RESEARCH ARTICLE

Expression of Human Epidermal Growth Factor Receptor (Her 2/neu) and Proliferative Marker Ki-67: Association with Clinicopathological Parameters in Gallbladder Carcinoma

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Abstract

Purpose: To evaluate the expression of Her2/neu and Ki-67 in benign and malignant gallbladder lesions, and to establish correlations with clinico-pathologic parameters. Materials and Methods: A retrospective analysis was conducted on formalin fixed paraffin embedded (FFPE) benign (n=25) and malignant gallbladder (n=25) tissue samples. Hematoxylin and eosin stained slides of each case were reviewed for: type of malignancy (whether adenocarcinoma, squamous cell carcinoma, or any other type), grade (well, moderate, and poor), depth of invasion, pre-neoplastic changes in adjacent mucosal epithelium like metaplasia and dysplasia. Immunohistochemistry for Her 2 neu and Ki-67 was performed and data analysis was conducted using SPSS 17 software. Chi-square test was used to compare categorical/dichotomous variables. P value of ≤0.05 was considered significant. Results: The difference of Her 2 neu expression and Ki67 index between benign and malignant groups was found to be statistically significant. Her2/neu positivity did not have any significant correlation with various clinicopathological parameters other than liver involvement. 5 cases of gallbladder cancer showed both Her2/neu and Ki67 positivity. Ten cases were Ki67 positive but Her2/neu negative while one case was Her2/neu positive but Ki67 negative. Conclusions: The present study demonstrated overexpression of Her2/neu and Ki67 in gallbladder cancer. A trend of decreasing Her2/neu expression with increasing grade of tumor was observed. Furthermore, greater Ki67 positivity was found in cases with lymph node metastasis and distant metastasis. Future studies with a larger number of patients will be required to precisely define the correlation of Her2/neu expression and Ki67 positivity with clinicopathological parameters. The results however are encouraging and suggest evaluation of Her2/neu as a candidate for targeted therapy.

Keywords: Gallbladder cancer - her 2 neu - Ki-67 - chronic cholecystitis

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Introduction

Gall bladder cancer (GBC) is the most frequent type of biliary tract cancers (BTC) and is the sixth most common gastrointestinal cancer worldwide, with an annual incidence of 2.2/100,000 and mortality rate of 1.7/100,000. However, the global rates for GBC demonstrate striking variability, reaching epidemic levels for some regions and ethnicities. High mortality rates have been reported from Chile, Peru, Nepal, India, Bolivia, Bangladesh, Japan, Korea and the Czech Republic. The basis for this variance possibly resides in differences in environmental exposure and intrinsic genetic predisposition to carcinogenesis (Rakicet al., 2014). GBC is one of the most common causes of cancer related mortality in women, in the northern and north eastern states of India. The carcinoma is twice more common in women and is the leading cancer among digestive tract cancers in women in northern Indian cities of Delhi and Bhopal (Dhir and Mohandas, 1999; Kapoor and McMichael, 2003). The Indian Council of Medical Research Cancer Registry has reported incidence rate of 4.5% in males and 10.1% in females per100,000 population in northern India (Ghosh and Thakurdas, 2015).

GBC is a highly malignant neoplasm presenting at advanced stages in majority of cases. Overall mean survival is a mere 6 months, while 5-year survival rate is only 5%. The most important prognostic factors are depth of invasion into the gall bladder wall and the presence of lymph node or distant organ metastasis. Risk factors for the development of GBC include the presence of gallstones, infection and the presence of an anomalous pancreatobiliary ductal junction (Hundal and Shaffer, 2014). Despite this potential for cure, less than 10% of patients have tumors that are resectable at the time of surgery, while nearly 50% have lymph node metastasis.

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Even with surgery, most tumors progress to metastatic disease (Goldin and Roa, 2009).

The role of systemic treatment in GBC is still unclear, although a few retrospective and small phase II studies have shown that the GBC patients may benefit from adjuvant therapy. Despite the available treatment modalities, the GBC still remains a therapeutic challenge. Therefore, a better understanding of the pathological molecular mechanisms of gall bladder carcinogenesis is important for improving the diagnosis, prognosis, and also for developing novel targeted therapies for patients with advanced GBC (Bizama et al. 2015).

Considering the significance of GBC in North India and the potential therapeutic impact of targeted therapy for patients with advanced GBC, we undertook this study with the aim to evaluate the expression of Her2/neu in benign and malignant gall bladder lesions, and to see its relation with clinico-pathologic parameters and a known tumor marker viz. Ki67. To add, very few reports are available from India on the expression of these biomarkers in chronic cholecystitis and GBC.

The aim of the present study was to evaluate the role of Her2/neu in gall bladder carcinogenesis with the following objectives: To study the expression of Her2/neu and Ki67 in benign and malignant gall bladder lesions (chronic cholecystitis & GBC respectively) by immunohistochemistry; To correlate Her2/neu protein expression with Ki67 index, a nuclear marker of cell proliferation in benign and malignant lesions and to study the correlation of Her 2/neu expression with the clinic-pathologic parameters like tumor grade, tumor stage and presence of gall stones.

Materials and Methods

The present study was carried out at the Department of Pathology, Hamdard Institute of Medical Sciences and Research, New Delhi in collaboration with Department of Pathology, King George's Medical University, Lucknow after obtaining ethical clearance.

Study design

A retrospective analysis was conducted on formalin fixed paraffin embedded (FFPE) benign (n=25) and malignant gall bladder (n=25) tissue samples. Hematoxylin and eosin stained slides of each case were reviewed for: type of malignancy (whether adenocarcinoma, squamous cell carcinoma or any other type), grade (well, moderate, and poor), depth of invasion, pre-neoplastic changes in adjacent mucosal epithelium like metaplasia and dysplasia. Representative sections from FFPE tissue blocks were selected for immunohistochemistry.

Immunohistochemistry

Immunohistochemistry for Her2/neu and Ki67 was performed concurrently on serial sections. Briefly, 4 µm sections were cut from all FFPE blocks and mounted on 3-aminopropyl triethoxysilane coated glass slides. Slides were incubated at 60oC for 1h and then deparaffinized and rehydrated in descending alcohol grades. Endogenous peroxidase blocking was done by using 3% H2O2. Antigen retrieval was performed at 98oC for 15 min in a microwave oven in Tris-EDTA buffer (pH 9.0). After adding primary antibody (anti-Her2/neu; Dako or anti Ki67; Dako) the slides were incubated for one hour at room temperature in a humidified chamber followed by detection using Dako Envision FLEX detection system (Dako, Denmark). Sections were then incubated with DAB chromogen followed by counterstaining with hematoxylin, dehydration and mounting with DPX for bright field microscopy. Carcinoma breast tissue was used as positive control. Negative control sections were processed by omitting primary antibodies.

Interpretation and immunoscoring

Cell membrane staining was used to assess, positivity for Her2/neu with criteria as used for breast cancer (Roa et al., 2014).

IHC 0 (Negative): No staining observed or membrane staining that is incomplete, faint/barely perceptible and within ≤ 10% of tumor cells.

IHC 1+ (Negative): Incomplete membrane staining that is faint/barely perceptible and within >10% of tumor cells.

IHC 2+ (Equivocal): Circumferential membrane staining that is incomplete and/or weak/moderate and staining > 10% of tumor cells or complete and circumferential membrane staining that is intense and within ≤ 10% of tumor cells.

IHC 3+ (Positive): Circumferential membrane staining that is complete, intense, and within > 10% of tumor cells.

The MIB-1 Index (Ki-67 labelling Index) was calculated as the percentage of positively stained tumor cell nuclei out of the total tumor cells counted (n=1000). A percentage >20% of stained cells was considered positive, regardless of the intensity of staining (Grau et al., 2004).

Statistical analysis

All Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 17 software for windows (SPSS, INC, Chicago, IL). The results are presented as mean ± SD and percentages. Chi-square test was used to compare categorical/dichotomous variables. P value of ≤0.05 was considered significant.

Results

The benign group, i.e. group 1 comprising of 25 cases of chronic cholecystitis, included 23 females and only 2 males, sex ratio being 11.5:1. Mean age was 39.36±5.68 (Range 28-52yrs). Most frequent histological diagnosis was chronic cholecystitis (n=20; 80%), followed by three cases of xanthogranulomatous cholecystitis and one case each of follicular and eosinophilic cholecystitis. The wall thickness of gall bladder ranged from 0.2 to 1.5cm, with mean of 0.46cm. All the three cases of xanthogranulomatous cholecystitis had a wall thickness more than 1cm.

The group 2 comprised of 25 cases of gall bladder malignancies. Most gall bladder carcinomas occurred in age group 51-70 years (n=16; 64%). Mean age was 54.40 years±14.08 (Range 28-75 years). The difference

in age between benign and malignant groups was statistically significant (p= 0.0001). Female to male ratio was 2.6:1 (18 females: 7 males). The most common histopathological type was conventional adenocarcinoma (72% cases). Based on differentiation, most of the tumors were moderately differentiated (48%) followed by well differentiated (32%).

An interesting observation in our study was that 44% of our cases (11/25) were discovered incidentally at histopathology with no clinical suspicion of malignancy. 40% cases (10/25) displayed perineural invasion while 60% cases (15/25) showed lymphovascular invasion. 56% cases (14/25) had lymph node metastasis while invasion into adjacent liver was found in 32% cases (7/25). Our study group comprised of almost equal number of patients from all tumor stages (Stages I to IV: 5,6,7,7 respectively).

Ki67 labelling index was found to be very low in the benign group with a mean of 2% (range 0% to 30%) (Figure 1). Only 4% cases (1/25) of benign group were considered positive (>20%), rest 96% were Ki67 negative. On the contrary, in the malignant group, the mean Ki67 index was 38% (range 0%-90%) (Figures 2,3). 60% cases (15/25) were found to be Ki67 positive (LI >20%). This difference of Ki67 index between benign and malignant

Table 1. Comparison of Ki67 LI and Her 2 neu Scoring between Benign and Malignant Groups

Index	Carcinoma (n=25)		Benign (n=25)		p-value
	No.	%	No.	%	
Ki67 index					
Negative	10	40	96	24	0.0001
Positive	15	60	4	1	
Her2/neu					
Negative	9	36	25	100	
1+	5	20	0	0	
2+	5	20	0	0	0.009
3+	6	24	0	0	

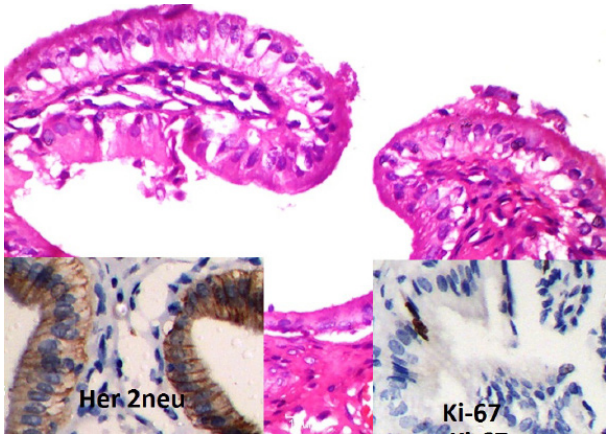

Figure 1. Moderately Differentiated Adenocarcinoma (Hematoxylin & Eosin, 400X); inset – Her/2 neu 2+ staining, inset- Ki67 LI 90%

Table 2. Correlation of Ki67 and Her 2 neu with Various Clinicopathological Parameters in Gall Bladder Carcinoma Cases

	Positive (LI>20%)		Negative (LI<20%)		p-value	Her2/neu Positive(3+)		Her2/neu negative		p-value
	No.	%	No.	%		No.	%	No.	%	
Stage										
I	2	40	3	60	0.38	1	20	4	80	0.2
II	3	50	3	50		3	50	3	50	
III	4	57.1	3	42.9		2	28.6	5	71.4	
IV	6	85.7	1	14.3		0		7	100	
Incidental/Diagnosed										
Present	7	58.3	5	41.7	0.87	3	25	9	75	0.91
Absent	8	61.5	5	38.5		3	23.1	10	76.9	
Histopathology										
Adenocarcinoma	11	61.1	7	38.9	0.89	3	16.7	15	83.3	0.34
Pap. Adenocarcinoma	2	50	2	50		2	50	2	50	
Mucin. Adenocarcinoma	2	66.7	1	33.3		1	33.3	2	66.7	
Perineural Invasion										
Present	6	60	4	40	1	2	20	8	80	0.7
Absent	9	60	6	40		4	26.7	11	73.3	
Lymphovascular invasion										
Present	9	56.2	7	43.8	0.61	2	12.5	14	87.5	0.07
Absent	6	66.7	3	33.3		4	44.4	5	55.6	
LN status										
Present	10	71.4	4	28.6	0.18	2	14.3	12	85.7	0.19
Absent	5	45.5	6	54.5		4	36.4	7	63.6	
Liver involvement										
Present	5	62.5	3	37.5	0.86	0		8	100	0.05
Absent	10	58.8	7	41.2		6	35.3	11	64.7	
Distant metastasis										
Present	6	85.7	1	14.3	0.1	0		7	100	0.08
Absent	9	50	9	50		6	33.3	12	66.7	
Tumor Grade										
WDI	4	50	4	50		3	37.5	5	62.5	0.3
MDII	7	58.3	5	41.7	0.5	3	25	9	75	
PDIII	4	80	1	20		0	0	5	100	

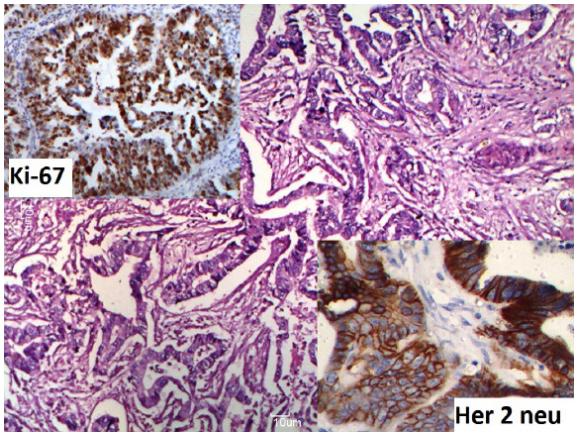

Figure 2. Moderately Differentiated Adenocarcinoma (Hematoxylin & Eosin, 400X); inset – Her/2 neu 2+ staining, inset- Ki67 LI 90%

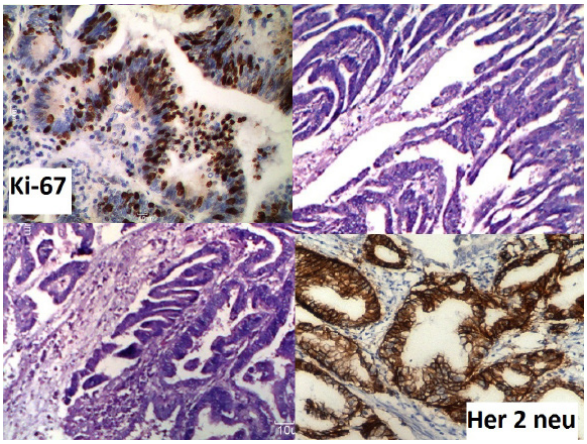

Figure 3. Papillary Carcinoma (Hematoxylin & Eosin, 400X); inset – Her/2 neu 3+ staining, inset- Ki67 LI 75%

Table 3. Correlation of Her2/neu with Ki67 in Both Benign and Malignant Cases

	Her2/neu positive(3+)		Her2/neu negative		p-value
	No.	%	No.	%	
Carcinoma cases					
Ki67					
Positive	5	83.3	10	52.6	0.18
Negative	1	16.7	9	47.4	
Benign					
Ki67					
Positive	0	0	1	4	NA
Negative	0	0	24	96	

group was statistically significant (p=0.0001) (Table 1).

Among female patients, 11/18(61%) were Ki67 positive, while among males 4/7(57%) were Ki67 positive. According to tumor grade, 50% of grade I, 58.3% of grade II and 80% of grade 3 tumors were Ki67 positive. Taking into account tumor stage, 40%, 50%, 57.1% and 85.7% tumors (stages I to IV respectively) were Ki67 positive. However, the difference was not statistically significant. 71.4% (10/14) cases of lymph node positive group were Ki67 positive, while in lymph node negative group, only 45.5% were Ki67 positive (non significant; p=0.18). Similarly, 85.7% of cases with distant metastasis were Ki67 positive compared to cases without distant metastasis, where 50% were Ki67 positive (non significant; p=0.10) (Table 2).

In group 1, none of the cases were Her2/neu positive (considering 3+ as positive) (figure 1). 88% cases were either 1+ or negative, while 3 cases (12%) showed 2+ positivity. In comparison, 24% cases (6/25) were Her2/neu positive in the malignant group (figures 2,3), which was statistically significant (p=0.009) (Table I).

Based on tumor grade, 37.5% of grade 1, 25% of grade 2 and none of grade 3 tumors were Her2/neu positive respectively. Considering tumor stages, 20% of stage I, 50% of stage II, 28.6% of stage III and none of stage IV cases were Her2/neu positive. 14.3% of the lymph node positive cases were Her2/neu positive compared to 36.4% of lymph node negative cases. None of the cases with distant metastasis were Her2/neu positive while 33.3% of cases without distant metastasis were Her2/neu positive. Her2/neu positivity did not have any significant correlation with tumor stage, grade, and lymph node metastasis; however, the difference in Her2/neu positivity between cases with liver involvement versus those without liver involvement was statistically significant (p=0.05) (Table 2).

In the present study, in the malignant group, 5 cases showed both Her2/neu positivity and Ki67 positivity. Ten cases were Ki67 positive but Her2/neu negative while one case was Her2/neu positive but Ki67 negative (Table 3).

Discussion

Her2/neu (C-erbB2), a member of the epidermal growth factor receptor (EGFR) family, is gaining popularity as a candidate for targeted therapy in different cancers like breast (Mitri et al., 2012) and gastric cancer (Chua and Merrett, 2012). There is increasing evidence that overexpression of Her2/neu may play a significant role in the development of biliary tract carcinomas and its expression may be a marker for worse prognosis. Javle et al (2015) retrospectively reviewed cases of advanced GBC and cholangiocarcinoma with Her2/neu genetic aberrations or protein overexpression who received Her2/neu directed therapy between 2007 and 2014. The study concluded that Her2/neu blockade is a promising treatment strategy for GBC patients with gene amplification and deserves further exploration.

Ki67 (MIB-1) protein is expressed during the active phases of the cell cycle (G1, S, G2 and mitosis), but is absent from resting cells (G0). Ki67 labeling index (LI) is considered a marker for cell proliferation and the prognostic impact of Ki67 LI has been reported in various solid cancer tissues. High LI predicts early recurrence after surgery for GBCs. Results of the study conducted by Artico et al (2010) have suggested that Ki67 expression could be utilized as a prognostic factor for the evaluation of clinicopathological progression of GBC.

The present study was undertaken to depict the role of Her2/neu and Ki67 in gall bladder carcinoma. Both benign and malignant cases were studied to compare the difference of expression in both the cases. The mean age of patients in the malignant group was 54.40±14.08 years which was significantly higher than that in benign group

viz. 39.36±5.68 years (p value=0.0001). This observation is comparable to that of Chaube et al (2006), who reported a mean age of 53.62±12.4 years (range 30 to 70 years) in their GBC cases.

Reports available in literature have indicated a higher incidence of GBC in females as compared to males (Dhir and Mohandas, 1999; Kapoor and McMichael, 2003). The number of female patients was higher than male patients in both benign and malignant groups in the present study. The difference, though apparently significant could not reach a statistically significant value (p=0.06). This may be attributed to small sample size.

Ki67 LI was markedly higher in malignant group as compared to benign group (p=0.0001). While 60% malignant cases showed Ki67 positive staining, only 4% were Ki67 positive in benign group. As expected Her2/neu positivity was also significantly higher in malignant group (6/25; 24%) in comparison to the benign group (0/25) (p= 0.009). This is in accordance to the findings of Toledo et al (2012) who observed absence of Her2/neu positivity in benign cases.

Her2/neu positivity ranges from 2 % to 46.5 % across worldwide literature (Roa et al., 2014; Chaube et al., 2006; Toledo et al., 2012; Kim et al., 2001; Matsuyama et al., 2004; Nakazawa et al., 2005; Puhalla et al., 2007; Kawamoto et al., 2007; Harder et al., 2014; Kumari et al., 2012; Doval et al., 2014 . This marked variation could be due to the different scoring systems adopted by different authors as pointed out in Table 4. Moreover, some authors have considered 2+ as well as 3+ score (cytoplasmic as well as membranous) as positive while others considered only 3+ (strong membranous staining) to be Her2/neu

Table 4. Comparative Analysis of Available Literature on Her2/neu Expression with Results of Present Study in Gall Bladder Carcinoma

S.no	Author	No. of Cases	Her2/neu scoring on IHC	FISH Done or not	Results	Conclusion
1	Kim et al., 2001	71	1+: 5-33% cells stained positive 2+: 34-66% 3+:>67%cells	Not Done	1+: 64% 2+: 27% 3+:9%	High Her2/neu expression correlated with poor survival
2	Matsuyama et al., 2004	43	Score 1+ to 3+(Based on membrane staining)	Not Done	1+: 2.3% 2+: 4.7% 3+: 4.7%	Authors did not find any correlation with grade
3	Nakazawa et al., 2005	89	Based on intensity of staining.(1+ cytoplasmic/ 1+ to 3+ membrane)	Yes	2+: 9% 3+: 6.7%	A significant correlation was found between Her2/neu expression and metastatic LN status in BTC.
4	Chaube et al., 2006	78	Both cytoplasmic and membrane staining considered	Not Done	Positive in 25%	Inverse correlation observed with tumor grade. Her2neu as an early event in carcinogenesis
5	Puhalla et al., 2007	55	Membranous staining (positive or negative)	Not Done	Positive in 12%	Correlation found with advanced tumor stages
6	Kawamoto et al., 2007	77	3+ : Complete membranous staining>10%	Yes	2+ and 3+:2,31%	There was strong correlation between Her2neu, IHC and FISH positivity
7	Harder et al., 2009	34	Membranous scoring from 1+ to 3+ only when ≥10% cells show staining	Yes	1+: 21% 2+: 18% 3+: 3%	All 3+ staining were confirmed on FISH
8	Toledo et al., 2012	22	Membrane staining in >30% of cells	Yes	33% adenoCa 90% CIS 91.7% IM	All cases turned out to be FISH negative
9	Kumari et al., 2012	97	3+ : if complete membrane staining in >10% cells 2+: incomplete membrane staining	Not Done	3+: 10% 2+: 4%	Her2/neu expression was more in WD and stage II to IV tumors c-erbB2 tumors better median survival than c-erbB2 negative
10	Roa et al., 2014	187	Criteria same as for breast: 3+ complete membrane staining in >30% cells	Not Done	3+: 12.8%(considered positive) More in WD adv stage cancer	Her2/neu overexpression seen in 14% of advanced GBCa, this group may benefit from inhibitors of Her2neu
11	Doval et al., 2014	50	Same scoring as breast cancer	Not Done	Overexpression in 4%	Her2/neu expression no correlate with stage, differentiation, nodal and distant metastasis.
12	Present study, 2015	25	Same scoring as breast Cancer.	Not Done.	3+: 24% (considered positive) 2+: 20% 1+/Negative: 56%	Her2/neu overexpression was seen in 24% of malignant cases. Her2/neu expression did not correlate with clinicopathological parameters in GBC, except liver involvement.

Table 5. Comparative Analysis of Available Literature on Ki67 with Results of Present Study in Gall Bladder Carcinoma

S.no	Author	No. of cases	Results and conclusion
1.	Shrestha et al., 1998	32	MIB LI found to be higher in cases with LN metastasis than without them. Patients with low MIB LI had a better prognosis than with higher MIB LI.
2.	Hui et al., 2002	37	High Ki67 index was significantly correlated with tumor lymphatic invasion. High Ki67 predicts early recurrence after surgery for GBC. 24/41(58.5%) were Ki67 positive.
3.	Grau et al., 2004	41	There was no difference between grade of differentiation and wall infiltrate. 5 years survival of patients with MIB-1 positive index Vs negative index was 9.2% *Vs* 27.7%.(Not significant)
4.	Roa et al., 2009	102-GBC	Staining Index of Ki67 was 19±25%(non tumoral cases) and 46±29% in GBC($p<0.01$).
		96-benign Gall bladder	75% cases had SI>20%.
5.	Kai et al., 2012	86	42 patients: low group 44 patients: High group Ki67 LI did not correlate with patient survival.
6.	Toledo et al., 2012	22	Cell Proliferation significantly higher in epithelia which had metaplasia(36.7%; p value=0.03) Carcinoma in situ(37.5%; p value=0.05) and cells of Invasive GBC(42%; p value=0.03)
7.	Doval et al., 2014	50	Ki67 LI was significantly higher in patients <40years of age and poorly differentiated tumors.
8.	Present study, 2015	Benign: 25 Malignant: 25	KI67 overexpression seen in 60% malignant cases. Ki67 expression did not correlate with any of the clinicopathological parameter.

positive (as in present study). This highlights the need to evolve a uniform consensus on scoring for Her2/neu staining in gall bladder cancers on similar lines as breast cancer. Her2/ neu positivity in our study was 24% (6/25) which is comparable to that reported by Chaube et al (2006) (25%), however, they considered both 2+ and 3+ score to be positive.

We did not find any statistical association between Her2/neu positivity and clinicopathological parameters like tumor stage, grade, lymph node metastasis or distant metastasis. This is in concordance with most of the available studies (Roa et al., 2014; Doval et al., 2014). Interestingly we found a significant correlation of liver involvement with Her2/neu positivity (p=0.05).

Similar to the findings of Chaube et al (2006), we did observe a trend in decrease in Her2/neu expression with increasing grade of tumor (37.5% cases of grade I, 25% cases of grade II and no case of grade III showed Her2/ neu positivity), the results however were not statistically significant. Unlike our findings, Puhalla et al (2007) observed that Her2/neu overexpression correlated with advanced tumor stage.

Contradictory to our findings as well as that reported by others (Kumari et al., 2012; Roa et al., 2014), Toledo et al. (2012) found Her2/neu overexpression in 90% carcinoma in situ and 33 % advanced cancers. This again reflects the marked variability observed by different authors in studies based on IHC. Roa et al. (2014) and Kim et al (2001) have reported a higher Her2/neu expression correlated with poor survival.

As with the results of Her2/neu expression, we could not find any statistically significant correlation of Ki67 with any of the clinicopathological parameters. Doval et al (2014) in his study found that Ki67 expression was significantly higher in patients of age group <40years and in patients with poorly differentiated tumors. In the present study most of the Ki67 positive patients were ≥40years of age with maximum Ki67 positivity (46.7%) in moderately differentiated tumors. Comparative evaluation of various studies on Ki-67 in biliary tract cancers is shown in Table 5.

Another intriguing finding was that Ki67 LI was higher in cases with lymph node metastasis (71.4%) than without lymph node metastasis (45%). A noteworthy finding was that out 6 cases with distant metastasis, 5(83.3%) of them stained positive for Ki67, this perhaps justifies, that more the proliferation, greater are the chances for metastasis.

Similar to the findings of Doval et al. (2014), we could not find a significant correlation between the two indices, in GBC patients.

The results of our work showed that besides clinicopathological factors, molecular biological factors may contribute to better tumor characterization and thus more precisely determine its clinical behavior.

One of the limitations of our study was the small sample size (n=25 for malignant tumors). This could be the reason we did not find any statistical association of Her2/neu with most of the clinicopathological parameters. Secondly, we relied on immunohistochemistry only. Recent reports in literature, have recommended use of florescent in situ hybridization for confirmation of all Her2/neu 2+ cases (on IHC).

In conclusions, The present study demonstrated overexpression of Her2/neu and Ki67 in gall bladder cancer. The mean age of the patients with GBC in our population was >50 years, with more than 70% of female patients being afflicted. Gall bladder carcinomas with liver involvement were found to express Her2/neu more than the uninvolved ones. A trend of decreasing Her2/neu expression with increasing grade of tumor was observed. Furthermore, greater Ki67 positivity was found in cases with lymph node metastasis and distant metastasis.

Future studies with larger number of patients will be required to precisely define the correlation of Her2/neu expression and Ki67 positivity with clinicopathological parameters. The results however are encouraging and suggest evaluation of Her2/neu as a candidate for targeted therapy.

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