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# HER2/NEU EXPRESSION IN COLORECTAL CARCINOMA: PREVALENCE AND ASSOCIATION WITH CLINICOPATHOLOGICAL CHARACTERISTICS IN A PAKISTANI COHORT

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## ABSTRACT

Colorectal cancer (CRC) represents a major and growing global health burden. Recent international cancer estimates place CRC among the most frequently diagnosed malignancies worldwide and among the leading causes of cancer-related mortality. The global burden is expected to increase substantially over coming decades, particularly in countries undergoing demographic, dietary, and lifestyle transitions [1]. Contemporary GLOBOCAN estimates continue to identify colorectal cancer as one of the three most commonly diagnosed cancers globally, emphasizing the importance of improving its prevention, molecular characterization, and treatment [1]. (IARC)

## INTRODUCTION

Colorectal carcinoma is a biologically heterogeneous disease characterized by considerable variation in its molecular alterations, histopathological characteristics, clinical behavior, and therapeutic response. Traditional clinicopathological parameters, including tumor location, histological type and grade, depth of invasion, lymph-node involvement, distant metastasis, lymphovascular invasion, and perineural invasion, remain fundamental for staging and clinical decision-making. However, advances in precision oncology have increasingly demonstrated that tumors with apparently similar morphological and pathological characteristics may possess substantially different molecular profiles and consequently respond differently to systemic therapy. Molecular characterization has therefore become increasingly integrated into the management of advanced CRC [2,3]. (PubMed)

Among the emerging molecular targets in CRC, human epidermal growth factor receptor 2 (HER2/neu) has attracted considerable attention. HER2, encoded by the *ERBB2* gene, is a member of the epidermal growth factor receptor family of transmembrane receptor tyrosine kinases. Activation of HER2-associated signaling promotes cellular proliferation, differentiation, and survival. Although HER2 overexpression and amplification are well-established therapeutic biomarkers in breast and gastric cancers, their clinical significance in CRC has historically been less clearly defined. Increasing evidence, however, supports the existence of a distinct HER2-positive molecular subgroup of CRC with potential therapeutic relevance [4,5]. (PubMed)

HER2 positivity occurs in only a minority of colorectal carcinomas, and reported prevalence depends considerably on the population studied, molecular characteristics of the tumors, assay methodology, and criteria used to define positivity. A recent systematic review and meta-analysis incorporating data from 52 studies using approved HER2 assays estimated an overall HER2-positive prevalence of approximately 4.1% in CRC. Importantly, HER2 positivity was enriched among RAS wild-type tumors, where the estimated prevalence increased to approximately 6.1%, and was also associated with microsatellite-stable and left-sided disease [4]. Large clinicopathological series have similarly demonstrated relatively low but clinically meaningful frequencies of HER2 overexpres sion and/or amplification [6]. (PubMed)

An important challenge is that assessment of HER2 in CRC has not historically been as standardized as in breast carcinoma. Immunohistochemistry (IHC) provides an accessible approach for evaluating HER2 protein expression in formalin-fixed paraffin-embedded tumor tissue, but interpretation may vary according to staining intensity, completeness of membranous staining, percentage of positive tumor cells, and the scoring system employed. Comparative investigations of IHC and in situ hybridization have demonstrated that the choice of scoring criteria can influence classification of HER2 status [7]. More recent evidence has therefore emphasized the need for unified and

CRC-specific approaches to HER2 IHC interpretation to improve reproducibility and identification of clinically relevant HER2-positive tumors [8]. (PubMed)

The importance of accurately identifying HER2-positive CRC has increased considerably with the development of HER2-directed therapies. HER2 is no longer merely an investigational pathological marker in advanced CRC but represents an actionable therapeutic alteration in appropriately selected patients. The phase II MOUNTAINEER study demonstrated clinically meaningful antitumor activity of combined tucatinib and trastuzumab in previously treated HER2-positive, RAS wild-type unresectable or metastatic CRC [9]. These findings subsequently supported regulatory approval of tucatinib in combination with trastuzumab for previously treated unresectable or metastatic RAS wild-type, HER2-positive CRC [10]. Contemporary reviews and clinical guidance therefore recognize HER2 testing as increasingly relevant to molecular stratification of metastatic CRC [2,5]. (PubMed)

Despite its emerging therapeutic relevance, the relationship between HER2 expression and conventional clinicopathological characteristics remains incompletely defined. Studies have reported differences in HER2 expression according to tumor location, RAS status, microsatellite status and other tumor characteristics, while the frequency of HER2-positive disease also varies considerably across populations and testing methodologies [4,6,7]. Recent institution-based studies continue to investigate the relationship between HER2 immunohistochemical expression and pathological characteristics, indicating that its clinicopathological significance remains an active area of investigation [11]. (PubMed)

Data regarding HER2 expression in CRC from Pakistan and comparable resource-constrained settings remain relatively limited. Establishing the local frequency of HER2 expression and determining its relationship with routinely available clinicopathological characteristics may therefore provide useful regional evidence. Such information could help characterize the HER2-positive CRC phenotype in the local population and provide a pathological basis for identifying patients who may warrant additional molecular evaluation as HER2-directed treatment strategies become increasingly incorporated into precision oncology.

Therefore, the present study aimed to determine the frequency of HER2/neu expression by immunohistochemistry in colorectal carcinoma and to evaluate its association with clinicopathological characteristics in a Pakistani cohort.

## MATERIALS AND METHODS

### Study Design and Participants

This retrospective cross-sectional study was conducted in the Department of Histopathology, Liaquat National Hospital, Karachi, over a period of six months following approval of the study protocol. The study included histologically confirmed cases of colorectal carcinoma identified from departmental records and archived formalin-fixed paraffin-embedded (FFPE) tissue blocks. The sample size was calculated using the WHO sample size calculator based on an anticipated HER2 positivity of 5%, a 95% confidence level, and a 5% margin of error. The minimum calculated sample size was 73 cases; however, 80–85 cases were planned to accommodate potential exclusions. A non-probability consecutive sampling technique was employed.

Adult patients of either sex with histologically confirmed colorectal carcinoma, available well-fixed tissue, and no history of preoperative treatment were eligible for inclusion. Both colorectal resection specimens and colonoscopic biopsies were considered. Poorly fixed specimens and post-treatment resection specimens were excluded.

Following approval from the Institutional Ethical Review Committee, eligible archival FFPE blocks were retrieved. Demographic and clinicopathological information was obtained from the corresponding pathology records and recorded on a standardized data collection proforma. The recorded variables included age, sex, tumor site, histological grade, pathological TNM stage, lymph-node status, and other available histopathological characteristics. Patient confidentiality was maintained throughout the study, and no direct patient contact was involved because the study utilized archived tissue and previously recorded clinical information.

### Histopathological and Immunohistochemical Evaluation

Existing hematoxylin and eosin (H&E)-stained sections were reviewed by the principal investigator and confirmed by a senior consultant histopathologist to verify the diagnosis and ensure the presence of representative viable tumor tissue. Tumors were categorized according to their anatomical location as right-sided colon, left-sided colon, or rectum. Histological grading was based on the extent of glandular formation, with tumors categorized as well differentiated (grade I), moderately differentiated (grade II), or poorly differentiated (grade III). Pathological staging was assessed according to the AJCC/UICC TNM system, incorporating the depth of primary tumor invasion ( pT), regional lymph-node involvement (pN), and distant metastasis (pM) when available. Lymphovascular invasion was defined by the presence of tumor cells within endothelial-lined lymphatic or blood vessels, whereas perineural invasion was defined by tumor involvement within or surrounding a nerve sheath.

Representative FFPE tumor blocks were selected for HER2/neu immunohistochemical staining using a ready-to-use HER2 antibody. HER2 expression was evaluated predominantly on the basis of lateral or basolateral membranous staining of tumor cells. Immunoreactivity was categorized using a four-tier scoring system: score 0, no staining; 1+, weak lateral or basolateral membranous staining; 2+, moderate lateral or basolateral membranous staining; and 3+,

strong complete or basolateral membranous staining. According to the predefined study criteria, scores of 2+ and 3+ were classified as HER2-positive, whereas scores of 0 and 1+ were considered HER2-negative.
All immunohistochemical findings were documented on the study proforma and subsequently correlated with the available clinicopathological characteristics. Standardized criteria for case selection, histopathological review, immunostaining, and interpretation were applied to minimize potential measurement and selection bias. The original protocol specifically states that H&E slides would be reviewed and representative blocks selected before standardized HER2 staining and scoring.

**Statistical Analysis**
Data will be analyzed using IBM SPSS Statistics [version to be inserted]. Continuous variables such as age will be summarized as mean ± standard deviation when normally distributed or median and interquartile range where appropriate. Categorical variables, including sex, tumor site, histological grade, TNM stage, lymph-node status, lymphovascular invasion, perineural invasion, and HER2/neu expression, will be presented as frequencies and percentages.
The overall frequency of HER2/neu expression will be calculated with an appropriate 95% confidence interval. HER2 IHC scores (0, 1+, 2+, and 3+) will initially be described separately to demonstrate the complete distribution of immunohistochemical expression. For the primary association analysis, cases will subsequently be categorized as HER2-negative (0/1+) and HER2-positive (2+/3+) according to the predefined study criteria. Associations between HER2 status and categorical clinicopathological characteristics will be evaluated using the Pearson chi-square test or Fisher's exact test where expected cell frequencies are small. Age will be compared between HER2 -positive and HER2-negative groups using an independent-samples *t*-test or Mann–Whitney *U* test, depending on data distribution. If the number of HER2-positive cases is sufficient to support reliable multivariable modelling, binary logistic regression will be performed to identify clinicopathological characteristics independently associated with HER2 positivity. Results will be reported as odds ratios (ORs) with 95% confidence intervals. A two-sided p-value $<0.05$ will be considered statistically significant.

**RESULTS**
A total of 90 patients with histologically confirmed colorectal adenocarcinoma were included. The mean age was 48.77 ± 13.10 years, with an age range of 20–70 years. Females constituted 53 (58.9%) cases and males 37 (41.1%). Moderately differentiated adenocarcinoma was the predominant histological grade, accounting for 65 (72.2%) cases, followed by poorly differentiated carcinoma in 13 (14.4%) and well-differentiated carcinoma in 6 (6.7%). Histological grade was recorded as not applicable in six cases.
Among the 67 cases with assessable pathological nodal status, 27 (40.3%) were N0, while 20 (29.9%) each were N1 and N2. Pathological T stage was similarly assessable in 67 cases; T3 was the predominant category (53/67, 79.1%), followed by T4 (8/67, 11.9%), T2 (5/67, 7.5%), and T1 (1/67, 1.5%). The non-assessable T and N entries corresponded predominantly to biopsy specimens and were not treated as pathological stage categories.
Specimen type was available for 89 cases. Of these, 66 (74.2%) were resection specimens and 23 (25.8%) were biopsy specimens.

**Table 1. Demographic and clinicopathological characteristics of the study population**

| Characteristic | n (%) |
|---|---|
| **Total cases** | **90 (100)** |
| **Age, years, mean ± SD** | 48.77 ± 13.10 |
| **Age range, years** | 20–70 |
| **Gender** | |
| **Female** | 53 (58.9) |
| **Male** | 37 (41.1) |
| **Histological grade** | |
| **Well differentiated** | 6 (6.7) |
| **Moderately differentiated** | 65 (72.2) |
| **Poorly differentiated** | 13 (14.4) |
| **Not applicable** | 6 (6.7) |
| **Pathological T stage*** | |
| **T1** | 1 (1.5) |
| **T2** | 5 (7.5) |
| **T3** | 53 (79.1) |
| **T4** | 8 (11.9) |
| **Pathological N stage*** | |

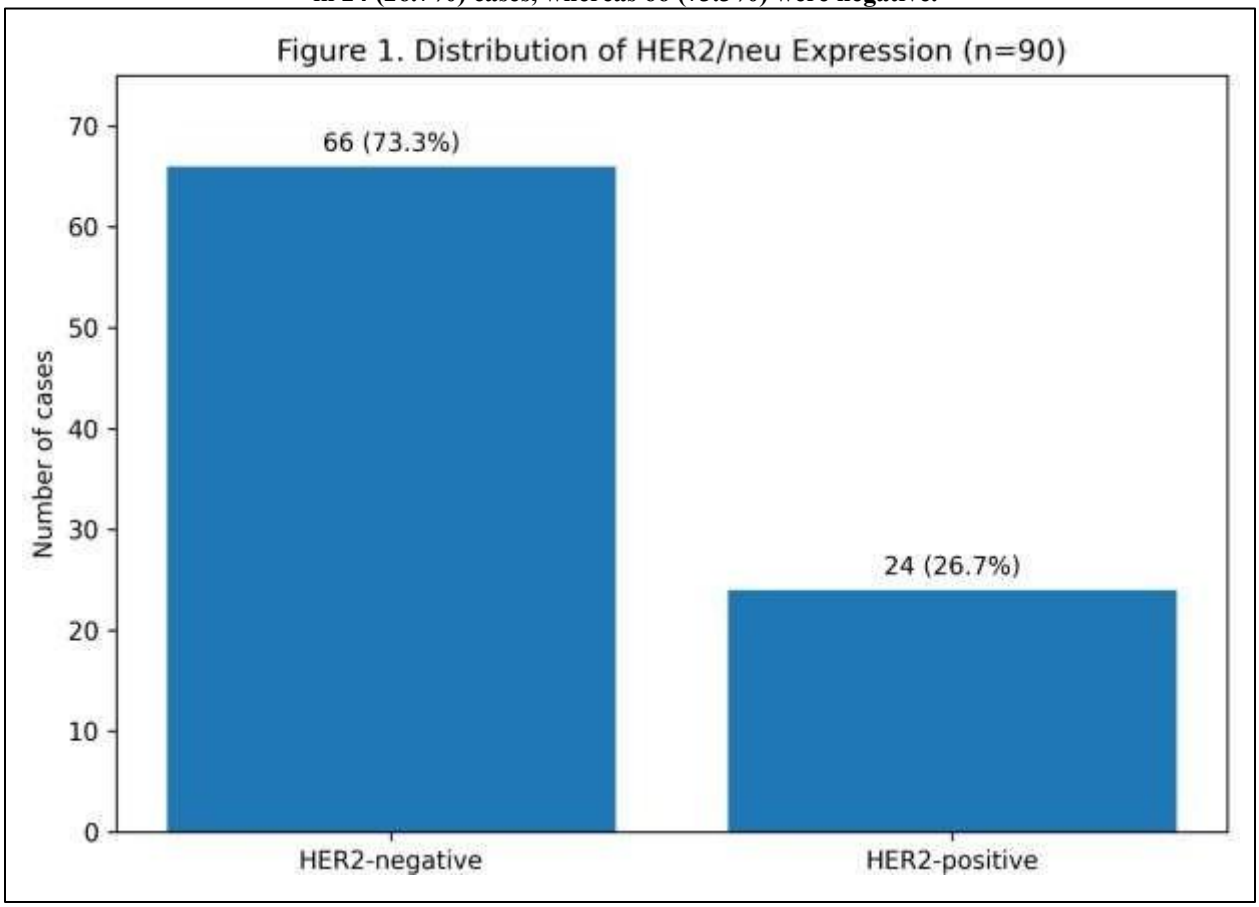
| | |
|---|---|
| **N0** | 27 (40.3) |
| **N1** | 20 (29.9) |
| **N2** | 20 (29.9) |
| **Specimen type†** | |
| **Resection** | 66 (74.2) |
| **Biopsy** | 23 (25.8) |

*Percentages calculated among 67 cases with assessable pathological staging.
†Specimen type available for 89 cases.

**HER2/neu Expression**

HER2/neu expression was positive in 24 of 90 cases (26.7%), while 66 (73.3%) were HER2-negative. The approximate 95% confidence interval for HER2 positivity was 18.6%–36.6%.

**Figure 1. Distribution of HER2/neu expression in colorectal adenocarcinoma. HER2 positivity was identified in 24 (26.7%) cases, whereas 66 (73.3%) were negative.**




**Association of HER2/neu Expression with Demographic and Histopathological Characteristics**

The mean age of HER2-positive patients was 49.13 ± 13.24 years, compared with 48.64 ± 13.15 years among HER2-negative patients, with no significant difference between the groups (p=0.887).

HER2 positivity was observed in 11/37 (29.7%) males and 13/53 (24.5%) females, with no significant association between sex and HER2 expression (p=0.633).

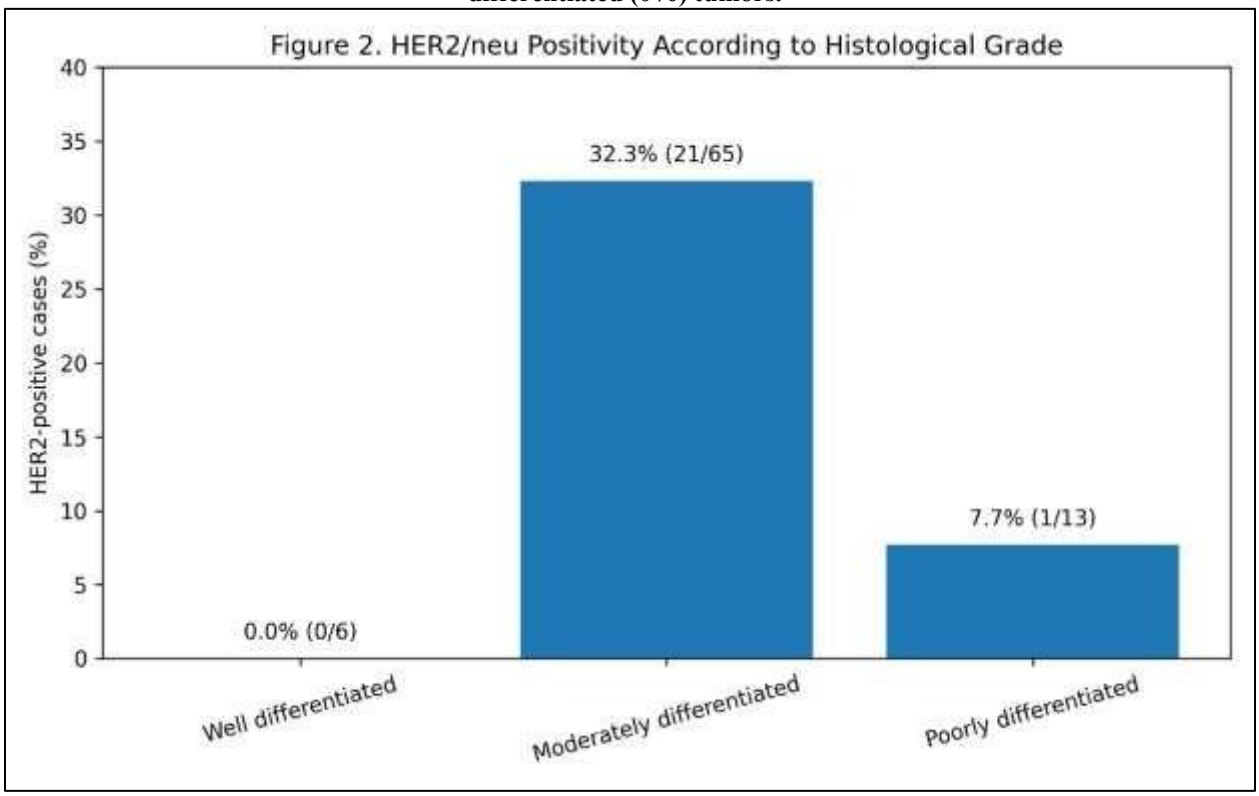
Histological grade demonstrated the most noticeable variation in HER2 expression. HER2 positivity was identified in 21/65 (32.3%) moderately differentiated tumors, compared with 1/13 (7.7%) poorly differentiated tumors and none of the six well-differentiated tumors. The overall three-category association approached but did not reach statistical significance (p=0.080).

**Table 2. Association of HER2/neu expression with demographic and clinicopathological characteristics**

| Variable | HER2 Positive | HER2 Negative | p-value |
|---|---|---|---|
| **Age, mean ± SD** | 49.13 ± 13.24 | 48.64 ± 13.15 | 0.887 |
| **Gender** | | | **0.633** |

| | | | |
|---|---|---|---|
| **Female** | 13/53 (24.5%) | 40/53 (75.5%) | |
| **Male** | 11/37 (29.7%) | 26/37 (70.3%) | |
| **Histological grade** | | | **0.080** |
| **Well differentiated** | 0/6 (0.0%) | 6/6 (100%) | |
| **Moderately differentiated** | 21/65 (32.3%) | 44/65 (67.7%) | |
| **Poorly differentiated** | 1/13 (7.7%) | 12/13 (92.3%) | |
| **N stage** | | | **0.816** |
| **N0** | 6/27 (22.2%) | 21/27 (77.8%) | |
| **N1** | 6/20 (30.0%) | 14/20 (70.0%) | |
| **N2** | 4/20 (20.0%) | 16/20 (80.0%) | |
| **T stage** | | | **0.397** |
| **T1** | 1/1 (100%) | 0/1 (0.0%) | |
| **T2** | 1/5 (20.0%) | 4/5 (80.0%) | |
| **T3** | 13/53 (24.5%) | 40/53 (75.5%) | |
| **T4** | 1/8 (12.5%) | 7/8 (87.5%) | |

**Figure 2. HER2/neu positivity according to histological grade. The highest HER2 positivity was observed among moderately differentiated tumors (32.3%), compared with poorly differentiated (7.7%) and well-differentiated (0%) tumors.**




**HER2 Expression According to Pathological Disease Extent**

To provide clinically more interpretable comparisons and reduce sparse individual-stage categories, pathological T and N stages were additionally dichotomized.

Among the six tumors classified as T1–T2, 2 (33.3%) were HER2-positive, compared with 14/61 (23.0%) T3–T4 tumors. Advanced T stage was not significantly associated with HER2 positivity (OR 0.60, 95% CI 0.10–3.60; p=0.623).

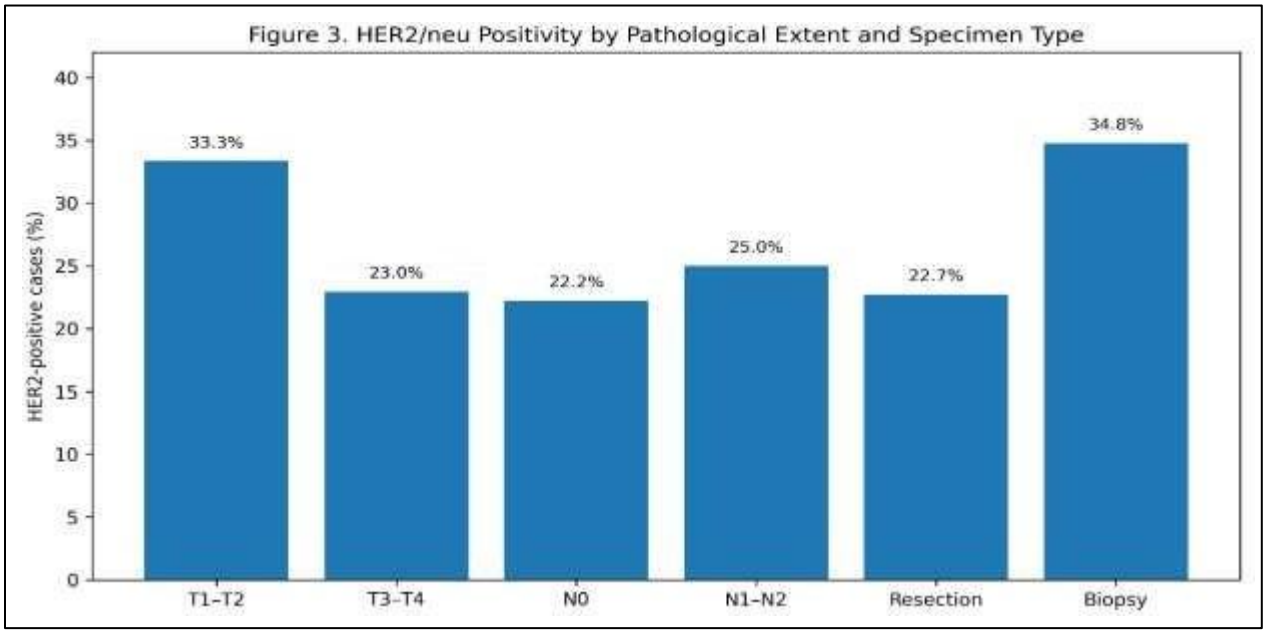
Among node-negative tumors, 6/27 (22.2%) were HER2-positive, compared with 10/40 (25.0%) node-positive (N1–N2) tumors. Nodal positivity was not associated with HER2 expression (OR 1.17, 95% CI 0.37–3.71; p=1.000).

HER2 positivity was numerically higher among biopsy specimens (8/23, 34.8%) than resection specimens (15/66, 22.7%). Biopsy specimens demonstrated an OR of 1.81 (95% CI 0.65–5.10) for HER2 positivity relative to resections; however, the difference was not statistically significant (p=0.278).

**Table 3. HER2/neu expression according to pathological disease extent and specimen type**

| Variable | HER2 Positive | HER2 Negative | OR (95% CI) | p-value |
|---|---|---|---|---|
| **T stage** | | | | |
| **T1–T2** | 2/6 (33.3%) | 4/6 (66.7%) | Reference | |
| **T3–T4** | 14/61 (23.0%) | 47/61 (77.0%) | 0.60 (0.10–3.60) | 0.623 |
| **Nodal status** | | | | |
| **N0** | 6/27 (22.2%) | 21/27 (77.8%) | Reference | |
| **N1–N2** | 10/40 (25.0%) | 30/40 (75.0%) | 1.17 (0.37–3.71) | 1.000 |
| **Specimen type** | | | | |
| **Resection** | 15/66 (22.7%) | 51/66 (77.3%) | Reference | |
| **Biopsy** | 8/23 (34.8%) | 15/23 (65.2%) | 1.81 (0.65–5.10) | 0.278 |

**Figure 3. HER2/neu positivity according to pathological T stage, nodal status, and specimen type. No statistically significant differences in HER2 expression were observed across these clinically grouped characteristics.**




**Relationship of Histological Grade with Pathological Extent**

An additional analysis was performed to characterize the pathological behavior of the cohort independently of HER2 expression. For this analysis, well- and moderately differentiated tumors were grouped together and compared with poorly differentiated tumors.

A notable association was identified between histological grade and nodal involvement. All 9 assessable poorly differentiated tumors were node-positive (100%), compared with 30/57 (52.6%) well/moderately differentiated tumors. The association was statistically significant (Fisher's exact p=0.008).

In contrast, histological differentiation was not significantly associated with dichotomized pathological T stage. All nine assessable poorly differentiated tumors were T3–T4; however, T3–T4 disease was also highly prevalent among well/moderately differentiated tumors (51/57, 89.5%), and the difference was not significant (p=0.585).

**Table 4. Relationship between histological differentiation and pathological disease extent**

| Pathological characteristic | Well/Moderately differentiated | Poorly differentiated | p-value |
|---|---|---|---|
| **Nodal status** | | | **0.008** |
| **N0** | 27 (47.4%) | 0 (0%) | |
| **N1–N2** | 30 (52.6%) | 9 (100%) | |
| **T stage** | | | **0.585** |
| **T1–T2** | 6 (10.5%) | 0 (0%) | |
| **T3–T4** | 51 (89.5%) | 9 (100%) | |

HER2/neu immunohistochemical positivity was identified in approximately one-quarter (26.7%) of the study population. HER2 expression was not significantly associated with age, sex, pathological T stage, nodal stage, or

specimen type. Histological grade demonstrated the strongest HER2-related trend, although it did not reach statistical significance. Importantly, independent characterization of the cohort demonstrated a significant association between poor histological differentiation and lymph-node positivity (p=0.008), with all assessable poorly differentiated tumors showing nodal involvement.

**DISCUSSION**

The present study evaluated HER2/neu expression in 90 cases of colorectal adenocarcinoma and examined its relationship with available clinicopathological characteristics. HER2/neu positivity was identified in 24 cases, corresponding to an overall frequency of 26.7%. No statistically significant association was observed between HER2 status and age, sex, lymph-node status, or pathological T stage. Histological grade demonstrated the most noticeable variation in HER2 expression, with positivity occurring predominantly among moderately differentiated tumors; however, this association did not reach statistical significance (p=0.080). These findings highlight the heterogeneity of HER2 expression in colorectal carcinoma and emphasize the importance of assay methodology and scoring criteria when interpreting reported prevalence.

The frequency of HER2 positivity observed in the present study was considerably higher than estimates reported in contemporary studies using more restrictive or molecularly confirmed definitions of HER2-positive colorectal carcinoma. A recent systematic review and meta-analysis of 52 studies involving 17,589 patients estimated an overall HER2-positive prevalence of approximately 4.1%, which increased to approximately 6.1% among RAS wild-type tumors [4]. Similarly, studies incorporating both immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) have demonstrated substantially lower frequencies of unequivocally HER2-positive disease than the frequency observed in our cohort [7]. This difference is clinically important because the prevalence of HER2-positive CRC varies according to the patient population, molecular background, assay methodology, and criteria used to define positivity [4,7].

The relatively high frequency of HER2 positivity in the current study should particularly be interpreted in the context of the predefined immunohistochemical scoring criteria. Both IHC 2+ and 3+ tumors were classified as HER2 - positive, without confirmatory in situ hybridization for IHC 2+ cases. Contemporary approaches generally distinguish unequivocal HER2 overexpression from equivocal immunoreactivity, with molecular confirmation playing an important role in selected IHC 2+ tumors. Sun et al. demonstrated that classification of HER2 status in colorectal carcinoma varied according to the IHC scoring system employed and that concordance between IHC and FISH was particularly relevant when interpreting intermediate staining [7]. More recent evidence has similarly emphasized the need for standardized criteria for HER2 assessment in colorectal carcinoma [8]. Therefore, the 26.7% positivity observed in our study is most appropriately interpreted as HER2 immunohistochemical positivity according to the predefined study criteria, rather than as the prevalence of molecularly confirmed ERBB2-amplified colorectal carcinoma.

Age was not associated with HER2 expression in the present study. The mean age of HER2-positive patients was 49.13 ± 13.24 years compared with 48.64 ± 13.15 years among HER2-negative patients (p=0.887). Similarly, HER2 positivity was somewhat more frequent among males than females (29.7% versus 24.5%), but this difference was not statistically significant (p=0.633). These findings suggest that basic demographic characteristics may have limited value for predicting HER2 status. Contemporary evidence indicates that HER2 positivity may instead be more strongly related to the molecular characteristics of colorectal carcinoma. In the recent meta-analysis, HER2 positivity was substantially more frequent among RAS wild-type tumors than RAS-mutant tumors and was also associated with microsatellite-stable and left-sided disease [4]. Thus, molecular characteristics may be more informative than age or sex in identifying patients likely to harbor clinically relevant HER2 alterations.

Histological differentiation demonstrated the most noteworthy clinicopathological pattern in the present study. HER2 positivity was detected in 21 of 65 moderately differentiated tumors (32.3%), compared with only one of 13 poorly differentiated tumors (7.7%), while none of the six well-differentiated tumors were HER2-positive. Although the overall association did not reach statistical significance (p=0.080), this finding suggests potential variation in HER2 expression according to tumor differentiation. The result should, however, be interpreted cautiously because most tumors in the cohort were moderately differentiated and relatively few cases were represented in the other histological grades.

Recent investigations have suggested that different degrees of HER2 expression may represent clinicopathologically distinct CRC groups. Wu et al. compared HER2-high, HER2-low, and HER2-zero colorectal cancers and demonstrated differences in selected clinicopathological characteristics between these expression categories [12]. Their findings indicate that HER2 expression may not have a simple linear relationship with histological differentiation or tumor aggressiveness. Similarly, Hashimoto et al. evaluated more than 1,000 colorectal cancers and identified HER2-amplified tumors in 2.5% and HER2-low tumors in 4.6% of cases, demonstrating distinct molecular characteristics between these categories [13]. These observations support evaluation of the complete spectrum of HER2 expression rather than treating all immunoreactive tumors as a biologically homogeneous group.

No significant association was demonstrated between HER2 status and regional lymph-node involvement in the present study (p=0.816). HER2 positivity was observed in 22.2% of N0, 30.0% of N1, and 20.0% of N2 tumors, with no consistent increase in HER2 positivity with advancing nodal disease. Likewise, HER2 expression was not significantly associated with pathological T stage (p=0.397). Most assessable tumors were T3, among which HER2 positivity was observed in 24.5%. Although the single T1 tumor was HER2-positive, this isolated observation cannot be considered evidence of greater HER2 expression in early-stage disease because of the extremely small number of T1 cases.

The absence of significant relationships between HER2 expression and T or N categories is consistent with the concept that HER2 alterations cannot necessarily be predicted from conventional pathological characteristics. Hashimoto et al. found relatively few conventional clinicopathological differences between HER2-amplified and non-amplified colorectal cancers, although important molecular differences were evident [13]. In contrast, Wu et al. reported that HER2-high tumors were more frequently associated with stage III disease and perineural invasion and demonstrated differences in disease-free survival [12]. The variability among studies may reflect differences in population characteristics, tumor stage distribution, HER2 scoring systems, molecular confirmation, and classification of HER2-high, HER2-low, and HER2-zero tumors.

The current findings should not be interpreted as evidence regarding the prognostic role of HER2 because survival outcomes were not evaluated. The study was cross-sectional and did not include longitudinal information regarding recurrence, progression, disease-free survival, or overall survival. Consequently, neither the presence nor absence of an association between HER2 expression and pathological stage can establish its prognostic significance. Wu et al. reported poorer disease-free survival among patients with HER2-high tumors and identified HER2-high expression as an adverse factor for disease-free survival in their cohort [12]. Nevertheless, the prognostic significance of HER2 remains less established than its emerging role as a predictive therapeutic biomarker, and longitudinal studies incorporating standardized HER2 assessment and appropriate molecular characterization are required.

From a clinical perspective, the greatest contemporary importance of HER2 in colorectal carcinoma is its emergence as an actionable therapeutic biomarker. The phase II MOUNTAINEER study demonstrated clinically meaningful activity of combined tucatinib and trastuzumab in previously treated patients with HER2-positive, RAS wild-type unresectable or metastatic colorectal cancer [9]. These findings supported regulatory approval of tucatinib in combination with trastuzumab for appropriately selected patients with previously treated HER2-positive, RAS wild-type advanced CRC [10]. Therefore, accurate identification of HER2-positive disease is becoming increasingly relevant to treatment selection rather than being solely of pathological or prognostic interest.

The therapeutic significance of HER2 has been reinforced by subsequent evidence. The final analysis of the MOUNTAINEER trial, published in 2026, reported a confirmed objective response rate of 39.3% among 84 patients treated with tucatinib plus trastuzumab, with a median duration of response of 15.2 months. Median progression-free survival was 8.1 months and median overall survival was 23.9 months [14]. These results strengthen the rationale for reliable HER2 testing in advanced colorectal carcinoma and demonstrate that identification of the HER2-positive molecular subgroup can have direct therapeutic consequences.

The biological context of HER2 positivity is also important when considering targeted therapy. Contemporary genomic evidence indicates that ERBB2-amplified colorectal carcinoma represents a distinct molecular subgroup and that concurrent RAS/RAF alterations may influence HER2 expression heterogeneity and responsiveness to HER2-directed treatment [15]. This further supports the integration of HER2 testing with broader molecular profiling rather than interpretation of HER2 immunohistochemistry in isolation. Future studies in the local population should therefore incorporate KRAS, NRAS, BRAF and MSI/MMR status where feasible.

The present study has several limitations. First, it was a single-center retrospective study with a relatively modest sample size, which reduced statistical power, particularly for comparisons involving smaller histological and TNM categories. Second, HER2 status was determined by immunohistochemistry, with both 2+ and 3+ staining classified as positive according to the predefined protocol. The absence of confirmatory ISH/FISH testing for IHC 2+ cases limits direct comparison with contemporary molecularly defined HER2-positive CRC cohorts and may partly explain the comparatively high prevalence observed. Third, molecular information including KRAS, NRAS, BRAF and MSI/MMR status was unavailable. Tumor location, lymphovascular invasion, and perineural invasion were also not available in the analyzed dataset. Finally, absence of longitudinal follow-up prevented assessment of recurrence, progression, disease-free survival, and overall survival.

Despite these limitations, the study provides useful local evidence regarding HER2 immunohistochemical expression in colorectal adenocarcinoma. The relatively high proportion of tumors meeting the study-defined IHC positivity threshold highlights the importance of standardized CRC-specific HER2 assessment and molecular confirmation of equivocal cases. Furthermore, the absence of significant associations with age, sex, T stage, or nodal status suggests that HER2-positive tumors cannot be reliably identified on the basis of these conventional clinicopathological characteristics alone. As HER2-directed therapies become increasingly integrated into precision treatment of advanced colorectal cancer, larger prospective studies combining standardized IHC assessment with ISH or genomic

confirmation, RAS/BRAF and MSI/MMR profiling, and longitudinal clinical outcomes are warranted to define the true prevalence and clinical significance of HER2-positive colorectal carcinoma in the Pakistani population.

## CONCLUSION

HER2/neu immunohistochemical positivity was observed in 26.7% of colorectal adenocarcinomas in the present cohort. HER2 expression was not significantly associated with age, sex, pathological T stage, or lymph-node status, while histological grade showed a nonsignificant trend toward association. The relatively high frequency of HER2 positivity should be interpreted in light of the study-defined criteria, which classified both IHC 2+ and 3+ cases as positive without confirmatory molecular testing. These findings support the need for standardized HER2 assessment and confirmatory testing of equivocal cases, particularly as HER2-directed therapies become increasingly relevant in selected patients with colorectal carcinoma.

**Ethical Approval:** The study was conducted following approval from the Institutional Ethical Review Committee of Liaquat National Hospital, Karachi. The study utilized archived tissue specimens and existing pathological records with no direct patient interaction.

**Confidentiality:** Patient-identifying information was not included in the study dataset, and confidentiality was maintained throughout data collection, analysis, and reporting.

**Conflict of Interest:** The authors declare no conflict of interest.

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**Data Availability:** The data supporting the findings of this study are available from the corresponding author upon reasonable request.

## REFERENCES

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2024: GLOBOCAN estimates of incidence and mortality worldwide for 185 countries for 36 cancers. **CA Cancer J Clin.** 2024;74(3):229–263.
2. Cervantes A, Adam R, Roselló S, Arnold D, Normanno N, Taïeb J, et al. Metastatic colorectal cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. **Ann Oncol.** 2023;34(1):10–32. doi:10.1016/j.annonc.2022.10.003. (PubMed)
3. Yoshino T, Cervantes A, Bando H, Martinelli E, Oki E, Xu RH, et al. Pan-Asian adapted ESMO Clinical Practice Guidelines for the diagnosis, treatment and follow-up of patients with metastatic colorectal cancer. **ESMO Open.** 2023;8(3):101558. doi:10.1016/j.esmoop.2023.101558. (PubMed)
4. Sawada K, et al. Systematic literature review and meta-analysis of HER2 amplification, overexpression, and positivity in colorectal cancer. 2023. (PubMed)
5. Lentz RW, et al. HER2-positive metastatic colorectal cancer. **Curr Treat Options Oncol.** 2024. doi:10.1007/s11864-024-01183-7. (PubMed)
6. Wang XY, et al. Human epidermal growth factor receptor 2 overexpression and amplification in patients with colorectal cancer: a large-scale retrospective study in Chinese population. 2022. (PubMed)
7. Sun Q, Li Q, Gao F, Wu H, Fu Y, Yang J, et al. HER2 overexpression/amplification status in colorectal cancer: a comparison between immunohistochemistry and fluorescence in situ hybridization using five different immunohistochemical scoring criteria. **J Cancer Res Clin Oncol.** 2023;149(2):579–592. doi:10.1007/s00432-022-04230-8. (PubMed)
8. Evans MG, Krause HB, Xiu J, Elliott A, Lou E, Ghani H, et al. Evidence for unified assessment criteria of HER2 immunohistochemistry in colorectal carcinoma. **Mod Pathol.** 2025;38(2):100654. doi:10.1016/j.modpat.2024.100654. (PubMed)
9. Strickler JH, Cercek A, Siena S, André T, Ng K, Van Cutsem E, et al. Tucatinib plus trastuzumab for chemotherapy-refractory, HER2-positive, RAS wild-type unresectable or metastatic colorectal cancer (MOUNTAINEER): a

multicentre, open-label, phase 2 study. **Lancet Oncol.** 2023;24(5):496–508. doi:10.1016/S1470-2045(23)00150-X. (PubMed)
10. Strickler JH, et al. FDA approval summary: tucatinib with trastuzumab for advanced unresectable or metastatic, chemotherapy refractory, HER2-positive RAS wild-type colorectal cancer. **Clin Cancer Res.** 2023. doi:10.1158/1078-0432.CCR-23-1041. (PubMed)
11. Recent institution-based evidence evaluating HER2/neu expression and clinicopathological characteristics in colorectal carcinoma. 2024. (PubMed)
12. Wu Z, Cheng Y, Wang H, Liu D, Qi X, Wang C, et al. Distinct clinicopathological features and prognostic values of high-, low-, or non-expressing HER2 status in colorectal cancer. *Cancers (Basel).* 2023;15(2):554. doi:10.3390/cancers15020554.
13. Hashimoto T, Takayanagi D, Yonemaru J, Naka T, Nagashima K, Machida E, et al. A comprehensive appraisal of HER2 heterogeneity in HER2-amplified and HER2-low colorectal cancer. *Br J Cancer.* 2023;129(7):1176–1183. doi:10.1038/s41416-023-02382-z.
14. Strickler JH, Cercek A, Siena S, André T, Ng K, Van Cutsem E, et al. Tucatinib plus trastuzumab for chemotherapy-refractory, HER2-positive, RAS wild-type metastatic colorectal cancer (MOUNTAINEER): final analysis. *Nat Commun.* 2026;17(1):1068. doi:10.1038/s41467-025-67824-z.
15. Study evaluating RAS/RAF co-mutation, ERBB2 copy number, HER2 heterogeneity, and response to HER2-directed therapy in colorectal cancer. *Clin Cancer Res.* 2024. doi:10.1158/1078-0432.CCR-23-2581.