

HER2 and COX-2 Expression in Colorectal Cancer and their Distribution in Different Categories Based on Tumour Border Configuration

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Abstract

Introduction: Colorectal cancer is the third most common cancer in men and the second in women worldwide. HER2 localization in these tumours has not been adequately investigated. As in breast and gastric cancers, identifying HER2 positive tumours has the potential of predicting the prognosis and developing newer modalities of treatment.

Objectives: To determine the frequency of HER2 and COX-2 expression in colorectal cancer by immunohistochemistry and to study their distribution in different categories of tumour border configuration.

Methods: Fifty (50) patients of colorectal cancer operated at GTB Hospital, Delhi were included. Two representative paraffin blocks from each case were stained with immunohistochemistry using monoclonal antibodies against pan CK, COX-2 and HER2. Immunohistochemistry was evaluated qualitatively and quantitatively.

Results: HER2 positivity showed a variable pattern with 9 (18%) showing a cytoplasmic staining and 2 (4%) showed a combined cytoplasmic-membranous pattern with none of the cases showing pure membranous pattern. 48 (96%) cases were positive for COX-2 immunostaining. On the basis of tumour border configuration, 13 (26%) belonged to 0 - 25%, 16 (32%) to 25 - 70%, 14 (28%) to 70 - 90% and 7 (14%) to > 90% infiltrating margins category. HER2 or COX-2 expression did not correlate significantly with tumour border configuration.

Conclusion: The pattern of HER2 positivity in the study does not support use of Herceptin in colorectal cancer as an exclusive membranous positivity was not seen in any of the cases. A high COX-2 positivity possibly suggests a role of COX-2 inhibitors in colorectal cancer. However, both these markers were not significantly associated with tumour border configuration.

Keywords: *Colorectal Cancer; Tumour Border Configuration; HER2; COX-2; CK*

Abbreviations

IHC: Immunohistochemistry; CK: Cytokeratin; COX: Cyclooxygenase

Introduction

Colon cancer is the third most common cancer in men and the second most common in women worldwide. However, this low incidence rate was associated with a low 5-year relative survival rate [1].

HER2, a member of the human epidermal growth factor receptor family is a proto-oncogene located at position 17q12 on chromosome 17. It regulates cell growth, survival and differentiation via multiple signal transduction pathways and participate in cellular proliferation and differentiation [2]. Amplification or overexpression of this oncogene has been shown to play an important role in the development and progression of breast, gastric, oesophageal, ovarian and colorectal cancer.

Two patterns of HER2 staining are observed in colorectal cancer- membranous and cytoplasmic. It was suggested that cytoplasmic HER2 is a truncated or different protein, based on the fact that a 155kD peptide was found, in contrast to the 185kD HER2 found on the membrane [3].

COX-2 is an inducible enzyme unlike COX-1, which is expressed constitutively. COX-2 is not expressed on normal tissue. Its expression is induced by oncogenes, growth factors and cytokines. COX-2 overexpression is believed to induce tumour angiogenesis, damage immune system and promote tumour invasion [4].

Recently there has been a proposal for an alternate scoring system based on tumour border configuration which classifies colorectal cancer on the basis of percentage of infiltrating margins from 0 - 25%, 25 - 70%, 70 - 90% and >90% [5]. A higher percentage of infiltrating margins correlated with adverse features such as higher grade, higher TNM classification, lymphatic and vascular invasion [5].

Earlier the margins were classified into pushing and infiltrating [6]. A pushing tumour border has reasonably well circumscribed margins. Features of infiltrating tumour border configuration include:

- The loss of a clear boundary between tumour and host tissues.
- The inability to define the limits of tumour invasion and/or distinguish host tissue from malignant tissue by naked eye examination of a microscopic slide.
- The wide dissection of tumour through the full thickness of the muscularis propria without a stromal response.
- The dissection of mesenteric adipose tissue by small glands or irregular clusters or cords of cells.
- The presence of perineural invasion.

Aim of the Study

Our study aimed to determine the distribution of COX-2 and HER2 in surgically excised colorectal specimens and correlate its expression with tumour border configuration and clinicopathological parameters.

Materials and Methods

The study had an observational, descriptive, part prospective and part retrospective study design and it was conducted in the Department of Pathology, UCMS and GTB Hospital, Delhi, India.

Inclusion and exclusion criteria

Fifty (50) patients of colorectal cancer who underwent resection surgery at GTB Hospital, Delhi from January 2010 to March 2017 were included in the study. Patients who received any kind of neo-adjuvant chemotherapy were excluded.

All tissue samples were retrieved from the archives of the Department of Pathology, histologically typed according to the WHO Classification of Colorectal Tumours, graded according to the Universal Grading System [7] and staged as per College of American Pathologists (CAP) guidelines [8].

Ethical clearance

Ethical clearance from the Institutional Ethics Committee was duly obtained before commencement of the study.

Immunohistochemical methodology

Immunostaining was performed on two representative tissue sections from tumours of all patients using three antibodies:

- COX-2 monoclonal antibody, clone RBT COX-2 (BSB 5358, ready to use).
- HER2 monoclonal antibody, clone RBT HER2 (BSB 2036, ready to use).
- CK monoclonal antibody, clone AE1/AE3 (DAKO FLEX, ready to use), using labelled DAKO EnVision kit based on polymer chain two step indirect technique, with DAB as chromogen. A positive control of proliferative endometrium for COX-2 and HER2 positive breast cancer for HER2 was run with each batch of staining for validity. Skin tissue was used as a positive control for CK.

Outcome measures – Evaluation of immunostaining

- The staining for COX-2 was evaluated in terms of the number of positive cells (< 1/3rd, 1/3rd to 2/3rd and > 2/3rd) and intensity.
- Staining for HER2 was evaluated for pattern of staining (membranous or cytoplasmic), percentage of positive cells (<1 0% or > 10%) and intensity of staining.
- Based on CK staining (cytoplasmic), tumours were classified into four categories based on the percentage of infiltrating margins, < 25%, 25 - 70%, 70 - 90% and > 90%.

Statistical analysis

The data obtained from the study were analysed using the Fisher’s exact test for significance. It was applied using Statistical Product and Service Solutions (SPSS) software v.24.0. Fisher’s exact test value and exact 2-sided significance (p-value) were calculated. The p-value less than 0.05 was taken as significant.

Results

Patient demographics

The patients were between the ages of 16 and 80 years with the mean and median age being 51 and 55 years respectively. Of the 50 cases included in the study, 15 (30%) were mucinous adenocarcinomas and the remaining 35 (70%) were adenocarcinomas. Out of the 50 patients, 16 patients (32%) had grade I tumours, 28 patients (56%) grade II and 6 patients (12%) had grade III tumours (Figure 1). Most patients presented at a late stage, with 24 patients (48%) diagnosed at stage III, 20 (40%) at stage II and 5 (10%) diagnosed at stage I. There was one patient of stage IV managed with surgical debulking.

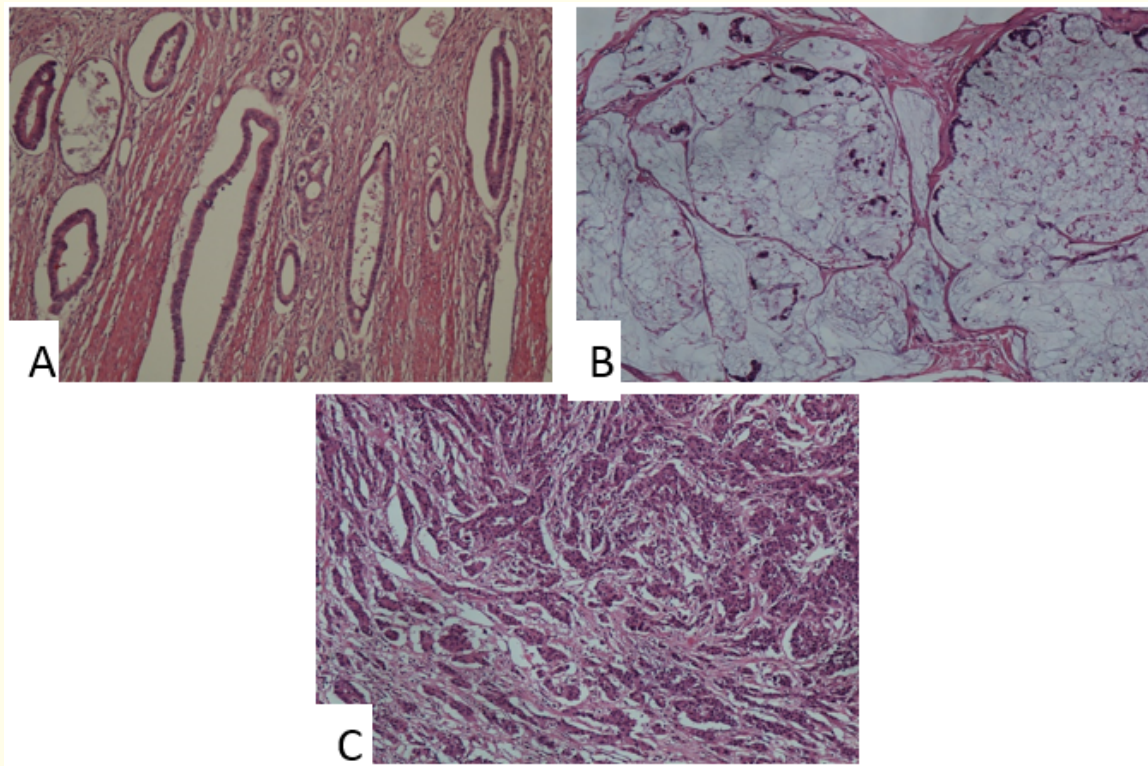


Figure 1: Tumor grade of colorectal cancer.
A) Low grade B) High grade C) Mucinous adenocarcinoma (200X magnification).

CK immunohistochemistry

The cases were divided into 4 categories based on percentage of infiltrating margins (Figure 2):

- 0 - 25%: 13/50 cases (26%)
- 25 - 70%: 16/50 cases (32%)
- 70 - 90%: 14/50 (28%)
- > 90%: 7/50 (14%).

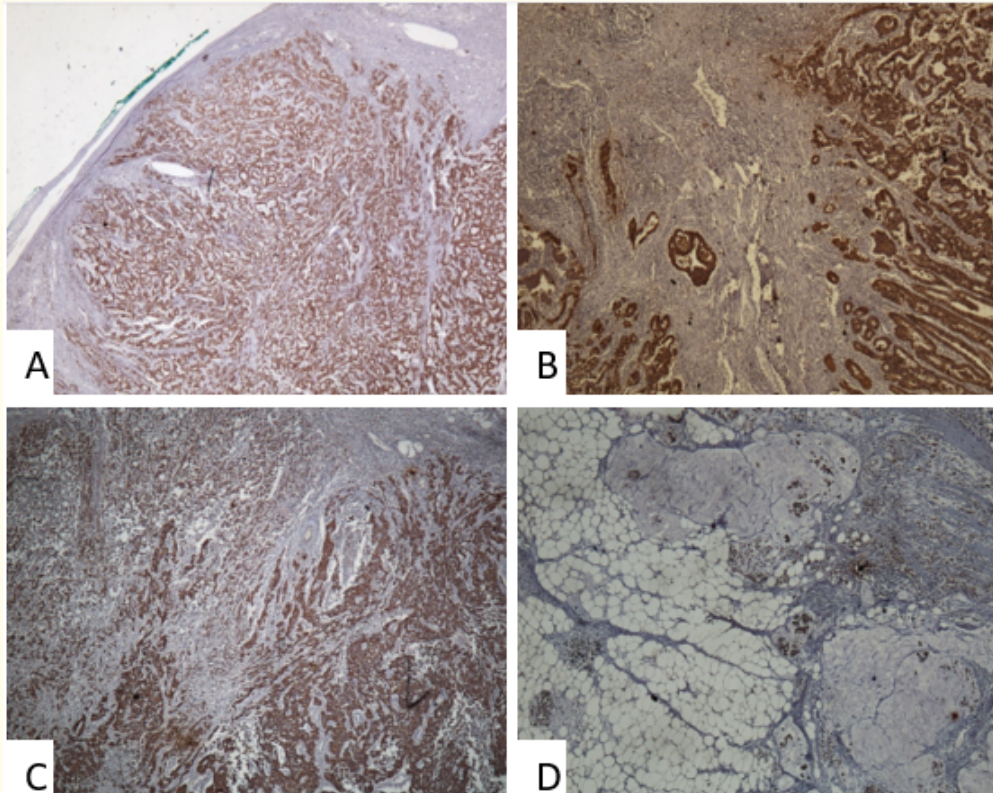


Figure 2: Percentage of infiltrating margins demonstrated based on CK IHC (200X magnification).
A) 0 - 25% B) 25 - 70% C) 70 - 90% D) >90%.

COX-2 immunohistochemistry

Based on the fraction of positive cells, the cases were classified into the following categories (Figure 3):

- None: 2 cases (4%) were negative for COX-2.
- < 1/3rd: None of the cases showed less than 1/3rd positivity.
- 1/3rd to 2/3rd: 3 cases (6%) fell in this category.
- > 2/3rd: In 45 cases (90%), more than 2/3rd cells were positive for COX-2.

Out of 50 cases, 22 cases (44%) showed intense staining, 26 cases (52%) had weak staining.

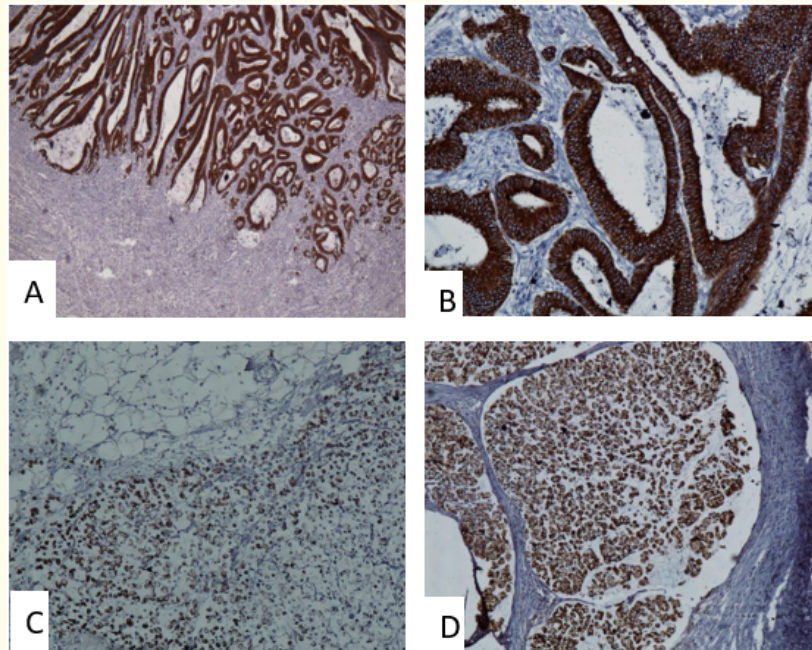


Figure 3: IHC staining for COX-2.

A) 3+ intensity at 40X magnification B) 3+ intensity at 200X C) 2+ intensity at 40X in mucinous adenocarcinoma D) 2+ intensity at 200X in mucinous adenocarcinoma.

HER2 immunohistochemistry

Of the 50 cases, 11 (22%) were HER2 positive. 9 (18%) showed a cytoplasmic pattern while 2 (4%) showed a combined membranous-cytoplasmic pattern. None of the cases showed a membranous pattern of staining with HER2. All positive cases had >10% cells positive (Figure 4).

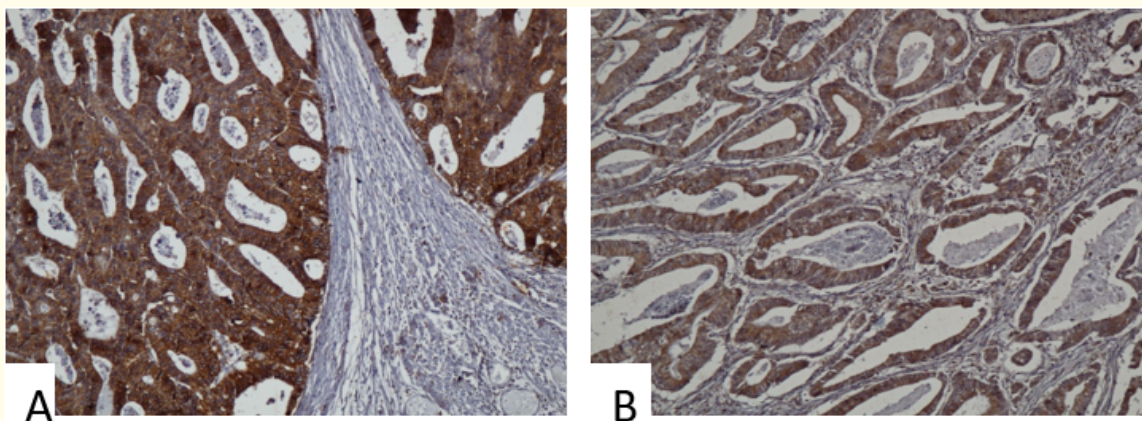


Figure 4: HER2 IHC at 200X magnification.

A) Membranous and cytoplasmic staining B) Cytoplasmic pattern of staining.

Correlation between tumour border configuration and HER2 and Cox-2 expression

No significant correlation was seen between tumour border configuration and HER2 (p value = 0.525) and COX-2 (p value = 1.000) (Table 1 and 2).

| | Tumour border configuration | | | | |
|----------|-----------------------------|----------|----------|-------|---------|
| HER2 | 0 - 25% | 25 - 70% | 70 - 90% | > 90% | p value |
| Positive | 4 | 4 | 3 | 0 | 0.525 |
| Negative | 9 | 12 | 11 | 7 | |

Table 1: Correlation of tumour border configuration with HER2 expression.

| | Tumour border configuration | | | | |
|----------|-----------------------------|----------|----------|-------|---------|
| COX-2 | 0 - 25% | 25 - 70% | 70 - 90% | > 90% | p value |
| Positive | 13 | 15 | 13 | 7 | 1.000 |
| Negative | 0 | 1 | 1 | 0 | |

Table 2: Correlation of tumour border configuration with COX-2 expression.

Correlation between tumour border configuration and histological type, grade and stage: A statistically significant correlation was not seen with histological subtype (p value = 0.747), grade (p value = 0.809) and stage (p value = 0.086). However, a trend was seen between higher tumour stage and increased percentage of infiltrating margins (Table 3).

| | Tumour border configuration | | | | |
|-------|-----------------------------|----------|----------|-------|---------|
| Stage | 0 - 25% | 25 - 70% | 70 - 90% | > 90% | p value |
| Low | 10 | 8 | 4 | 3 | 0.086 |
| High | 3 | 8 | 10 | 4 | |

Table 3: Tumour border configuration with tumour stage.

Correlation between HER2 and histological subtype, grade and stage: A statistically significant correlation was not seen with histological subtype (p value = 0.139), grade (p value = 1.000) and stage (p value = 0.641). However, a possible trend between histologic subtype and HER2 was seen (Table 4).

| | HER2 | | |
|----------------|----------|----------|---------|
| Subtype | Positive | Negative | p value |
| Adenocarcinoma | 10 | 25 | 0.139 |
| Mucinous | 1 | 14 | |

Table 4: HER2 expression with histological subtype of tumour.

Discussion

Unlike breast cancer, inspite of there being a number of studies on HER2 expression in colorectal carcinoma, there are no specific guidelines for positivity on IHC expression of HER2. This is one of the reasons for the varied percentage of positivity results of HER2 expression in colorectal cancer. Further there is paucity of literature on the distribution of HER2 and COX-2 in different categories based on tumour border configuration according to the percentage of infiltrating margins.

The mean age of patients in our study was 51 years, which was much lower than 63 - 70 years described in the Western population [9-11]. It could be explained on the basis of racial differences between the study populations. The study conducted by Ochs., *et al.* showed a different trend from our study with respect to histologic subtypes of cases, with 98% tumors being adenocarcinomas and remaining mucinous and signet ring [12]. Gill., *et al.* found a similar pattern with 77.5% adenocarcinomas [13]. The distribution of patients as per the histologic grade observed by us was in accordance with previous studies, with highest number of patients having grade 2 and lowest belonging to grade 1 [11]. Wu., *et al.* and Li., *et al.* showed a similar distribution except for a higher percentage of tumours in grade 3 [14,15]. The pathologic staging in our study showed a different trend, with Jesus., *et al.* showing a peak in patients with stage IV, followed by stages III, I and II [11].

The colorectal cancer cases were classified based on the percentage of infiltrating margins, which has not been studied previously. Increased percentage of infiltrating margins was seen to be associated with higher tumour grade although the association was not significant.

The HER2 positivity obtained by us were similar to the results of Ochs and Li., *et al.* [12,15]. Gill., *et al.* demonstrated a cytoplasmic positivity of 57.5% though a combined cytoplasmic membranous pattern was seen only in 2.5% cases [13]. Wu., *et al.* got a positivity of 46.2%, majority of which were membranous, using anti- human polyclonal HER2 antibody [14]. This could be attributed to use of a polyclonal antibody or the ethnic differences between the study populations. Also, the criteria for the quantification of HER2 positivity have not been clearly stated in the study.

The studies that evaluated gene amplification with HER2 protein overexpression showed a low HER2 positivity similar to our study [9,10,16-18]. IHC may be affected by tumour fixation, type of the primary antibody, technique of antigen retrieval and the detection system. The scoring system also varies among different studies. However, FISH is the best method of detecting gene amplification having a higher sensitivity and specificity.

Seo., *et al.* [16], Ochs., *et al.* [12], Tu., *et al.* [18], Nathanson., *et al.* [17] and Jesus., *et al.* [11] found no association of HER2 positivity with aggressive clinicopathological features like TNM stage, invasion depth, lymph node status and distant metastasis similar to our study. However, few studies [10,13-15,19] showed a different trend with correlation between high HER2 expression and clinicopathologic variables. This could be attributed to a smaller sample size and racial differences. There could be a possible difference in pathogenesis of colorectal cancer in the Indian population in comparison to the other ethnic groups.

COX-2 positivity and its lack of correlation with histological parameters was in concordance with studies by Wen Wu and Zafirellis., *et al.* [20,21]. However Wu., *et al.* demonstrated a similar COX-2 positivity with positive correlation with clinicopathological variables [14].

Based on the data obtained in the study, Herceptin therapy cannot be advocated, as Trastuzumab is active against the membranous HER2 and not the cytoplasmic HER2. The findings are quite contrary to studies done on the Chinese population. However, to further validate the data, more studies need to be done with a greater sample size and standardization of the guidelines to grade HER2 staining in colorectal cancer.

Conclusion

Based on the data obtained in the study, in context of the Indian population, Herceptin therapy cannot be advocated, as Trastuzumab is active against the membranous HER2 and not the cytoplasmic HER2. The findings are quite contrary to the studies done on the Chinese population.

THE COX-2 positivity of the study was 96% suggesting that the COX-2 inhibitors like celecoxib can be utilised in the treatment of COX-2 positive colorectal cancers. However, more research is needed to see the effectiveness of celecoxib group of drugs in cancer therapy.

The two markers evaluated in the study- HER2 and COX-2 could not be correlated with tumour border configuration. Although a significant correlation could not be established, HER2 positivity was found to be associated with conventional adenocarcinoma and with higher stage of tumour. A trend was also seen between higher grade of tumour and increased percentage of infiltrating margins although it was not significant.

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