

## Original Research Article

# A histopathological evaluation of gall bladder cancer with special reference to HER2/neu and E-cadherin in Southern part of Assam

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## ABSTRACT

**Background:** Gall bladder carcinoma (GBC) is an aggressive malignancy with high mortality and aggressive course. The signs and symptoms of gall bladder carcinoma are not specific and often present late poorer outcomes. Identifying biomarkers and cancer specific cellular targets will pave the way for novel therapeutic approaches and early diagnosis for gall bladder carcinoma. Proto-oncogenes (HER-2) and E-cadherin are commonly deregulated in gallbladder cancer (GBC). This study evaluates the prognostic significance of HER-2 and E-cadherin in GBC patients in Silchar medical college. Our main objective was to evaluate frequency of E-cadherin and HER-2/neu overexpression in GBC and to seek its correlation, if any with conventional clinicopathological parameters.

**Methods:** Total 168 cases were evaluated for gall bladder from 2018-2020. Immunohistochemical staining was done to evaluate HER-2 and E-cadherin protein expression. The criterion for HER-2 and E-cadherin positivity was set at 10% and >5% tumor cells showing complete, membranous staining. Clinicopathological correlations were drawn with major clinical outcomes.

**Results:** It was observed that out of 168 cases the male to female ratio is 1:5 with highest number of cases in the age group of 50-59, i.e., 70 cases with 41.7%. The most common location in this study was fundus with 69% of cases (116 out of 168), most commonly presented as biliary colic with 56 number of cases. Grading was also done in 168 cases where the greatest number of cases were moderately differentiated with 86 number of cases with a percentage of 51%. Expression of Her2Neu and E-cadherin was evaluated where highest number of cases were seen with 1+ score in the IHC expressions of both the markers with 81 and 61 number of cases respectively.

**Conclusions:** Our study shows the abnormal expression of HER-2 and E-Cadherin expression in gall bladder carcinoma patients and suggests that these two markers can be used for potential tool for early detection and targeted therapy in gall bladder carcinoma.

**Keywords:** Gall bladder cancer, Her2Neu, E-cadherin

## INTRODUCTION

Gallbladder carcinoma is one of the most common forms of digestive tract cancer. Surgical resection has been the curative procedure, and while early tumours may be treated with a simple cholecystectomy, the vast majority of cases are still advanced cases, in which hepatectomy and regional lymphadenectomy are necessary to enhance survival or even achieve a cure.<sup>1,2</sup> Despite the

advancements in diagnostic surgical techniques, there has been no change in the survival rate. This is primarily due to the delayed appearance of symptoms and the tumor's direct extension to nearby organs at the time of diagnosis.<sup>3</sup> Diagnosis is, therefore, often made at advanced stage with poorer outcomes. Identifying biomarkers and cancer specific cellular targets, that will pave the way for novel therapeutic approaches and early diagnosis for gall bladder carcinoma, is urgently needed.

Due to an increase in lifestyle-related modifiable risk factors, the disease burden in northern India is on the rise. With an annual incidence of 2.5 percent and a mortality rate of 2.75 percent, it is India's 14<sup>th</sup> most common malignancy.<sup>4</sup>

In preclinical studies, the oncogenic potential of human epidermal growth factor receptor 2 (HER2) has been identified, as well as in clinical environments. Among the four members of the HER family of proteins, HER2 is a proto-oncogene with catalytic kinase activity and functions.<sup>5,6</sup>

Reduced cell to cell adhesiveness helps cancer cells to break the histological structure of tumour tissue, allowing the tumour to dedifferentiate. The cell adhesion molecule E-cadherin is made up of a variety of components, each of which contains about 110 amino acid residues. The E-cadherin-catenin complex is formed by three cytoplasmic proteins (alpha-, beta-, and gamma-catenins), and it plays a vital role in cellular adhesion; loss of this function has been linked to tumour dedifferentiation and metastasis.<sup>7</sup>

The aims of our study are to evaluate clinical and epidemiological factors gallbladder carcinoma and also the tissue expression of the proteins of E-cadherin and HER-2/neu in tumor tissue.

## METHODS

The present study is a retrospective study conducted in the department of pathology, SMCH. We included 168 consecutive cases of cholecystectomy specimens from August 2018 to May 2020 that were operated with radiological suspicion of carcinoma or had prior cytology proving carcinoma. This study included both retrospective as well as prospective components. A gross evaluation of tumor was done in the resected specimens. After the preliminary study of all hematoxylin and eosin-stained sections, IHC staining was done for HER2/neu and E-cadherin in malignant tumor along with non-tumorous area. The relative frequency of HER2/neu and E-cadherin positivity was scored and correlated with other histological prognostic parameters of the tumor. Areas that were not involved by the tumor were also studied and negative controls were put while performing IHC staining in each batch. Controls were taken from the known cases of the breast specimen. HER2/neu and E-cadherin was graded as per criteria defined by the American society of clinical oncology and the college of American pathologists. The data thus collected was compiled and analyzed using SPSS version 21 for Mac (IBM Corporation, 2012).

For IHC, we followed the standard IHC protocol, starting with baking the slides and deparaffinization, rehydration with graded alcohol and then antigen retrieval was done. We then wash the slides with buffer and did peroxidase blocking. Primary antibody was added, and after washing the slide again, secondary antibody was added and

washed again. DAB chromogen was added and later counter staining was done with H and E.

HER2/neu scoring was done as follows: 0=No staining or membrane staining of <10% of epithelial cells, 1+=Faint/barely perceptible membrane staining in more than or equal to 10% of epithelial cells, in which only a part of the membrane was stained, 2+=Weak to moderate complete membrane staining in 10% of epithelial cells, 3+=Strong, complete membrane staining in >10% of epithelial cells.

For the evaluation of the protein E-cadherin expression: We considered the intensity of staining and also the number of positive cells.

The intensity of staining was on a scale from 0 to 3, 0 being negative, 1 was weak staining, 2 was intermediate staining and 3 was strong staining. For evaluating the number of positive cells, the scale ranges from 0 to 3, defined as follows: 0=less than 5% of the neoplastic cells were stained, 1=where 5-25%, 2=where 26-50% and 3=where more than 50%.

The definitive score was calculated by the score for the intensity of staining multiplied by the score for the number of stained cells. In this manner, the negative group (0) was defined when the product from multiplication was 0, products of 1 to 3 were weakly positive (1+), products of 4 to 5 were moderately positive (2+) and products greater than or equal to 6 were strongly positive (3+).

### *Inclusion criteria*

All clinically diagnosed and histopathologically confirmed cases were included in the present study.

### *Exclusion criteria*

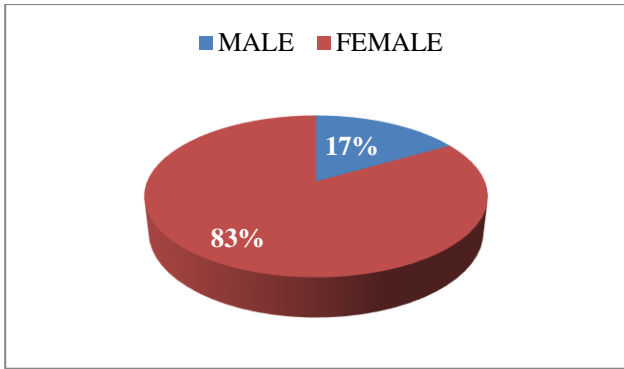
The non-neoplastic lesions were not included.

Samples were collected using stratified random sampling technique. All the data were analyzed using Microsoft excel 2013 and figures were drawn using Microsoft word 2013. An approval for this study was obtained from the institutional ethical committee.

## RESULTS

We conducted the study on total 168 gall bladder carcinoma cases collected over a span of 2 years and we intended to study the various clinical, morphological and histological parameters related to the tumour along with E-cadherin and HER-2/neu expression in the tumour tissue.

We tried to figure out the gender preponderance in gall bladder carcinoma.



**Figure 1: Gender distribution according to case number.**

In the Figure 1, we showed the gender distribution in our study in a simplified way. In our study period, total 168 cases of gall bladder carcinoma were received. Out of which, 140 were females and rest were males. So, the male:female ratio is around 1:5.

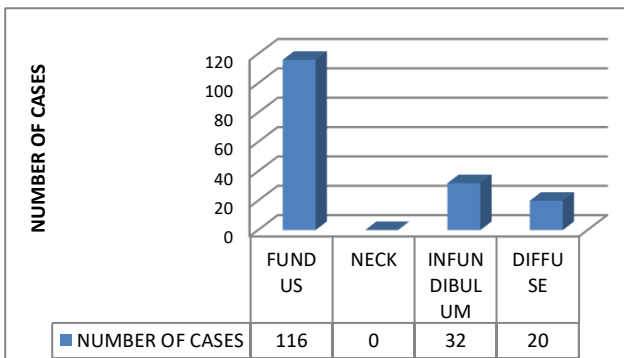
We tried to determine the distribution of cases according to age group.

**Table 1: Number of cases distributed according to age group.**

Age (years)	Number of cases	Percentage
<20	2	1.1
20-29	8	4.8
30-39	13	7.7
40-49	28	16.7
50-59	70	41.7
60-69	37	22
>70	10	6
<b>Total</b>	<b>168</b>	<b>100</b>

In the Table 1, it shows that the incidence of gallbladder carcinoma is highest in the age group 50-59 years of age i.e., around 41.7% according to our study.

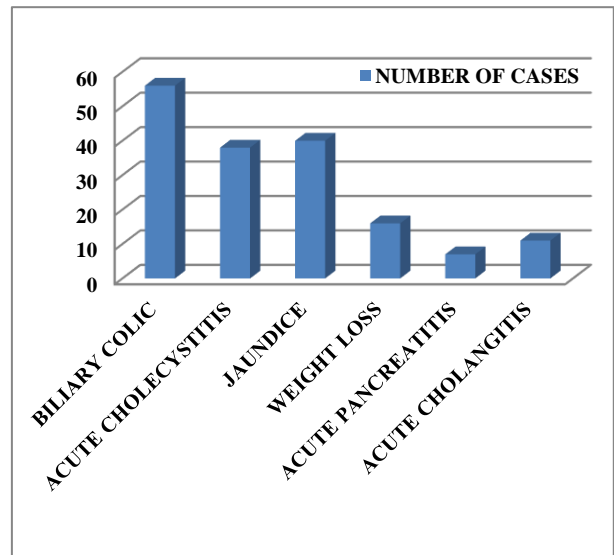
The distribution of cases according to the site of lesion were determined.



**Figure 2: Distribution of cases according to site of lesion.**

In the Figure 2, we explained case distribution according to site of lesion. Maximum cases are found to be in the fundus area of gall bladder, followed by infundibulum. Around 20 cases out of 168 cases (12%) shows diffuse involvement of the gall bladder whereas, there was no tumour noted in the neck region of gall bladder.

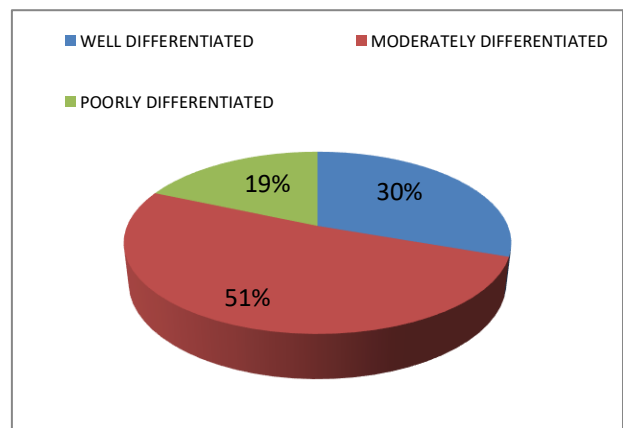
In our study, patients presented with various clinical characteristics. So, we tried to figure out the case distribution according to clinical features.



**Figure 3: Case distribution according to clinical characteristics.**

In Figure 3, we found that maximum patients presented with biliary colic (56 out of 168 patients) and jaundice (40 out of 168 patients). 38 patients presented with acute cholecystitis, 16 patients complained of weight loss and 11 patients had acute cholangitis and 7 patients presented with acute pancreatitis

We distributed the cases according to histologic grades of the tumours.



**Figure 4: Distribution of cases according to grade of tumour.**

Figure 4 shows that around 51% of cases were moderately differentiated, 30% were well differentiated and 19% were poorly differentiated tumours.

We evaluated the expression of HER-2/neu in our study.

**Table 2: Distribution of cases according to HER-2/neu expression.**

Expression of HER-2/neu	Number of cases	Percentage
0	33	20
1+	81	48
2+	40	24
3+	14	8
<b>Total</b>	<b>168</b>	<b>100</b>

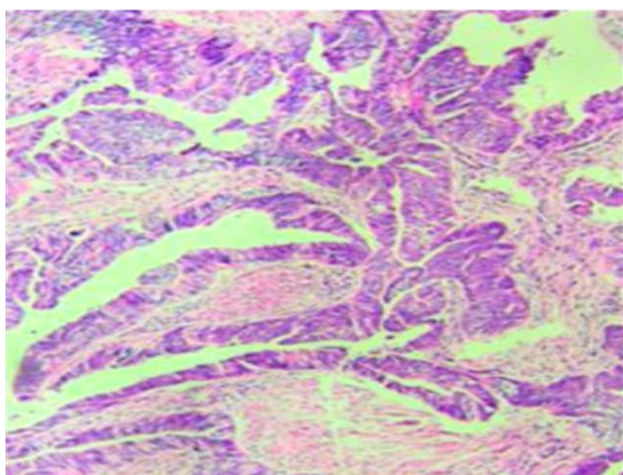
In the Table 2, we found that majority of cases (48%) have score 1+, while 20% and 24% cases have score zero and 2+. Almost 8% of cases in our study showed score 3+.

We also evaluated the expression of E-cadherin in our study group.

**Table 3: Distribution of cases according to E-Cadherin expression.**

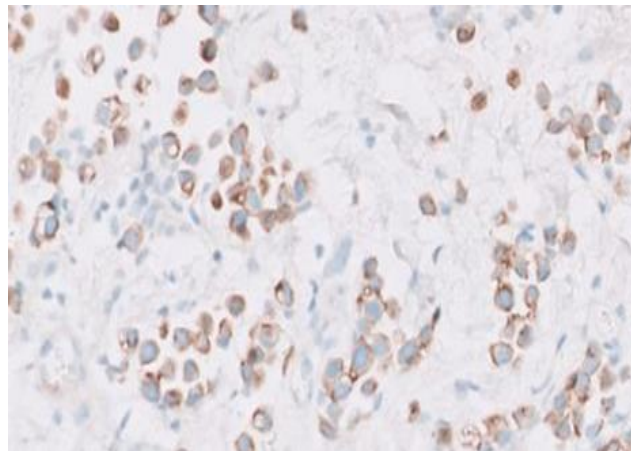
Expression of E-cadherin	Number of cases	Percentage
0	40	23.8
1+	61	36.3
2+	52	31
3+	15	8.9
<b>Total</b>	<b>168</b>	<b>100</b>

Table 3 explains that 61 cases, i.e., 36.3% cases showed 1+ E-cadherin expression score, 40 cases showed zero score, 52 cases showed 2+ score and 15 cases showed 3+ score for E-cadherin expression.



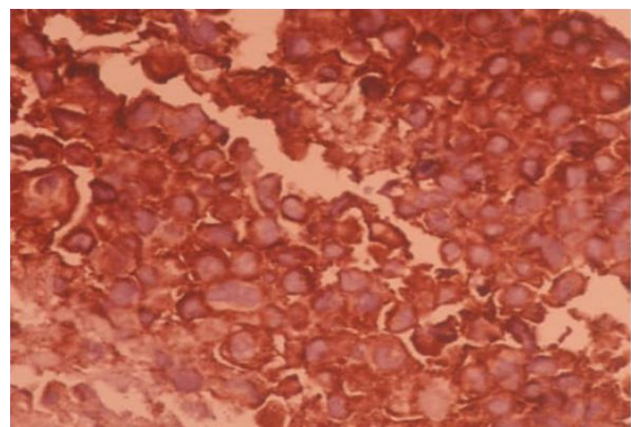
**Figure 5: Well differentiated adeno carcinoma of gall bladder (40X, H and E).**

The photomicrograph in the Figure 5 shows prominent gland formation, nuclear crowding, nuclear hyperchromatic, increased N:C ratio.



**Figure 6: E-cadherin expression score 2+ (40X).**

The photomicrograph in the Figure 6 shows moderate membrane staining in 40% of cells.



**Figure 7: HER-2/neu expression of score 3+ (100X).**

The Figure 7 shows strong and complete membranous staining for HER-2/neu in almost 90% of the cells.

## DISCUSSION

The present study titled “A histopathological evaluation of gall bladder cancer with special reference to Her2neu and E-cadherin in southern part of Assam” was carried out at the department of pathology in Silchar medical college of Assam and we included 168 consecutive cases of cholecystectomy specimens from August 2018 to May 2020 that were operated with radiological suspicion of carcinoma or had prior cytology proving carcinoma.

The present study showed that there is a preponderance of female patients (83.3%) in gall bladder carcinoma with male:female ratio being 1:5. We studied several other research papers and found that our result is also in



consistent with the study by Renato et al, Jain et al and Kostantinidis et al.<sup>8-10</sup>

In the study by Nahar et al, it is found that maximum incidence of gall bladder carcinoma was in the age group of 51-60 years.<sup>11</sup> We also found similar results in our study. 41.7% or 70 cases are found to be in 50-59 years of age group. A. Duffy et al found that the median age of presentation of gall bladder carcinoma is 67 years.<sup>12</sup>

Gall bladder has four anatomic parts, viz., fundus, body, infundibulum and neck. While conducting our study, we noticed that most common location of the tumour being the fundus of the gall bladder (69%), followed by the infundibulum (19%). Majority of the patients presented in the hospital with biliary colic (56 patients or around 33%) followed by 40 patients who came with jaundice. This result is in accordance with the result of the study by Renato et al.<sup>8</sup> They also found the highest incidence of gall bladder carcinoma in fundus region.

Gall bladder carcinoma is usually detected late because the symptoms are vague and often present late in the course of the disease. So, we tried to determine the common clinical presentation of gall bladder carcinoma in the present study. 38 patients presented with acute cholecystitis, 16 patients complained of weight loss and 11 patients had acute cholangitis and 7 patients presented with acute pancreatitis. We looked for similar parameters in other studies and found that our result is in accordance with the result of the study by Renato et al.<sup>8</sup>

We determined the histologic grade of the tumour according to the degree of gland formation and differentiation. We found out that around 51% of cases or 86 out of 168 cases were moderately differentiated, 30% were well differentiated and 19% were poorly differentiated tumours. Singh et al also found similar results in their study on gall bladder carcinoma.<sup>13</sup>

Ashai et al studied the HER2/neu expression in correlation to advanced tumour stage of gall bladder carcinoma and showed that 46% cases were score 1+, 23% were score 2+, 9% cases were 3+ and 19% cases were negative.<sup>14</sup> We also evaluated HER-2/neu expression in gall bladder carcinoma and found similar results. Majority of cases (48%) have score 1+, while 20% and 24% cases have score zero and 2+. Almost 8% of cases in our study showed score 3+. Whereas Jan Harder et al showed that in their study, HER2 expression was as follows: 72/124 (58%) were negative, 26 (21%) 1+, 22 (18%) 2+ and 4 (3%) 3+ score.<sup>15</sup>

Renato et al found that E-cadherin score 0=9 cases, score 1+=15 cases, score 2+=12 cases and score 3+=4 cases.<sup>8</sup> This is in accordance with our study. Here, 61 cases, i.e., 36.3% cases showed 1+ E-cadherin expression score, 40 cases showed zero score, 52 cases showed 2+ score and 15 cases showed 3+ score for E-cadherin expression. But

Xu et al found that E-cadherin expression was positive in 40.8% cases and negative in 59.2% cases.<sup>16</sup>

### **Limitations**

The limitation of this study is that it was a hospital-based study with small sample size. To validate our findings, further research with bigger sample size is needed

### **CONCLUSION**

The increasing global incidence, late presentation leading to poor prognosis and lack of effective therapy make the management of gall bladder carcinoma really challenging. Our study shows the abnormal expression of HER-2 and E-cadherin expression in gall bladder carcinoma patients in Southern Assam and suggests that these two markers can be used for potential tool for early detection of gall bladder carcinoma and also can be used for targeted therapy in gall bladder carcinoma

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