

Original Research Article

HISTOPATHOLOGICAL FEATURES OF GASTROINTESTINAL ADENOCARCINOMA AND HER-2 NEU EXPRESSION BY IMMUNOHISTOCHEMISTRY: A CROSS-SECTIONAL STUDY

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ABSTRACT

Background: Gastrointestinal (GI) adenocarcinomas are among the most common and lethal cancers worldwide. HER-2 neu overexpression is a validated therapeutic target, yet its prevalence across all GI adenocarcinoma subsites in an Indian tertiary care setting remains poorly characterized.

Materials and Methods: A cross-sectional, hospital-based study enrolled endoscopic biopsies and surgical resection specimens of GI adenocarcinomas over two years (January 2023-December 2024). Tumors were classified as per the 2019 WHO Classification. HER-2 neu status was assessed by immunohistochemistry (IHC) using a four-step Hoffmann scoring system (0, 1+, 2+, 3+), with score 3+ defined as positive and 2+ as equivocal.

Results: Out of the 252 GI specimens received, 41 were diagnosed as adenocarcinoma (n=41). Mean age was 62.5 years (SD 11.9); males predominated (70.7%; M:F ratio 2.4:1). Colorectal adenocarcinoma was the most common site (56.1%), followed by gastric (26.8%), small intestinal (9.8%), and esophageal (7.3%). Well-differentiated tumors accounted for 51.2%. HER-2 positivity (score 3+) was found in 6 cases (14.6%), equivocal staining (2+) in 12 (29.3%), and negativity in 23 (56.1%). Gastric adenocarcinoma showed the highest site-specific positivity (36.4%; 4 of 11 cases). All HER-2 positive gastric cases were intestinal-type as per Lauren's classification. Well-differentiated tumors had the highest positivity (5 of 6 positive cases).

Conclusion: HER-2 neu positivity was 14.6% across GI adenocarcinomas, consistent with published literature. Gastric adenocarcinoma showed the highest site-specific rate. Positivity correlated with intestinal histological type and well-differentiated grade. Routine HER-2 IHC testing across all GI adenocarcinoma subsites will help identify patients eligible for targeted anti-HER-2 therapy.

Keywords: Gastrointestinal adenocarcinoma; HER-2 neu; Immunohistochemistry; Gastric adenocarcinoma; Lauren's classification; Targeted therapy.

INTRODUCTION

Gastrointestinal (GI) malignancies are among the most common and lethal cancers worldwide. Their epidemiology is shifting, with rising incidence in several subsites driven by dietary changes, rising obesity rates, and evolving exposure to established risk factors.^[1] Colorectal cancer is the third most common cancer globally and the second leading

cause of cancer-related death, with over 1.9 million new cases recorded in 2018.3 Gastric adenocarcinoma ranks fourth in cancer mortality worldwide and is the second most common cause of cancer death in India, with incidence highest in East Asia, Eastern Europe, and South America.^[2] Esophageal and small bowel adenocarcinomas add to this burden, each with distinct pathogenetic pathways, risk profiles, and molecular characteristics.^[1]

Adenocarcinoma is the predominant histological type across all GI subsites, encompassing a spectrum of morphologies including tubular, papillary, mucinous, signet-ring cell and poorly cohesive types, classified according to the 2019 WHO Classification of Digestive System Tumors. Histological grade (well, moderate and poor differentiation) is a key prognostic parameter that also informs the likelihood of response to therapy. Accurate subtyping is clinically important, as particular morphological subtypes correlate with distinct molecular profiles and eligibility for biomarker-directed treatment.

One such biomarker is HER-2 (human epidermal growth factor receptor 2), encoded by the HER2 gene on chromosome 17q21. The HER-2 protein is a 185-kilodalton transmembrane tyrosine kinase receptor belonging to the epidermal growth factor receptor (EGFR/ErbB) family, comprising four members: HER1 (EGFR), HER2 (ErbB2), HER3, and HER4. Unlike other family members, HER-2 has no known direct-binding ligand; it functions as the preferred dimerization partner within the receptor family. When HER2 gene amplification drives receptor overexpression, constitutive dimerization and downstream signalling occur without ligand binding. HER2-EGFR heterodimers promote tumor cell proliferation and invasion; HER2-HER3 dimers amplify survival, metabolic activity, and motility.^[5] Sustained activation of these pathways leads to unchecked cell growth, reduced apoptosis, and enhanced invasiveness, the molecular basis of aggressive behavior in HER-2 positive tumors.

HER-2 overexpression or gene amplification has been identified in breast, ovarian and multiple GI malignancies.⁴ In GI adenocarcinomas, gastric cancer carries the highest documented HER-2 positivity rates, typically 10-20% by IHC, with lower rates in esophageal, colorectal and small bowel adenocarcinomas. HER-2 positivity in gastric cancer is most strongly associated with intestinal-type histology as per Lauren's classification and with chromosomally unstable molecular subtypes, the same groups most likely to harbor actionable gene amplification.

The clinical relevance of HER-2 in GI cancers was established by the Trastuzumab for Gastric Cancer (ToGA) trial, an international open-label randomized phase.^[3] study, which demonstrated that trastuzumab, a humanized anti-HER-2 monoclonal antibody, added to standard chemotherapy significantly prolonged overall survival in patients with HER-2 positive advanced gastric or gastroesophageal junction adenocarcinoma (median overall survival 13.8 versus 11.1 months).^[5] This trial established HER-2 testing as a prerequisite for treatment planning in advanced disease and opened the pathway for evaluating anti-HER-2 therapy across other GI subsites where HER-2 amplification has since been identified.

HER-2 status is most commonly assessed by IHC, which is widely available, reproducible when standardized, and cost-effective relative to

fluorescence in situ hybridization (FISH). In GI tumors, a validated four-step scoring system (0, 1+, 2+, 3+) applies distinct criteria for biopsy and resection specimens; strong, complete, or basolateral membranous staining in at least 10% of tumor cells defines a score of 3+ (positive).^[4] Score 2+ (equivocal) requires reflex FISH testing. Accurate application of these criteria is essential: misclassification denies eligible patients access to proven treatment or exposes ineligible patients to unnecessary drug toxicity.

Despite the established utility of HER-2 testing, comprehensive data from the Indian subcontinent encompassing all GI adenocarcinoma subsites in a single study are limited. Most published Indian series focus on gastric adenocarcinoma in isolation. Understanding the full landscape of HER-2 expression across esophageal, gastric, small bowel, and colorectal adenocarcinomas is increasingly important as the therapeutic indication for anti-HER-2 agents expands beyond gastric cancer to include HER-2 amplified colorectal and other GI cancers. A cross-site analysis provides a more complete picture of the therapeutic opportunity available in this population.

This study was undertaken to document the histopathological features of GI adenocarcinomas from all four subsites at a tertiary care teaching hospital and to evaluate HER-2 neu expression by IHC in relation to patient demographics, anatomical site, histological grade, and tumor histological type, with the aim of identifying subgroups most likely to benefit from anti-HER-2 targeted therapy.

MATERIALS AND METHODS

The present cross-sectional, analytical, hospital-based study was conducted in the Department of Pathology, Bharati Vidyapeeth (Deemed to be University) over a period of 2 years. Endoscopic biopsies and surgical resection specimens of gastrointestinal tract tumours diagnosed as adenocarcinoma on histopathology were included. Approval was obtained from the institutional research ethics committee for the storage and future research use of hospital records, requisition forms, slides and paraffin blocks, along with the relevant clinical details.

The sample size was calculated by power analysis for a single proportion using SPSS, applying the formula $n = Z^2PQ/d^2$, with a prevalence (P) of 0.5783, Q of 0.4217, an absolute precision (d) of 0.2 and a two-tailed level of significance of 5% ($Z = 1.96$). This yielded a minimum required sample size of 23 cases. All specimens received in the department were fixed in 10% neutral buffered formalin. After gross examination, the required number of sections were taken, processed and embedded in paraffin according to standard protocol. Sections of 3–4 μ m thickness were stained with hematoxylin and eosin. Based on gross and microscopic architectural features, lesions

diagnosed as adenocarcinoma according to the WHO Classification of Digestive System Tumours (2019) were selected. All slides were reviewed and tumor characteristics were recorded. Representative paraffin blocks were then retrieved from the departmental archives for immunohistochemical (IHC) staining for HER-2/neu, performed according to the institutional standard operating procedures.

For IHC, sections were cut onto charged slides, incubated at 60–70°C for one hour, deparaffinized in xylene and rehydrated through graded alcohols to distilled water. Heat-induced antigen retrieval was performed in a microwave retrieval system for 15–20 minutes using a buffer of appropriate pH. Endogenous peroxidase activity was blocked with hydrogen peroxide, after which the primary antibody was applied for 30 minutes in a moist chamber. Detection was carried out using the PolyExcel target binder and HRP polymer system, followed by visualisation with DAB chromogen. Sections were counterstained with hematoxylin, dehydrated, cleared and mounted. Buffer washes were performed between successive steps. A known case of breast carcinoma with HER-2/neu overexpression served as the positive control in all runs.

HER-2/neu expression was interpreted using the Hoffmann scoring system in accordance with College of American Pathologists guidelines, on a four-tier scale. Staining in fewer than 10% of tumor cells was scored as 0, faint incomplete membranous or basolateral staining in more than 10% of cells as 1+, weak to moderate complete or basolateral staining in more than 10% of cells as 2+, and strong complete or

basolateral membranous staining in 10% or more of cells as 3+. In biopsy specimens, a cluster of at least five stained evaluable tumor cells was required for scoring. Scores of 0 and 1+ were considered negative, 2+ equivocal and 3+ positive for HER-2/neu overexpression. HER-2/neu expression was then compared with histopathological features of the tumor, with particular reference to the degree of differentiation, special histological type and anatomical site.

Inclusion Criteria

- Endoscopic biopsies of gastrointestinal tract diagnosed as adenocarcinoma on histopathology
- Wide excision, segmental resection, and radical resection specimens from these sites diagnosed as adenocarcinoma

Exclusion Criteria

- All benign tumours of the gastrointestinal tract
- All malignant tumours of the gastrointestinal tract other than adenocarcinoma
- Carcinomas involving the anal canal

RESULTS

Out of the 252 gastrointestinal tract specimens received during the two-year study period, 41 were histopathologically diagnosed as adenocarcinoma and formed the study cohort.

The study population ranged in age from 27 to 79 years, with the 61–70-year group accounting for the largest proportion of cases. Males constituted 70.7% of cases, with a male-to-female ratio of 2.4:1 [Table 1].

Table 1: Age and gender distribution of the study population (n=41).

Variable	n	Percentage (%)
Age distribution (years)		
<=30	1	2.4
31-40	2	4.9
41-50	2	4.9
51-60	10	24.4
61-70	17	41.5
71-80	9	22.0
Total	41	100.0
Mean +/- SD (years)	62.5 +/- 11.9	
Gender distribution		
Male	29	70.7
Female	12	29.3
Total	41	100.0

Colorectal adenocarcinoma was the most frequent site, accounting for more than half of all cases, followed by gastric adenocarcinoma [Table 2].

Table 2: Site-wise distribution of gastrointestinal adenocarcinomas (n=41).

Anatomical Site	n	Percentage (%)
Esophagus	3	7.3
Stomach	11	26.8
Small intestine	4	9.8
Colorectum	23	56.1
Total	41	100.0

Well-differentiated adenocarcinoma was the predominant grade across all sites [Table 3].

Table 3: Distribution of gastrointestinal adenocarcinomas by histological grade (n=41).

Histological Grade	n	Percentage (%)
Well differentiated	21	51.2
Moderately differentiated	14	34.2
Poorly differentiated	6	14.6
Total	41	100.0

Site-wise grade analysis showed that well-differentiated tumors predominated in the colorectal group, while all three esophageal cases were moderately differentiated [Table 4].

Table 4: Site-wise histological grade distribution of gastrointestinal adenocarcinomas (n=41).

Site	Well differentiated n (%)	Moderately differentiated n (%)	Poorly differentiated n (%)	Total
Esophagus	0 (0)	3 (7.3)	0 (0)	3
Stomach	5 (12.2)	3 (7.3)	3 (7.3)	11
Small intestine	3 (7.3)	1 (2.4)	0 (0)	4
Colorectum	13 (31.7)	7 (17.1)	3 (7.3)	23
Total	21 (51.2)	14 (34.1)	6 (14.6)	41

On IHC analysis, HER-2 neu was positive (score 3+) in 6 cases (14.6%), equivocal (score 2+) in 12 (29.3%), and negative (score 0 or 1+) in 23 (56.1%) [Table 5].

Table 5: HER-2 neu IHC score distribution in gastrointestinal adenocarcinomas (n=41). Scoring by Hoffmann 4-step criteria; score 0/1+ = negative, 2+ = equivocal, 3+ = positive.

IHC Score	Interpretation	n	Percentage (%)
0	Negative	14	34.1
1+	Negative	9	22.0
2+	Equivocal	12	29.3
3+	Positive	6	14.6
Total		41	100.0

Gastric adenocarcinoma had the highest site-specific HER-2 positivity rate; esophageal adenocarcinoma showed no positive cases in this series [Table 6].

Table 6: HER-2 neu expression by anatomical site (n=41). Hoffmann IHC scoring criteria applied.

Site	Total n	HER-2 Negative/Equivocal n (%)	HER-2 Positive (3+) n (%)	Positivity rate (%)
Esophagus	3	3 (100.0)	0 (0)	0
Stomach	11	7 (63.6)	4 (36.4)	36.4
Small intestine	4	3 (75.0)	1 (25.0)	25.0
Colorectum	23	22 (95.7)	1 (4.3)	4.3
Total	41	35 (85.4)	6 (14.6)	14.6

Well-differentiated tumors showed the highest HER-2 positivity rate; no poorly differentiated tumor was positive [Table 7].

Table 7: HER-2 neu expression by histological grade (n=41). Hoffmann 4-step IHC scoring criteria applied.

Grade	Total n	Negative (0/1+) n (%)	Equivocal (2+) n (%)	Positive (3+) n (%)
Well differentiated	21	9 (42.9)	7 (33.3)	5 (23.8)
Moderately differentiated	14	11 (78.6)	2 (14.3)	1 (7.1)
Poorly differentiated	6	3 (50.0)	3 (50.0)	0 (0)
Total	41	23 (56.1)	12 (29.3)	6 (14.6)

Among the 11 gastric adenocarcinoma cases, 8 were intestinal type and 3 were diffuse type per Lauren's classification. All four HER-2 positive gastric cases were intestinal type; none of the diffuse-type cases tested positive [Table 8].

Table 8: HER-2 neu expression in gastric adenocarcinoma by histological type per Lauren's classification (n=11). Hoffmann IHC scoring criteria applied.

Histological Type (Lauren's classification)	Total n	HER-2 Negative/Equivocal n (%)	HER-2 Positive (3+) n (%)	Positivity rate (%)
Intestinal type	8	4 (50.0)	4 (50.0)	50.0
Diffuse type	3	3 (100.0)	0 (0)	0
Total	11	7 (63.6)	4 (36.4)	36.4

DISCUSSION

GI adenocarcinomas frequently present at advanced stages, often because early-stage disease produces no distinctive symptoms. Identifying molecular markers that guide therapy is therefore a clinical priority. HER-2 neu holds particular value among available markers given the proven survival benefit of trastuzumab in HER-2 positive gastric cancer. As anti-HER-2 agents continue to receive approval across additional GI subsites, accurate HER-2 assessment across the full GI tract becomes increasingly relevant to patient selection.

The mean age of GI adenocarcinoma patients in this study was 62.5 years, with the 61–70-year group accounting for the largest proportion of cases (41.5%). Shabbir A et al⁶ reported a considerably lower mean age of 46 years in a South Asian gastric cancer cohort, a difference that may reflect regional variation in *H. pylori* exposure patterns, tumor subtype distribution, or access to endoscopic screening. In contrast, Sayadnejad N et al,⁷ reported a mean of 60.42 years, Gupta P et al⁸ found 57.1 years in an Indian gastric adenocarcinoma series, and Rajagopal I et al,⁹ observed a mean of 65.65 years in gastric and gastroesophageal junction adenocarcinomas, findings broadly consistent with the present study.

Males accounted for 70.7% of cases (M:F ratio 2.4:1). Male predominance in GI adenocarcinoma is a consistent finding across published series,^{6–9} likely reflecting sex-based differences in exposure to tobacco use, alcohol consumption, *H. pylori* infection, and high-salt diets.

Colorectal adenocarcinoma was the most frequent site (56.1%), followed by gastric (26.8%), small intestinal (9.8%), and esophageal (7.3%). This pattern reflects the global distribution of GI cancer incidence. The predominance of colorectal adenocarcinoma in this hospital-based cohort is consistent with the rising colorectal cancer incidence trend observed in India, attributed in part to dietary westernization and reduced physical activity.

Well-differentiated adenocarcinoma was the most common grade (51.2%), followed by moderate (34.1%) and poor differentiation (14.6%). The predominantly well and moderately differentiated profile may reflect the high proportion of intestinal-type colorectal and gastric adenocarcinomas in the cohort, as these subtypes tend toward glandular differentiation.

HER-2 positivity (IHC score 3+) was found in 6 of 41 cases (14.6%). This rate falls within the range consistently reported in comparable studies. Parasa GD et al,¹⁰ found 11.26% positivity in a mixed GI adenocarcinoma cohort at an Indian tertiary care hospital, which most closely mirrors the design of the present study. Marx AH et al,¹¹ reported 16% positivity in gastric cancer. Gomez-Martin C et al¹² found 10.1%, and Kunz PL et al,¹³ found 11.24% in gastric and gastroesophageal junction

adenocarcinomas. These results collectively confirm that HER-2 positivity across GI adenocarcinomas typically falls within the 10–16% range, and the present finding of 14.6% aligns with this estimate. The equivocal rate (score 2+) was 29.3%; FISH testing would be required for these cases before clinical decisions are made.

Gastric adenocarcinoma showed the highest site-specific HER-2 positivity (36.4%; 4 of 11 cases), followed by small intestinal adenocarcinoma (25%; 1 of 4) and colorectal adenocarcinoma (4.3%; 1 of 23). No esophageal case was positive, though the esophageal subgroup was small (n=3). The gastric predominance is consistent with previously published Indian data reporting positivity rates of 24.5% and 26.7% in gastric adenocarcinoma, respectively.^{8,9}

All four HER-2 positive gastric adenocarcinomas were intestinal-type as per Lauren's classification; none of the three diffuse-type cases was positive. Hofmann M et al,¹⁴ established in their validation study that HER-2 positivity is predominantly found in intestinal-type gastric tumors (83.3% versus 26% in diffuse type). This has been corroborated in subsequent series reporting that over 90% of HER-2 positive gastric cancers are intestinal type.¹¹ The intestinal subtype is characterized by chromosomal instability and EGFR pathway activation, the molecular context that creates susceptibility to HER2 gene amplification. Diffuse-type tumors arise through a distinct oncogenic pathway and are very unlikely to harbor HER-2 overexpression. Lauren's subtyping should therefore accompany HER-2 IHC in all gastric adenocarcinoma reports, to optimize resource allocation and focus molecular testing on the subtype with meaningful positivity rates.

Five of the six HER-2 positive cases (83.3%) were well-differentiated, with one moderately differentiated case; no poorly differentiated tumor was positive. Among gastric adenocarcinomas specifically, 75% of positive cases were well-differentiated. Dewan K et al,¹⁵ reported comparable findings in Indian gastric carcinoma, with well-differentiated tumors showing the highest HER-2 positivity. This pattern is mechanistically consistent with the predominance of intestinal-type morphology, which tends toward better glandular differentiation, in HER-2 positive gastric cancer.

CONCLUSION

HER-2 neu positivity was found in 14.6% of GI adenocarcinomas in this study, consistent with published literature. Gastric adenocarcinoma carried the highest site-specific positivity rate (36.4%), with all positive gastric cases being intestinal-type by Lauren's classification. Well-differentiated tumors showed the greatest HER-2 positivity. These findings support routine HER-2 IHC testing across all GI adenocarcinoma subsites, not restricted to gastric cancer, to identify the full proportion of patients

eligible for anti-HER-2 targeted therapy. Larger prospective multicenter studies with FISH confirmation of equivocal cases are warranted.

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