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EVALUATION OF HER2/NEU STATUS IN GASTRIC CARCINOMA USING IMMUNOHISTOCHEMISTRY AND FISH TECHNIQUES

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Abstract

Background:
Gastric carcinoma remains a major global health concern, with a high mortality rate due to late diagnosis and limited targeted therapies. Overexpression or amplification of the HER2/neu (Human Epidermal Growth Factor Receptor 2) gene has been identified in a subset of gastric cancers and is associated with aggressive tumor behavior and poor prognosis. Accurate determination of HER2 status is essential for selecting patients eligible for HER2-targeted therapy. This study aims to evaluate HER2/neu expression in gastric carcinoma using Immunohistochemistry (IHC) and confirmatory Fluorescence In Situ Hybridization (FISH) techniques.

Materials and Methods:
A total of 60 histologically confirmed gastric carcinoma cases were retrospectively analyzed. Formalin-fixed paraffin-embedded (FFPE) tissue sections were subjected to HER2/neu IHC staining. Scoring was done according to the modified Hofmann criteria for gastric cancer. Cases showing IHC scores of 2+ (equivocal) or 3+ (positive) were further evaluated using FISH to assess HER2 gene amplification.

Results:
Out of 60 cases, HER2/neu overexpression (IHC 3+) was observed in 12 cases (20%), while 18 cases (30%) showed equivocal staining (2+). Negative expression (0 and 1+) was seen in 30 cases (50%). Among the 18 equivocal cases, FISH detected HER2 gene amplification in 10 cases (55.6%). Overall, HER2 positivity confirmed by either IHC 3+ or FISH amplification was noted in 22 cases (36.7%). HER2 positivity was more frequent in intestinal-type gastric carcinoma and tumors located at the gastroesophageal junction.

Conclusion:
HER2/neu expression is present in a significant proportion of gastric carcinoma cases, highlighting its potential as a therapeutic target. Combining IHC with FISH provides a more reliable assessment of HER2 status, especially in equivocal cases. Routine evaluation of HER2 is recommended in gastric cancer for better prognostication and treatment planning.

Keywords: Gastric carcinoma, HER2/neu, Immunohistochemistry, Fluorescence In Situ Hybridization, Targeted therapy, Gene amplification

Introduction

Gastric carcinoma is one of the most common malignancies worldwide and ranks as the third leading cause of cancer-related mortality, particularly in Eastern Asia and parts of South America and Eastern Europe (1). Despite advancements in surgical techniques and chemotherapeutic regimens, the overall prognosis of

advanced gastric cancer remains poor, with a 5-year survival rate below 30% in most countries (2). Molecular characterization of gastric cancer has paved the way for targeted therapies, aiming to improve outcomes in selected patient populations.

One such promising molecular target is the human epidermal growth factor receptor 2 (HER2/neu), a transmembrane tyrosine kinase receptor that plays a pivotal role in cell proliferation, differentiation, and survival (3). Amplification or overexpression of the HER2 gene has been extensively studied in breast cancer and has shown considerable therapeutic relevance with the introduction of trastuzumab (4). Similar therapeutic implications have been observed in a subset of gastric cancers that exhibit HER2 overexpression or gene amplification (5).

Immunohistochemistry (IHC) is widely used as the initial screening method for assessing HER2 expression in gastric carcinoma due to its accessibility and cost-effectiveness (6). However, given the heterogeneity of HER2 expression in gastric tissue and the limitations of IHC in equivocal cases, Fluorescence In Situ Hybridization (FISH) is often employed as a confirmatory technique to evaluate HER2 gene amplification at the genomic level (7). According to guidelines by the College of American Pathologists and the American Society of Clinical Oncology, HER2 testing in gastric cancer should incorporate both IHC and FISH to ensure diagnostic accuracy (8).

This study aims to evaluate the HER2/neu status in gastric carcinoma cases using IHC and FISH techniques, with a focus on assessing the concordance between the two methods and identifying the clinicopathological correlations of HER2 positivity.

Materials and Methods

This retrospective observational study was conducted in the Department of Pathology at a tertiary care hospital. A total of 60 formalin-fixed, paraffin-embedded (FFPE) tissue blocks from patients diagnosed histologically with gastric adenocarcinoma were selected over a period of year. Clinical and histopathological details including age, sex, tumor location, and histological subtype were retrieved from pathology records.

Immunohistochemistry (IHC):

Tissue sections of 3–4 µm thickness were cut from FFPE blocks and mounted on poly-L-lysine-coated slides. After deparaffinization and rehydration, antigen retrieval was carried out using citrate buffer (pH 6.0) in a microwave oven. Endogenous peroxidase activity was blocked using hydrogen peroxide. The slides were incubated with primary anti-HER2/neu antibody (monoclonal, clone CB11) followed by a secondary antibody conjugated with horseradish peroxidase. Diaminobenzidine (DAB) was used as the chromogen and hematoxylin was applied for counterstaining.

HER2 IHC scoring was done as per the modified Hofmann criteria for gastric carcinoma. The results were categorized as 0, 1+, 2+ (equivocal), and 3+ (positive). Cases with 2+ or 3+ scores were selected for further FISH analysis.

Fluorescence In Situ Hybridization (FISH):

FISH was performed on tissue sections using a dual-color probe for HER2 gene and centromere 17 (CEP17). Following deparaffinization and pretreatment, the sections were denatured and hybridized overnight with the probe mixture. Post-hybridization washes were followed by counterstaining with DAPI. The slides were examined under a fluorescence microscope. HER2 gene amplification was defined based on the HER2/CEP17 signal ratio, with a ratio ≥ 2.0 considered positive.

Statistical Analysis:

Data were compiled and analyzed using SPSS software version 25.0. The relationship between HER2 expression and clinicopathological variables was evaluated using the Chi-square test, with a p-value <0.05 considered statistically significant.

Results

A total of 60 patients with histologically confirmed gastric adenocarcinoma were included in the study. The age of the patients ranged from 32 to 79 years, with a mean age of 56.4 ± 10.2 years. There was a male predominance with 38 males (63.3%) and 22 females (36.7%).

HER2/neu Expression by Immunohistochemistry:
Out of the 60 cases, 12 cases (20%) showed strong membranous staining (IHC 3+), while 18 cases (30%) were scored as equivocal (IHC 2+). 16 cases (26.7%) were scored as IHC 1+, and 14 cases (23.3%) showed no expression (IHC 0) (Table 1).

Table 1. Distribution of HER2 Expression by IHC (n = 60)

IHC Score	Number of Cases	Percentage (%)
0	14	23.3
1+	16	26.7
2+	18	30.0
3+	12	20.0

HER2 Amplification by FISH:
All 30 cases with IHC scores of 2+ or 3+ were subjected to FISH analysis. Among the 18 equivocal cases (2+), 10 cases (55.6%) showed HER2 gene amplification. In the 12 cases with IHC 3+, 11 cases (91.7%) exhibited gene amplification. Thus, a total of 21 out of 60 cases (35%) were confirmed HER2 positive based on FISH (Table 2).

Table 2. FISH Results in IHC 2+ and 3+ Cases (n = 30)

IHC Score	Number of Cases	FISH Positive	FISH Negative
2+	18	10 (55.6%)	8 (44.4%)
3+	12	11 (91.7%)	1 (8.3%)

Correlation with Clinicopathological Features:
HER2 positivity (by IHC 3+ or FISH-confirmed 2+) was more commonly observed in tumors of the intestinal type (71.4%) compared to diffuse type (28.6%). Additionally, HER2 overexpression was seen predominantly in tumors located at the gastroesophageal junction (GEJ) (61.9%) compared to distal stomach (38.1%) (Table 3).

Table 3. Association Between HER2 Positivity and Clinicopathological Variables

Variable	HER2 Positive (n=21)	HER2 Negative (n=39)	*p*-value
Gender (Male)	15 (71.4%)	23 (59.0%)	0.31
Tumor Type (Intestinal)	15 (71.4%)	12 (30.8%)	0.01*
Tumor Location (GEJ)	13 (61.9%)	9 (23.1%)	0.02*

These findings suggest that HER2 overexpression is significantly associated with intestinal-type histology and tumors located at the gastroesophageal junction.

Discussion
The evaluation of HER2/neu status in gastric carcinoma has gained clinical significance due to the emergence of HER2-targeted therapies. In the present study, HER2 overexpression (IHC 3+ or FISH-amplified 2+) was observed in 35% of gastric carcinoma cases, which aligns with findings from previous studies that report HER2 positivity rates ranging from 10% to 38% in gastric cancers (1,2).
Our study confirmed the utility of immunohistochemistry as a primary screening method, with FISH used for confirming equivocal (2+) cases. This is consistent with the guidelines proposed by the College of American Pathologists and the American Society of Clinical Oncology, which recommend a dual-testing algorithm to ensure diagnostic precision (3). The high concordance between IHC 3+ and FISH positivity (91.7%) further supports the reliability of IHC as a predictive test when results are unequivocal (4,5).
HER2 positivity was significantly higher in intestinal-type adenocarcinomas compared to diffuse-type, a finding supported by earlier reports (6,7). This difference may be attributed to variations in tumor biology, cellular adhesion mechanisms, and HER2 gene regulation between the histological subtypes (8). The association of HER2 positivity with gastroesophageal junction (GEJ) tumors, as observed in this study, has also been documented in prior literature and may be linked to the embryological origin and molecular behavior of GEJ tumors (9,10).
The rate of HER2 gene amplification among IHC 2+ cases in our study was 55.6%, indicating that nearly half of the equivocal cases may benefit from further evaluation through FISH. This finding is in agreement with

multiple studies where FISH confirmed gene amplification in 40–60% of IHC 2+ cases (11,12). It highlights the importance of not relying solely on IHC, especially in borderline or ambiguous cases.

Trastuzumab, a monoclonal antibody targeting HER2, has shown significant survival benefit in HER2-positive advanced gastric cancers when used in combination with chemotherapy (13). Therefore, accurate detection of HER2 status is essential not only for prognostication but also for therapeutic decision-making. Several clinical trials and real-world studies have demonstrated improved response rates and prolonged progression-free survival in HER2-positive gastric cancer patients receiving targeted therapy (14,15).

The variation in HER2 expression reported across studies could be due to multiple factors including tumor heterogeneity, different scoring systems, pre-analytical conditions, and inter-observer variability in IHC interpretation. To mitigate such issues, standardized protocols and strict adherence to scoring criteria are necessary, especially in centers without access to molecular techniques.

Our study reinforces the clinical relevance of HER2 assessment in gastric carcinoma and supports the routine implementation of HER2 testing using a combination of IHC and FISH. Larger multi-centric studies and integration of HER2 testing with other molecular biomarkers may further refine patient stratification and expand the scope of personalized therapy in gastric malignancies.

Conclusion

HER2/neu overexpression and gene amplification are observed in a significant subset of gastric and gastroesophageal junction adenocarcinomas, particularly those with intestinal-type histology and GEJ location. Combined use of immunohistochemistry and FISH enhances diagnostic accuracy, guiding the selection of patients who may benefit from HER2-targeted therapies. Routine assessment of HER2 status should be incorporated into the diagnostic workflow for gastric cancer.

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