Original Research

EVALUATION OF HER2/NEU EXPRESSION IN GALLBLADDER LESIONS USING IMMUNOHISTOCHEMISTRY

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ABSTRACT

Gallbladder carcinoma (GBC) is an aggressive malignancy with poor prognosis due to late presentation. Identification of molecular targets such as HER2/neu may improve therapeutic strategies. This descriptive case–control study (n = 78) evaluated HER2/neu expression in gallbladder carcinoma and its association with clinicopathological parameters. HER2/neu expression was assessed using immunohistochemistry and scored as 0, 1+, 2+, or 3+ based on membranous staining. Scores of 0/1+ were considered negative, 2+ as equivocal, and 3+ as positive. The mean age of GBC patients was 58.09 ± 13.7 years, with female predominance. HER2 positivity (3+) was observed in 29.3% of cases, while 2+ equivocal expression was noted separately. Although HER2 expression was higher in GBC compared to controls, the difference was not statistically significant ($p > 0.05$). No significant association was observed with tumor grade, stage, or metastasis. HER2/neu overexpression in a subset of GBC cases suggests potential for targeted therapy; however, further validation with molecular techniques is required.

Keywords: HER2/Neu, Gallbladder Carcinoma, Immunohistochemistry, ERBB2.

INTRODUCTION

Gallbladder carcinoma (GBC) is the most common malignancy of the biliary tract and represents a major health concern, particularly in Northern India, where incidence rates are among the highest worldwide (1,2). The disease is often diagnosed at an advanced stage because of its non-specific clinical manifestations and the absence of effective early screening strategies, resulting in poor prognosis and limited survival outcomes (3).

Several risk factors, including gallstones, chronic inflammation, and environmental influences, are known to contribute to its pathogenesis; however, the molecular mechanisms underlying tumor progression remain incompletely understood (4).

Recent advances in molecular oncology have emphasized the role of genetic and protein alterations in carcinogenesis. One such molecule is the human epidermal growth factor receptor-2 (HER2/neu), also known as ERBB2, a transmembrane tyrosine kinase receptor that regulates cellular proliferation, differentiation, and survival (5). Overexpression or amplification of HER2/neu has been widely reported in several malignancies, particularly breast and gastric cancers, where it functions as both a prognostic biomarker and a therapeutic target (6,7).

In gallbladder carcinoma, HER2/neu expression has been reported with variable frequency, ranging from approximately 10% to 30%, reflecting biological heterogeneity as well as differences in methodological approaches used for its detection (8). While some studies have suggested an

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association between HER2/neu overexpression and aggressive tumor behaviour, advanced disease stage, and poor prognosis, other investigations have not demonstrated a consistent relationship with clinicopathological parameters (9,10). These discrepancies may be related to differences in study design, sample size, and immunohistochemical scoring systems. However, in GBC, HER2 expression shows variable prevalence, and its clinicopathological significance remains unclear. Additionally, limited data are available from high-incidence regions like North India. Therefore, this study aimed to evaluate HER2/neu expression in gallbladder lesions and assess its association with clinicopathological parameters.

MATERIALS AND METHODS

Study Design and Ethical Approval: This descriptive case–control study was conducted in the Department of Pathology in collaboration with the Departments of General Surgery, Oncosurgery, and Radiotherapy at Motilal Nehru Medical College and Associated Hospitals, Prayagraj, India, from January 2023 to March 2025. The study protocol was approved by the Institutional Ethics Committee (IEC), MLN Medical College & Associated Hospitals, Prayagraj (Approval No.: ECR/922/Inst/UP/RR-22). All procedures were carried out in accordance with the principles of the Declaration of Helsinki[11], and written informed consent was obtained from all participants.

Study Population: A total of 78 gallbladder specimens were included, comprising 58 histopathologically confirmed gallbladder carcinoma (GBC) cases and 20 benign controls diagnosed as chronic cholecystitis, including xanthogranulomatous cholecystitis.

Inclusion and Exclusion Criteria: Patients aged 14–65 years undergoing surgical intervention for gallbladder lesions and providing informed consent were included. Cases with incomplete data, lack of consent, or metastatic disease were excluded.

Histopathological Examination: All specimens were fixed in 10% neutral buffered formalin, processed by routine paraffin-embedding techniques, and sectioned at 3–5 µm thickness. The sections were stained with hematoxylin and eosin and examined under light microscopy. Tumor classification and grading were carried out according to the World Health Organization (WHO) criteria, and staging was determined using the American Joint Committee on Cancer (AJCC) 8th edition TNM staging system. Histopathological parameters, including depth of invasion, lymphovascular invasion, perineural invasion, and lymph node involvement, were systematically evaluated (3,9).

Immunohistochemistry (IHC): HER2/neu expression was assessed on paraffin-embedded tissue sections using standard immunohistochemical techniques. The procedure included antigen retrieval, blocking of endogenous peroxidase activity, and incubation with the primary antibody. Breast carcinoma tissue was used as a positive control. HER2/neu expression was evaluated based on the intensity of membranous staining and the proportion of tumor cells showing positivity. The scoring system was defined as follows: 0/1+ (negative), 2+ (equivocal), and 3+ (positive). Scores of 2+ and 3+ were considered indicative of overexpression, while equivocal cases were recommended for further confirmation using fluorescence in situ hybridisation (FISH) (4,8,11).

Statistical Analysis: Statistical analysis was performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Categorical variables were expressed as frequencies and percentages, whereas continuous variables were presented as mean ± standard deviation. Associations between categorical variables were evaluated using the Chi-square test or Fisher's exact test, while continuous variables were compared using the independent t-test or Mann–Whitney U test, as appropriate. Multivariate logistic regression analysis was performed where applicable. A p-value of <0.05 was considered statistically significant (10,12).

RESULTS

1. Study Population and Demographic Profile: This present study consists of a total of 78 patients(comprising 58 cases of gallbladder carcinoma (GBC) and 20 benign controls. The mean age of patients with GBC was 58.09 ± 13.7 years, which was significantly higher than that of controls (37.2 ± 11.5 years) ($p < 0.001$), indicating a strong association between increasing age and malignancy. Age group distribution also differed significantly between cases and controls ($\chi^2 = 22.67$, $p < 0.001$) (Table 1).

A female predominance was observed in both groups (77.5% in cases vs. 85% in controls), with no statistically significant difference ($\chi^2 = 0.15$, $p = 0.699$). Abdominal pain was the most common presenting symptom (65.5% in cases vs. 80% in controls), followed by jaundice (27.7% vs. 15%) and vomiting (6.8% vs. 5%), with no significant association between symptom profile and disease status ($\chi^2 = 1.50$, $p = 0.472$). Gallstones were present in 60.3% of cases and 80% of controls, although this difference was not statistically significant ($\chi^2 = 1.74$, $p = 0.187$) (table 1).

Table 1: Demographic Profile, Clinical Features, and Gallstone Incidence in Study Population

Parameter	Cases (n = 58)	Controls (n = 20)	χ2 Test	P-Value
Age Group (years)			22.67 (df = 2)	< 0.001
< 30	0 (0.0%)	7 (35.0%)		
30–50	39 (67.2%)	10 (50.0%)		
> 50	19 (32.8%)	3 (15.0%)		
Mean Age (years)	58.09 ± 13.7	37.2 ± 11.5	—	< 0.001
Gender			0.15 (df = 1)	0.699
Female	45 (77.5%)	17 (85.0%)		
Male	13 (22.5%)	3 (15.0%)		
Clinical Features			1.50 (df = 2)	0.472
Abdominal pain	38 (65.5%)	16 (80.0%)		
Jaundice	16 (27.7%)	3 (15.0%)		
Vomiting	4 (6.8%)	1 (5.0%)		
Gallstones			1.74 (df = 1)	0.187
Present	35 (60.3%)	16 (80.0%)		
Absent	23 (39.7%)	4 (20.0%)		

2. Histopathological Evaluation and Tumor Staging: Histopathological analysis confirmed that adenocarcinoma was the predominant histological type, accounting for 96.5% (55/58) of the GBC cases. Among the adenocarcinoma subtypes, the biliary pattern was most frequent (48.4%), followed by the intestinal subtype (23.2%). However, in terms of differentiation, moderately differentiated tumors were the most common (45.5%), followed by poorly differentiated (27.5%) and well-differentiated tumors (24.0%). Perineural invasion, lymphovascular invasion, and lymph node metastasis were identified in 27.6%, 25.9%, and 17.2% of the cases, respectively (**Figure 1**).

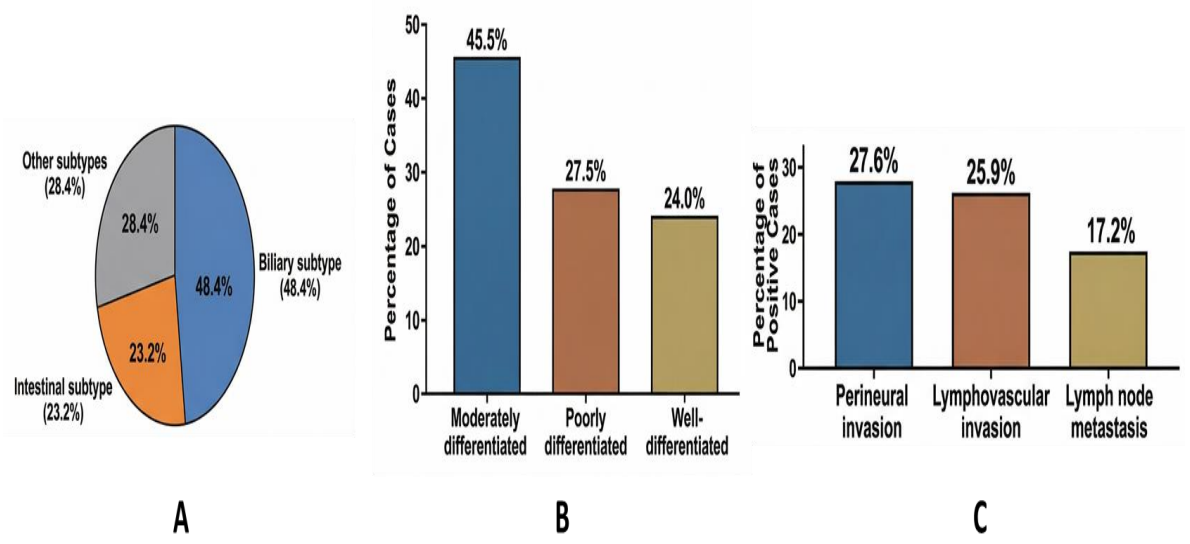

Figure 1. Histopathological Characteristics of Gallbladder Carcinoma (GBC)

(A) Distribution of adenocarcinoma subtypes among GBC cases, showing predominance of the biliary subtype followed by the intestinal and other variants, representing 96.5% of all tumors. (B) Proportional distribution of tumor differentiation, including well-differentiated, moderately differentiated, and poorly differentiated adenocarcinomas (n = 55), with moderately differentiated tumors forming the largest group. (C) Frequency of major prognostic pathological features observed in GBC, including perineural invasion (27.6%), lymphovascular invasion (25.9%), and lymph node metastasis (17.2%).

However, the analysis of tumor extension into the gallbladder wall (n=52) indicated that the majority of tumors had invaded the muscular layer (46.1%), followed by perimuscular connective tissue (30.7%) and liver invasion (17.3%). TNM pathological staging (n=53) demonstrated that most tumors were classified as pT1b (52.8%) and pT2a (34.0%), reflecting a predominance of early to moderately advanced disease stages in this cohort (**Figure 2**).

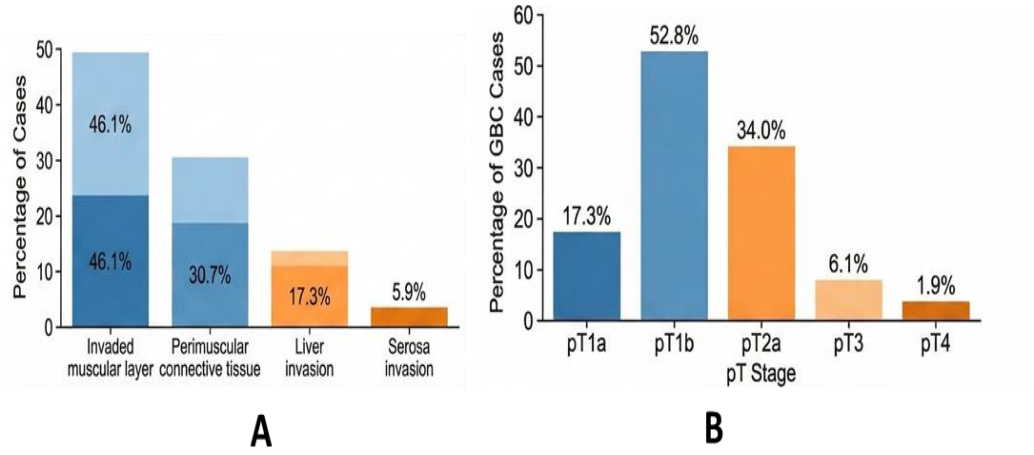

Figure 2. Tumor Extension and Pathological Staging in Gallbladder Carcinoma (GBC)

(A) Stacked bar chart illustrating the proportional distribution of tumor invasion depth in GBC cases (n = 52). The majority of tumors show invasion up to the muscular layer, followed by involvement of the perimuscular connective tissue and liver infiltration.

(B) Bar chart representing the distribution of TNM pathological stages (pT1b, pT2a, pT3, and pT4) among GBC cases (n = 53), demonstrating that the highest proportion of tumors is classified as pT1b. Values are presented as percentages of the total cases.

3. Immunohistochemical Analysis of HER2/neu Expression: Immunohistochemical evaluation of HER2/neu expression in the gallbladder carcinoma (GBC) cohort demonstrated variable membranous staining patterns and intensities (Figure 3A). HER2/neu positivity, defined as an immunohistochemical score of 2+ or 3+, was identified in 29.3% (17/58) of GBC cases, compared with 10.0% (2/20) of the control samples. However, the difference in the prevalence of HER2/neu expression between the two groups was not statistically significant ($\chi^2 = 2.05$, $p = 0.152$) (Figure 3B).Among the HER2/neu-positive tumors, membranous staining represented the most frequent pattern, observed in 47.1% of cases. This was followed by a combined membranous and basolateral staining pattern in 35.3% of cases. In contrast, basolateral staining was more commonly identified in the control group. Despite these variations in staining distribution, the difference in staining patterns between the groups did not reach statistical significance ($\chi^2 = 5.34$, $p = 0.069$).

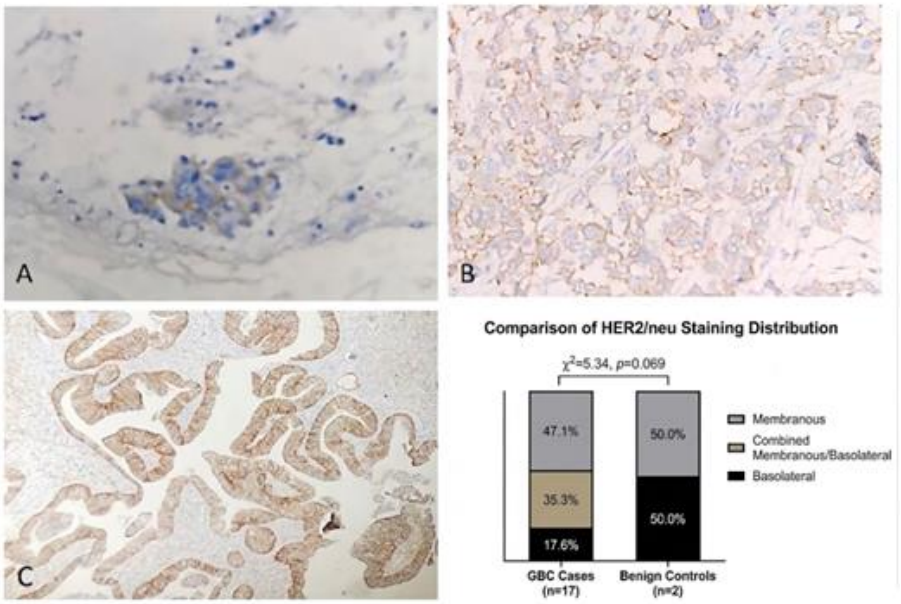

Figure 3. HER2/Neu Immunohistochemical Expression in the Study Cohort

(A) Representative photomicrographs showing HER2/neu IHC scoring patterns: Score 1+ (faint/incomplete membranous staining, ×100), Score 2+ (weak–moderate complete membranous staining, ×100), Score 3+ (strong circumferential membranous staining, ×100), and Score 3+ with basolateral/membranous positivity (×400). (B) Distribution of HER2/neu positivity (score 2+/3+) and staining patterns (membranous, combined membranous–basolateral, basolateral) in positive GBC cases (n = 17) compared with controls (n = 2) ($\chi^2 = 5.34$, p = 0.069).

4. Correlation of HER2/neu Expression with Clinicopathological Features: Analysis of the association between HER2/neu expression and tumor characteristics showed that HER2 positivity was more frequently observed in moderately differentiated adenocarcinomas (23.5%) compared with well-differentiated (11.0%) and poorly differentiated tumors (5.4%). However, this difference was not statistically significant ($\chi^2 = 2.06$, p = 0.357) (Figure 4).

No significant association was identified between HER2/neu expression and lymph node metastasis, perineural invasion, or lymphovascular invasion (p > 0.05). Although HER2/neu positivity appeared slightly higher in advanced tumor stages (29.7%), the relationship between HER2/neu expression and pathological stage was not statistically significant ($\chi^2 = 0.00$, p = 1.00).

HER2/neu overexpression was detected in a subset of gallbladder carcinoma cases; however, no significant correlation with the evaluated clinicopathological parameters was observed.

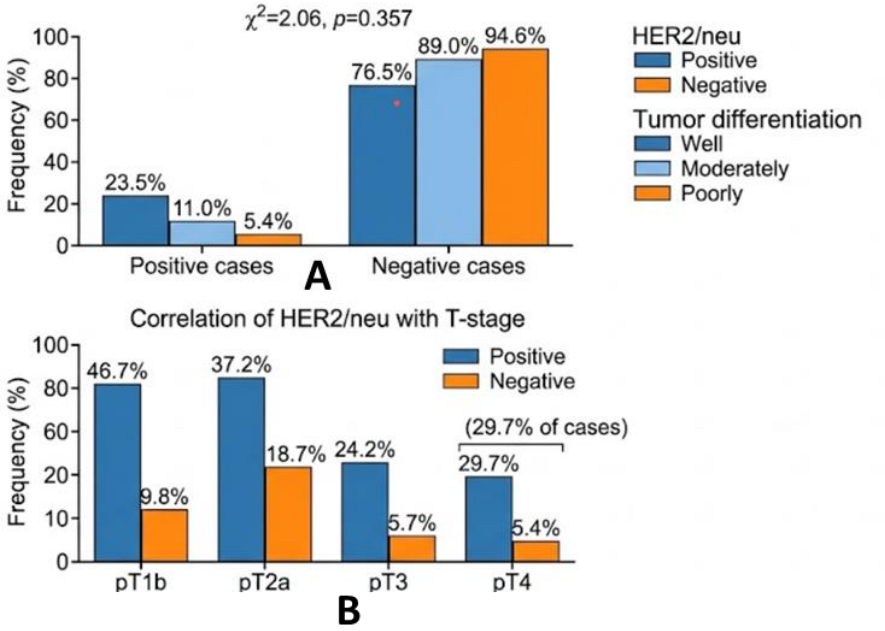

Figure 4. Correlation of HER2/Neu Expression with Clinicopathological Features

(A) Distribution of HER2/neu positivity (score 2+/3+) across tumor differentiation grades (well, moderately, and poorly differentiated), showing a higher frequency in moderately differentiated tumors (23.5%) ($\chi^2 = 2.06$, p = 0.357). (B) Bar chart illustrating the frequency of HER2/neu expression across pathological stages (pT1b, pT2a, pT3, pT4). Although HER2 positivity was noted in some advanced stages (29.7%), no statistically significant association with tumor stage was identified. Values are presented as percentages.

DISCUSSION

The demographic profile observed in the present study is consistent with the established epidemiological pattern of gallbladder carcinoma (GBC) as a disease predominantly affecting older individuals with a marked female preponderance. The mean age of 58.09 years in our cohort is comparable to previous reports from North India by Shukla et al. (1) and Misra et al. (2), who documented mean ages of 56.4 and 54.2 years, respectively. The female-to-male ratio of 3.4:1 in our study is slightly higher than the 2.5:1 reported by Hundal et al. (3), but remains in line with the global trend, which is often attributed to hormonal influences and the higher prevalence of cholelithiasis among females.

The primary objective of this study was to evaluate HER2/neu overexpression, which was identified in 29.3% of GBC cases. This prevalence is comparable to that reported by Chaube et al. (4) in a similar North Indian population (26.5%). In contrast, lower rates have been documented in studies from Japan (Nakazawa et al., 15.7%) and Europe (Harder et al., 10%), suggesting possible geographic and

biological variability in HER2/neu expression across different populations. Such variation may reflect underlying genetic differences, environmental factors, or methodological inconsistencies in HER2 assessment.

An additional observation in our study was the relatively higher frequency of basolateral and "U-shaped" staining patterns among HER2-positive cases. Similar staining characteristics have been described by Hofmann et al. (8) in gastric carcinoma, indicating that scoring criteria adapted from gastric cancer (e.g., ToGA guidelines) may be more appropriate for gallbladder carcinoma than conventional breast cancer-based scoring systems. Furthermore, the predominance of the biliary subtype in our cohort (48.4%) is in agreement with the morphological patterns reported by Goldin and Roa (9) in the Asian population.

In the present study, no statistically significant association was observed between HER2/neu expression and tumor stage, lymph node metastasis, or other adverse pathological features ($p > 0.05$), which is consistent with the findings of Pignochino et al. (10). While some earlier studies have suggested a correlation between HER2 overexpression and poor prognosis, such an association was not evident in our cohort. The presence of HER2 expression across both early and relatively advanced stages may indicate its involvement in tumor biology; however, the current data are insufficient to establish its role as an early molecular event in gallbladder carcinogenesis.

These findings suggest that, although HER2/neu overexpression is present in a subset of GBC cases, its clinicopathological significance remains uncertain. Nevertheless, it may still hold therapeutic relevance, and further studies incorporating larger cohorts and molecular validation techniques are required to better define its role in targeted therapy.

CONCLUSION

This study highlights that **HER2/neu** is overexpressed in a significant proportion (29.3%) of gallbladder carcinoma cases in the North Indian population. While HER2/neu positivity did not show a statistically significant correlation with tumor grade, TNM stage, or lymph node metastasis, its presence across various histological subtypes—predominantly the biliary adenocarcinoma subtype—suggests it is an early molecular driver in gallbladder carcinogenesis. Given the aggressive nature of GBC and the lack of effective systemic therapies, our findings indicate that nearly one-third of GBC patients could potentially benefit from anti-HER2 targeted therapies. Future clinical trials are warranted to evaluate the efficacy of Trastuzumab and other HER2-inhibitors in this specific patient subset.

Limitations: The study is limited by a relatively small sample size and a single-centre design. HER2/neu assessment was performed only using immunohistochemistry without confirmatory FISH testing, and the absence of follow-up data limits prognostic evaluation.

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Conflict of Interest: The authors declare that they have no competing interests.

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