ANALYSIS OF IMMUNOHISTOCHEMICAL EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) IN COLORECTAL CANCER

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ABSTRACT

Objective: To evaluate immunohistochemical expression of EGFR in colorectal cancer (CRC).
Study Design: Descriptive cross sectional study.
Place and Duration of Study: Histopathology Department, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from Mar 2017 to Aug 2017.
Methodology: A total of 100 cases of histologically confirmed CRC were retrieved from archive of Histopathology department, AFIP Rawalpindi. Patients' age, gender, histologic type and grade were noted. Immunohistochemistry for EGFR was applied and results were recorded. Data was analyzed using SPSS version 22. Descriptive statistics, frequencies and percentages were calculated.
Results: A total of hundred (n=100) patients were enrolled. Mean age of the study patients was 54.3 ± 16.3 years. The group of patients consisted of 68 (68%) men and 32 (32%) women. The majority of primary tumours were located in the rectum 39, (39%), followed by ascending colon 16 (16%), rectosigmoid junction 14 (14%), cecum 13 (13%), sigmoid colon 9 (9%), transverse colon 5 (5%) and descending colon 4 (4%). Most frequent histologic type was adenocarcinoma 80 (80%), followed by mucinous adenocarcinoma 12, (12%) and signet ring cell carcinoma 8 (8%). Most tumours were moderately differentiated 47 (47%), followed by well differentiated 34 (34%) and poorly differentiated 19 (19%). EGFR expression was found in 39 cases (39%). Among adenocarcinoma 41% (n=33/80) were EGFR positive and among mucinous adenocarcinoma 50% (n=6/12) were EGFR positive. All signet ring cell carcinoma cases were EGFR negative. Among well differentiated CRCs 41% (n=14/34) were EGFR positive, among moderately differentiated 40% (n=19/47) were EGFR positive and among poorly differentiated 46% (n=6/13) were EGFR positive.
Conclusion: A significant percentage of CRC expressed EGFR but no statistically significant correlation was seen between EGFR expression and clinicopathological variables. Estimation of EGFR expression status may help to select the patients with CRC for targeted therapy, which is likely to improve the response rates.

Keywords: Anti-EGFR therapy, Colorectal cancer, Epidermal growth factor receptor, Immunohistochemistry.

INTRODUCTION

Colorectal cancer is a major cause of tumour related mortality globally being the fourth leading cause of cancer related mortality with >1.3 million new diagnoses and 694,000 deaths in 2012[1-4]. All over the world, colorectal cancer (CRC) makes 10% of cancer burden annually affecting one million individuals hence being the second most frequently diagnosed cancer in females (195,400 new cases) and the third in males (217,400 new cases)[5-7]. Patients with locally invasive CRC have better outcome showing 5-year survival rate upto 80–90%, which decreases to 60-70% in node-positive cases and less than 10% in patients with distant metastases[8]. Recently there has been an improvement in median survival of patients with metastatic CRC of 24 to 30 months, mainly because of introduction of new therapeutic agents such as anti-EGFR targeted monoclonal antibodies namely cetuximab or panitumumab and vascular endothelial growth factor targeted monoclonal antibody bevaci-zumab[9].

EGFR (also known as ERBB1/HER1) is a member of ERBB family of receptor tyrosine kinases (RTK) and plays a significant role in tumour pathophysiology and management[10,11]. It is a 170 kD transmembrane protein with an intracellular tyrosine kinase domain[12]. EGFR signaling pathway is activated through the binding

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of various ligands to the receptor's extracellular domain which induces homo- or heterodimerization of EGFR and autophosphorylation on tyrosine residues in its intracellular domain[13,14]. The ultimate consequence of EGFR activation and initiation of intracellular signaling pathways are tumour proliferation with inhibition of apoptosis, promotion of angiogenesis, metastasis and tumour invasiveness[13,15]. It has been reported in literature that EGFR is expressed in about 60-80% of colorectal cancers and latest developments in targeted treatment perspectives of CRC have recognized the significance of anti-EGFR targeted therapies for EGFR-positive cases[16-18]. Anti-EGFR antibodies including cetuximab and panitumumab have been authenticated as appropriate drugs in various human malignancies including CRC and are presently being utilized as first, second- or third-line agents for the management of metastatic CRC[19-21]. Therefore, assessment of EGFR expression status is important in the setting of the administration of anti-EGFR agents, including monoclonal antibodies (mAbs) and small molecule tyrosine kinase inhibitors[2]. Most of the studies show that EGFR-targeted treatments have been used for treating CRC, but the value of EGFR immunoexpression to predict the efficacy of adjuvant treatment is controversial and the EGFR antagonist cetuximab has proven effective even against EGFR-negative tumours as well[8]. Therefore EGFR gene amplification appears to be a more promising predictive biomarker[9,20]. However the EGFR gene copy number estimated by fluorescence in situ hybridization (FISH) correlates better with the clinical response of patients than the quantitative PCR[19]. The disparity between the EGFR expression status and the immunohistochemical staining might be attributed to faulty and inappropriate procedures in the immunohistochemical staining, specimen fixation and storage or the delay between specimen fixation and immunohistochemical evaluation[2]. The assessment of EGFR expression can also be affected by immunoreactivity of normal tissues, variable postivity of tumours for EGFR in various regions of colon and heterogeneity of reactivity within the tumour itself[7,22]. And the plus points of using immunohistochemistry for evaluation of EGFR expression status are that it is rapid, economical, easily available in all routine laboratories and retains tissue context[23].

The evidence from local population is limited and there is hardly any study on evaluation of EGFR status among cases of colorectal cancer. Present study was designed to study the EGFR status in CRC through immunohisto-chemical analysis and to determine the number of patients who can benefit from targeted therapy.

METHODOLOGY

This descriptive cross-sectional study was carried out at department of histopathology, Armed Forces Institute of Pathology, Rawalpindi, from March to August 2017 after taking approval from the Institutional Review Board. A total of 100 specimens of colorectal cancer including small biopsies (endoscopic, colonoscopic and proctoscopic) and surgical resections (colectomies and APRs) from patients of all ages and both genders which were diagnosed on routine histopathology as CRC were included in the study. Other colorectal tumours such as neuroendocrine tumours, lymphomas and metastatic tumours, poorly processed and poorly fixed tissues and scanty specimens were excluded. Immunohistochemical analysis of EGFR was performed on formalin-fixed, paraffin embedded CRC tissue. Tissue blocks were sectioned at 3µm thickness and deparaffinized in xylene and rehydrated with decreasing concentration of ethanol. Heat induced epitope retrieval in Tris/EDTA buffer at pH 9.0 was used for ready to use primary antibody EFGR (BioSB antibody). EGFR expression status was analyzed using the defined criterion of membranous and/or cytoplasmic staining of >1% tumour cells of varying intensities. Specific membranous/cytoplasmic immunostaining in less than 1% of tumour cells was defined as EGFR negative[1]. Data was analyzed by using SPSS version 22. Mean and SD were calculated for quantitative variables whereas frequencies and percentages were calculated for qualitative vari-

ables. Associations were calculated and *p*-value <0.05 was taken as significant.

RESULTS

A total of hundred (n=100) patients were included. Mean age of the patients was 54.3 ± 16.3 years with a range of 17 to 85 years. Males were affected more 68 (68%) while females being 32 (32%) and male to female ratio was 2.1:1. The commonest primary tumour site was rectum 39 (39%) followed by ascending colon 16 (16%), rectosigmoid junction 14 (14%), cecum 13 (13%), sigmoid colon 9 (9%), transverse colon 5 (5%) and descending colon 4 (4%). The most frequent histological type was adenocarcinoma 80 (80%) followed by mucinous adenocarcinoma 12 (12%) and signet ring cell carcinoma 8 (8%). The commonest histologic grade was moderately differentiated 47 (47%) followed by well differentiated 34 (34%) and poorly differentiated 19 (19%). EGFR expression was found in 39 (39%) cases. Among adenocarcinoma 41% (n=33/80) cases were positive for EGFR and among mucinous adenocarcinoma 50% (n=6/12) were positive for EGFR. All signet ring cell carcinoma were EGFR negative. Among well differentiated cases of CRCs 41% (n=14/34) were EGFR positive, among moderately differentiated 40% (n=19/47) were EGFR positive and among poorly differentiated 46% (n=6/13) were EGFR positive. No association was found between immunohistochemical expression of EGFR and various clinicopathological variables such as patients' age, gender, histologic tumour type or histologic grade (table).

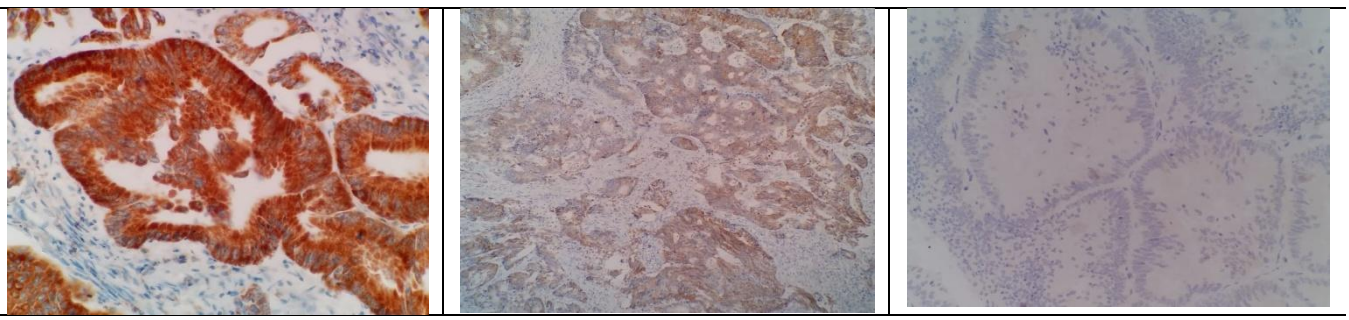

Figure-1: Immunohistochemistry Showing Slides A: Strong EGFR Positivity. B: Weak EGFR Positivity. C: Negative EGFR Expression.

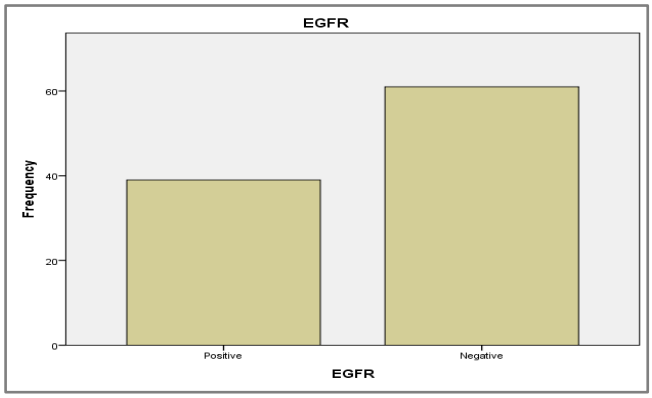

Figure-2: EGFR expression in study sample.

DISCUSSION

Mean age of the patients in our study was 54.3 ± 16.3 years with a range of 17 to 85 years. Rokita *et al* reported that CRCs were common in 5th and 6th decades and the medain age was 65 years (range: 45-78 years) whereas Theodoropoulos *et al* reported that mean age was 64.9 ± 8.75 years[2,7]. Zlatian *et al* showed an increased incidence of disease in patients over 55 years whereas Rego *et al* showed that mean age of 62.5 years (median 64 years, range 26-85)[14,22]. All these studies showed that mean age was almost ten years more than in our population.

Our study showed that males were affected more being 68 (68%) while females being 32 (32%) and male to female ratio was 2.1:1. Rokita *et al* reported that the percentage of male patients having CRC was more than female patients i.e 52.5% males (n=95/181) versus 47.5% females (86/181) which was also the case in our study[2]. According to Theodoropoulos *et al* precentage of male patients was 68.3% (n=112/164) and female

patients was 31.7% (n=52/164) which were almost exactly the same as in our study[7]. Zlatian *et al* and Rego *et al* both reoprted quite similar percentages of gender distribution, male patients being 54% (n=27/50) and 52% (203/388) whereas female patients being 46% (23/50) and 48% (n=185/388) respectively[14,22].

Analysis of primary tumour sites in our study showed rectum to be the commonest primary tumour site followed by ascending colon, rectosigmoid junction, cecum, sigmoid colon, transverse colon and descending colon respectively. However contrary to our findings Rokita *et al* repored that the commonest primary tumour site was the sigmoid colon 38.75 (n=70/181) followed by other parts of clon 37% (n=67/181) and rectum being the least common site 24.359 (n=44/181)[2]. Theodoropoulos *et al* showed that 38% tumours (n=62/164) were located on the right side of colon, 32% (n=52/164) were located on left side and 30% (n=50/164) were located in the rectum[7]. According to Zlatian *et al* and Rego *et al* proximal part of the colon was the primary tumour site in 62% (31/50) and 51% (n=198/388) of the cases whereas distal part of the colon was the primary tumour site in 38% (19/50) and 49% (n=190/388) of the cases respectively[14,22].

Our study showed that the most frequent histologic type was adenocarcinoma followed by mucinous adenocarcinoma and signet ring cell carcinoma respectively. Rokita *et al* also demonstrated the adenocarcinoma to be the commonest histologic type being 45.3% (n=82/181) followed by mixed 28.7% (n=52/181), unclassified 20.4% (n=37/181), mucinous 3.95 (n=7/181) and cylindrocellular 1.7% (n=3/181)[2]. Zlatian *et al* also documented the adenocarcinoma to be the commonest histologic type being 82% (n=41/50), followed by mucinous 16% (n=8/50) and signet ring cell type 2% (n=1/50)[22]. These results were similar to the results in our study.

Analysis of histologic grades showed that the commonest histologic grade was moderately differentiated 47 (47%) followed by well differentiated 34 (34%) and poorly differentiated 19 (19%). Rokita *et al* showed that the most frequent histologic grade was poorly differentiated/unknown 92.3% (n=167/181) followed by well/moderately differentiated 7.7% (n=14/181) which was opposite to the findings in our study[2].

Table: Association between EGFR status and clinicopathological variables.

Study variables	Attributes	EGFR status			*p*-value Chi-Square
		Positive	Negative	Total	
Gender	Male	25 (25%)	43 (43%)	68 (68%)	0.504
	Female	14 (14%)	18 (18%)	32 (32%)	
Site	Cecum	6 (6%)	7 (7%)	13 (13%)	0.147
	Ascending colon	6 (6%)	10 (10%)	16 (16%)	
	Transverse colon	1 (1%)	4 (4%)	5 (5%)	
	Descending colon	4 (4%)	-	4 (4%)	
	Sigmoid colon	5 (5%)	4 (4%)	9 (9%)	
	Rectosigmoid	4 (4%)	10 (10%)	14 (14%)	
	Rectum	13 (13%)	26 (26%)	39 (39%)	
Type	Adenocarcinoma	33 (33%)	47 (47%)	80 (80%)	0.052
	Mucinous	6 (6%)	6 (6%)	12 (12%)	
	Signet ring cell	-	8 (8%)	8 (8%)	
Grade	Well differentiated	14 (14%)	20 (20%)	34 (34%)	0.76
	Moderately differented	19 (19%)	28 (28%)	47 (47%)	
	Poorly differentiated	6 (6%)	13 (13%)	19 (195)	

Theodoropoulos *et al* showed that the majority cases were well and moderately differentiated 87.8% (n=144/164) and poorly differentiated being only 20 (13.2%)[7]. Zlatian *et al* documented that the predominant grade was moderately differentiated 58% (n=29/50) followed by well differentiated 24% (n=12/50) and poorly differentiated 9 (18%)[22]. These findings were similar to those in our study. According to Rego *et al* 65% cases (n=253/388) were well/moderately differentiated and 35% (n=135/388) were poorly/ undifferentiated[14].

Literature review shows a great variability in results of EGFR expression in colorectal cancer ranging from as low as 38% to as high as 91.7%[8,9]. However our results of EGFR expression are comparable with most of the studies and fall within the range defined by different studies[2,7]. The rate of EGFR expression in our study was found to be 39% (n=39/100). The rate of EGFR expression in a study conducted by Rokita *et al* in 2013 was found to be 53% (n=96/181) and 43.9% (n=72/164) in another study conducted by Theodoropoulos *et al* in 2009 which was quite close to the percentage in our study[2,7]. Zlatian *et al* in 2015 stated similar results with EGFR expression being identified in 42% (n=21/50) of the cases[22]. Another study conducted by Rego *et al* in 2010 showed EGFR expression detected in 40% (n=157/388) cases which was almost the same as in our study[14].

No study conducted on immunohistochemical expression of EGFR in local population was found in literature however the results of our study were consistent with the majority of published studies. In our study EGFR expression was not correlated with other clinicopathological variables like histologic tumour type, tumour differentiation grade, patient gender or patient age. This is consistent with the published studies including Rokita *et al*, Theodoropoulos *et al*, Zlatian *et al*, Rego *et al* and Chen *et al*[2,7,9,14,22].

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Author's Contribution

Dr. Ghazala Sadaf has collected the data, analyzed and interpreted the results and wrote the initial manuscript. Dr. Muhammad Asif critically reviewed the manuscript. Dr. Rabia Ahmed conceived the idea and contributed in data analysis. Dr Muhammad Tahir Khadim reviewed and finally approved the manuscript for submission to the journal.

CONCLUSION

Our study showed that a significant number of patients (39%) with CRC showed immunohistochemical expression of EGFR and these are the patients who have the advantage of getting benefitted by anti-EGFR therapy. However in the light of our results and results of other studies conducted internationally, additional research to standardize EGFR determination methods is proposed. We further recommend that a thorough and comprehensive analysis is essential to take account of quantitative analysis of aberrant EGFR expression within the membranous and cytoplasmic compartments, combined with ligand expression, studying complicated molecular pathways involved in carcinogenesis, molecular analysis of genetic abnormalities such as gene copy number variation and mutational status.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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