size ≥5 cm in diameter. The most common histopathological type with EGFR positivity was low-grade adenocarcinoma, with 26 (55.02%) cases, followed by high-grade adenocarcinoma, with 4 (80%) cases in frequency. The majority of patients had invasion into the muscularis propria (T2), which was seen in 32 (55%) patients. Lymph node metastasis was seen in 38 (68.5%) cases. Six (10.3%) patients had metastasis at the time of diagnosis, which involved the liver as secondaries. CEA levels were <5 in 28 (48.3%) cases and >5 in 30 (51.7%) cases.

The expression of the EGFR marker in this study, which is expressed in the cytoplasm of tumour cells, showed a significant difference in the intensity of staining (negative, weak, high). The positive control was taken from squamous cell carcinoma of the cervix, where a positive reaction to the EGFR antibody was indicated by the appearance of a brown colour on the cytoplasmic membrane of the cell, as shown in [Table/Fig-1].

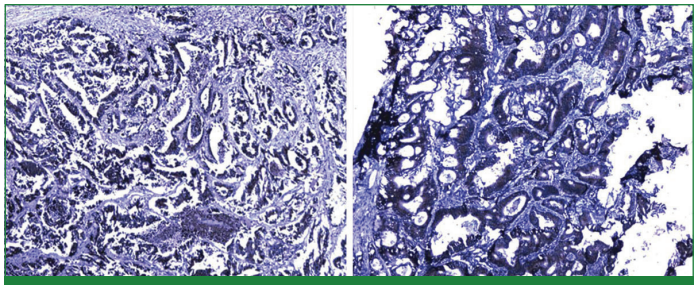


[Table/Fig-1]: The positive control reaction to EGFR antibody from squamous cell carcinoma of cervix.

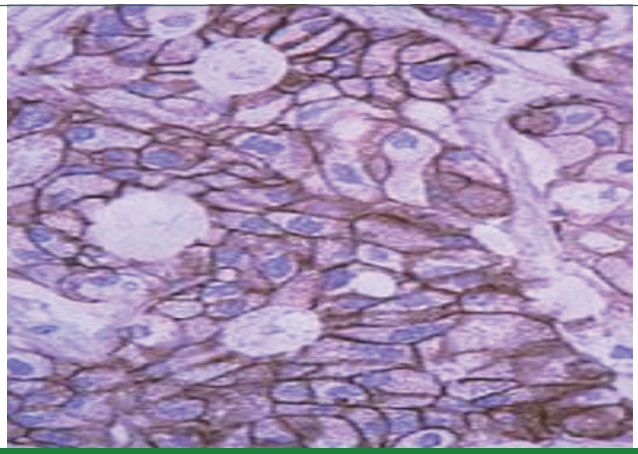
The IHC staining of EGFR expression showed negativity (cytoplasmic positivity) in neoplastic cells, as shown in [Table/Fig-2]. The immunoexpression of EGFR in neoplastic cells displayed a moderate intensity cytoplasmic pattern, as shown in [Table/Fig-3]. The immunoexpression of EGFR in neoplastic cells exhibited a strong intensity, as shown in [Table/Fig-4].



[Table/Fig-2]: Weak (score 1) EGFR expression (10x) in neoplastic cells.



[Table/Fig-3]: Moderate (score 2) EGFR expression (10x) in neoplastic cells.



[Table/Fig-4]: Strong (score 3) EGFR expression (40x) in neoplastic cells.

Age, sex, CEA levels, tumour site, tumour size (cm), histopathological diagnosis, tumour stage, lymph node involvement, and metastases were significantly associated with EGFR expression and thus appear to be prognostic factors for CRC outcome. The expression of EGFR with regard to clinicopathological parameters is described in [Table/Fig-5].

The percentage of patients with EGFR overexpression was higher in TNM stage T2 than in stage T4 CRCs. EGFR overexpression was associated with histopathological diagnosis and tumour staging, with a p-value of 0.03 and 0.001, respectively, which was significant.

Clinicopathological parameters		Negative	Low EGFR	High EGFR	p-value
Age (years)					
<70		4 (12%)	12 (36%)	17 (52%)	0.77
>70		4 (16%)	7 (28%)	14 (56%)	
Gender					
Male		4 (9%)	14 (33%)	25 (58%)	0.21
Female		4 (27%)	5 (33%)	6 (40%)	
CEA					
<5		1 (3%)	12 (43%)	15 (54%)	0.054
≥5		7 (23%)	7 (23%)	16 (54%)	
Tumour site					
Proximal colon		2 (8%)	7 (28%)	16 (64%)	0.33
Distal colon		5 (21%)	10 (41%)	9 (38%)	
Rectum		1 (11%)	2 (22%)	6 (67%)	
Tumour size (cm)					
<5		7 (20%)	9 (26%)	19 (54%)	0.14
≥5		1 (4%)	10 (44%)	12 (52%)	
Histopathology diagnosis					
Adenocarcinoma-low-grade		7 (15%)	14 (30%)	26 (55%)	0.03
Adenocarcinoma-high-grade		1 (20%)	0	4 (80%)	
Mucinous carcinoma		0	5 (100%)	0	
Signet ring cell carcinoma		0	0	1 (100%)	
TNM stage					
T	T1	1 (9%)	3 (27%)	7 (64%)	0.001
	T2	6 (19%)	7 (22%)	19 (59%)	
	T3	0	9 (100%)	0	
	T4	1 (17%)	0	5 (83%)	
N	N0	1 (5%)	11 (55%)	8 (40%)	0.056
	N1	6 (17%)	8 (22%)	22 (67%)	
	N2	1 (50%)	0	1 (50%)	
M	M0	7 (13%)	19 (37%)	26 (50%)	0.18
	M1	1 (17%)	0	5 (83%)	

[Table/Fig-5]: Clinicopathological parameters with EGFR expression using chi-square test.

DISCUSSION

EGFR, also known as HER (human EGF receptor), is present in all epithelial cells, stromal cells, glial cells, and smooth muscle cells. It has a multifunctional role in cell differentiation, migration, division, apoptosis, increased proliferative activity, and angiogenesis [10]. Overexpression of EGFR is common in many tumours, especially in CRC (60-80% of tumours), which is associated with a poor prognosis. Molecular markers (such as EGFR) have played a major theranostic role, in addition to being targets for anticancer therapy.

The main aim of the present study was to evaluate the expression of EGFR in CRC using IHC and analyse the level of EGFR expression in relation to clinicopathological aspects. The results of the present study were consistent with many other studies, as discussed below. The present study demonstrates a significant association between high EGFR expression and TNM tumour stage diagnosis.

CRC usually occurs in old age, mostly after the fifth decade of life. The common age group of CRC patients involved in this study was 61 to 70 years (28, 48.3%). This was similar to studies conducted by Elzouki AN, where the majority of cases (92 patients, 60.5%) were between 50 and 70 years of age [10]. In the present study, males (43, 74.1%) were more commonly affected than females. This was similar to studies conducted by Aljebreen AM, which involved a total of 118 patients, with 58% being male and 42% being female [11].

CEA is an independent prognostic factor in CRC. In the present study, CEA levels were >5 in 30 cases (51.7%), which was comparable to studies conducted by Spano JP et al., [12]. Additionally, 35 patients (60.3%) had tumour sizes <5 cm in diameter, which was similar to the study conducted by Spano JP et al. On the other hand, studies conducted by Abdulkareem FB et al., found that left-sided (distal colon) tumours (261, 62%) were more common than right-sided (proximal) ones (58, 14%) [13]. This was in contrast to the present study, where the majority of tumours (25, 43.1%) were located in the proximal colon.

In their study, Kaneez S et al., found that well-differentiated adenocarcinomas were more common (24, 41.37%), followed by moderate and poorly differentiated adenocarcinomas. Mucinous carcinoma and signet cell carcinoma were identified in 13 and 5 patients, respectively [14].

In the present study, 50 patients (86%) had positive EGFR expression in the tumour cells, while 8 patients (14%) had negative EGFR expression. This finding was similar to the study conducted by Liu J et al., [15]. CEA levels and tumour site did not show a positive correlation with EGFR status, with p-values of 0.054 and 0.33, respectively. Similarly, Spano JP et al., found no positive correlation between EGFR status and tumour site in their study involving 150 CRC patients [12].

In the present study, EGFR overexpression was associated with histopathological diagnosis and tumour infiltration, with p-values of 0.03 and 0.001, respectively. However, Galizia G et al., who studied 49 specimens of colorectal neoplastic tissue, did not identify a relationship between histological differentiation and EGFR expression [16]. This finding was in contrast to the present study.

The present study demonstrates a significant association between EGFR overexpression and TNM (T2) tumour stage at diagnosis,

highlighting a relationship between EGFR overexpression and tumour invasion [17]. Goldstein NS and Armin M analysed EGFR expression in Stage-IV CRCs and did not find a significant difference in marker overexpression between Stage-IV CRC cases [9]. In contrast, Spano JP et al., identified a relation between EGFR expression and the more advanced stages of tumour stage [12].

In their study, Doger FK et al., did not observe a relationship between the presence of lymph node metastases and EGFR expression [18]. The expression of EGFR was not related to neoplastic lymph node infiltration. This finding was comparable to the present study, which showed a p-value of 0.056. Additionally, in the present study, no significant relationship was found between EGFR expression and metastasis. Similar findings were reported by Scartozi et al., and Bralet MP et al. In contrast, Italiano A et al., identified higher expression of EGFR in patients with distant metastases [19-21].

Limitation(s)

The interpretation of EGFR expression in colorectal adenocarcinoma cases has been proven to be time-consuming and cost-effective.

CONCLUSION(S)

EGFR overexpression in colonic adenocarcinoma cases plays a role as a prognostic marker that correlates with a poor prognosis. However, the theranostic effect of CRC against EGFR has no role, and it needs to be evaluated using molecular studies. The immunohistochemical expression of EGFR in primary CRC predicts its expression in recurrence. The outcome of the present study helps substantiate the potential therapeutic effect of anti-EGFR treatment for colonic patients in the future.

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