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Research Article

Immunohistochemical expression of Her2-neu and Ki-67 in premalignant and malignant lesion of gall bladder and its correlation with survival time

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Abstract
Introduction: *Gall Bladder Carcinoma (GBC) is a diagnostic and a therapeutic challenge. Although it is increasing, chronic cholecystitis remains the most worldwide gall bladder lesions, harbouring many epithelial changes that may end in carcinoma.*
Aim: *To investigate the expression of HER2-neu and Ki-67 in malignant and non-malignant gall bladder lesions, and to evaluate its correlation with survival time.*
Materials and Methods: *In this retrospective as well prospective study include total of 154 cases of gall bladder in which 85 cases of malignant lesions and 47 cases of premalignant and premalignant like lesion (including dysplasia , metaplasia and xanthogranulomatous cholecystitis) along with 22 cases of chronic cholecystitis as control. The blocks were collected from the Department of Pathology of KGMU, Lucknow. Immunohistochemical staining results of HER2-neu and Ki-67 were analysed and correlated by Statistical Package for the Social Sciences (SPSS) version 15 and Chi-square test and for survival time interpretation use Kaplan-Meier survival curve and Log- rank test.*
Results: *Positive HER2-neu expression (+2, +3) was detected in 47.5% (19/40) of malignant cases and 12.5% (1/8) of dyspastic group, at the same time it was completely absent in the metaplastic and cholecystitis cases. Similarly Ki-67 Li expression was observed to be <10% in 75.3% of malignant and all the cases in premalignant and control groups. In malignant group, a total of 14 (16.5%) patients had Ki-67 Li expression in range 10-25% and remaining 7 (8.2%) had Ki-67 Li expression >25%.*
Conclusion: *HER2-neu, and Ki-67 are overexpressed in GBC cases compared with control and dysplastic group, these can be use as new therapeutic targeted agent in GBC.*
Keywords: *Gall bladder cancer, Her2neu expression, Ki-67, Chronic cholecystitis.*

Introduction

Gall bladder carcinoma (GBC) is an aggressive malignancy accounting for 1.2% of all new cancer related cases and 1.7% of all cancer related deaths worldwide[1]. The disease burden is on rising trend in northern India due to increase in lifestyle-

related modifiable risk factors. The associated risk factors for gallbladder carcinoma includes cholelithiasis, obesity, reproductive female factors, chronic infection of the gallbladder and environmental exposure to specific chemicals like heavy metals etc.[2,3] It is 14th most common malignancy in India with annual incidence of 2.5% and mortality rate of 2.75%.[4] In early stages GBC is usually asymptomatic, Majority of patients present in advanced stage when palliation remains the only possible therapeutic option. Due to limited intervention, the prognosis remains dismal(5). Better insight into the molecular pathogenesis is needed to develop an effective targeted therapy which can offer a hope of better survival in these patients. After successful implementation of HER-2/neu directed therapy in breast and gastric adenocarcinoma, interest on similar lines is generated in GBC as well.[6] Fluorescent in situ hybridization (FISH) and immunohistochemistry (IHC) are two commonly used modalities for HER-2/neu expression. Heterogeneity exists in the literature regarding frequency of HER-2-neu overexpression in GBC.[7] Prognostic role of HER-2/neu in GBC is also not clear. Some authors have found significant correlation of HER-2/neu overexpression with tumor grade and patient survival while few failed to establish any significant correlation[8,9]. In most instances, gallbladder cancer develops over 5 to 15 years, when metaplasia progresses to dysplasia, carcinoma in situ, and then, invasive cancer[10]. Recent molecular genetic studies have shown that selected proto-oncogenes and tumor suppressor genes are involved in the development and progression of gallbladder carcinoma, and a different spectrum of molecular genetic changes appears to be responsible for each of the different pre-neoplastic conditions[11,12].

Receptor tyrosine-protein kinase erbB-2, also known as CD340, proto-oncogene Neu, ErbB2 (human) is a protein that in humans is encoded by the ErbB2 gene, which is also frequently called HER2 (from human epidermal growth factor receptor 2) or HER2/neu. ErbB2 located at the long arm of chromosome 17 (17q12). HER2 is a member of the human epidermal growth factor receptor (HER/EGFR/ERBB) family. c-erbB2 is gaining popularity as a candidate for targeted therapy in different cancers. Although started as Herceptin, the drug against c-erbB2 for breast cancer is now being explored in gastrointestinal cancers. There is an increasing evidence that over-expression c-erbB2 (HER2-neu) may play an important role in the development of biliary tract carcinomas[13,14]. Antigen Ki-67 is a protein that corresponds to a nuclear non-histone protein expressed by cells in the proliferative phases G1, G2, M, and S. In general, there is a good correlation between Ki-67 staining and mitotic count. There is a good correlation of Ki67 labeling index and the morphologic aggression indicators of hyperplastic, dysplastic and malignant diseases of the gallbladder in addition to its prognostic significance[15,16]. The aim of this study is to assess the proliferative activity of the gallbladder epithelium utilizing Ki-67 score in neoplastic and non-neoplastic diseases of gall bladder.

Aims and Objective

To evaluate expression of Her-2neu and Ki-67 in pre-malignant and malignant lesions of gall bladder and correlated with survival time.

Material and Methods

Prospective and Retrospective case control two year study.

Study Sample: Tissue samples of primary gall bladder lesions picked up after the surgery, suspected on the basis of clinicoradiological findings from Department of Pathology in King George's University Lucknow. Total of 154 cases of gall bladder was studied in which 85 cases of malignant lesions and 47 cases of premalignant and premalignant like lesion (including dysplasia, metaplasia and xanthogranulomatous cholecystitis) along with 22 cases of chronic cholecystitis as control.

Inclusion and Exclusion Criteria: All gall bladder carcinoma and premalignant lesions along with chronic cholecystitis as controls cases included. Secondary gall bladder carcinomas (Metastatic), Post chemotherapy or post radiotherapy gallbladder malignancies were excluded.

Hematoxylin and Eosin section staining and Histopatholigical typing :

Histological types were classified in accordance with World Health Organization guidelines and carcinomas were divided into well, moderately and poorly differentiated groups.

Immunohistochemistry: Immunohistochemistry was performed with antibodies to HER2- neu manufactured by Dako (FLEX Monoclonal mouse Anti-Human HER2- neu , Clone DAK- HER2-neu) and Ki-67 manufactured by Dako (FLEX Monoclonal mouse Anti-Human HER2- neu or Anti-Human Ki-67 , Clone DAK- HER2-neu).

Immunohistochemistry Control: Known 3+ positive case of carcinoma breast tissue was used as positive control for HER-2neu.

Immunohistochemistry Interpretation: For the interpretation of IHC, Cell membrane staining was used to assess, positivity for Her2/neu with criteria as used for breast cancer. Scoring was done as follows:

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+ (Positive): 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.

Immunohistochemical evaluation of Ki-67 was done by the use of Dako (trade mark) Flex monoclonal antihuman Ki-67 and evaluated under light microscopic observation at x 400 magnification. In the immunostaining analysis, positive brown nuclei were detected and discriminated from negative blue hematoxyline nuclei. Ki-67 labelling index (MIB-1 index) was calculated as the percentage of positively stained tumour cell nuclei out of the total tumour cells counted (n=1000). A percentage of stained cells was considered positive regardless of the intensity of staining.

Survival time interpretation: For survival time interpretation we use Kaplan-Meier survival curve and Log- rank test. Mortality rate up to a maximum follow up period of 19 months.

Statistical Analysis: The statistical analysis was done using SPSS (statistical package for social sciences) version 15.0 statistical analysis software. The values were represented in Number (%) and Mean±SD. The results of ki-67 were expressed in mean, range and Chi square test and level of significance ie P 10% of tumor cells.

Results

The study was carried out with an aim to evaluate the HER2/neu and Ki-67 expression in carcinoma gallbladder, premalignant and chronic cholecystitis and to carry out survival time correlation. For this purpose a case-control study was carried out in which a total of 154 subjects were enrolled. Group-wise distribution of subjects enrolled in the study has been shown in **[Table 1**] below:

Table 1: GroupWise Distribution of Subjects enrolled in the study

SN	Group	Description	No. of cases	Percentage
1.	Malignant	Cases with malignant lesions of gall bladder	85	55.2
2.	Premalignant	Cases with premalignant and premalignant like lesions of gall bladder	47	30.5
3.	Controls	Specimen obtained from patients undergoing cholecystectomy	22	14.3

Out of 154 subjects enrolled in the study, a total of 85 (55.8%) were cases of malignant lesions of gallbladder. There were 47 (30.5%)specimen obtained from patients with premalignant and premalignant like lesions of gall bladder while remaining 22 (14.3%) were specimen obtained from patients undergoing cholecystectomy – these patients comprised the control group of study.

Table 2: Distribution of cases according to Diagnostic Type in each group

SN	Type	No. of cases	Percentage
1.	**Malignant (n=85)**		
	Adenocarcinoma (Well differentiated)	34	40.0
	Adenocarcinoma (Moderately differentiated)	40	47.1
	Adenocarcinoma (Poorly differentiated)	5	5.9
	Adenocarcinoma sarcomatoid differentiation	1	1.2
	Adenocarcinoma with Xanthogranulomatous cholecystitis	5	5.9
2.	**Premalignant & premalignant like lesions (n=47)**		
	Dysplasia	1	2.1
	Xanthogranulomatous cholecystitis	31	66.6
	Antral metaplasia	14	29.8
	Intestinal metaplasia	1	2.1
3.	**Control (n=22)**		
	Chronic cholecystitis	22	100

Among malignant cases (n=85), [**Table 2**] maximum were moderately differentiated adenocarcinoma (n=40; 47.1%) followed by well differentiated adenocarcinoma (n=34; 40%). A total of 5 (5.9%) cases each were poorly differentiated adenocarcinoma and adenocarcinoma with xanthogranulomatous cholecystitis respectively. There was 1 (1.2%) case of adenocarcinoma with sarcomatoid differentiation.

Among premalignant cases, majority (n=31; 66.6%) were xanthogranuloma cholecystitis followed by antral metaplasia (n=14; 29.8%). There was 1 (2.1%) case each with dysplasia and intestinal metaplasia respectively.

Table 3: Her2-neu Expression levels in different groups

SN	Group	Expression							
		0		1+		2+		3+	
		No.	%	No.	%	No.	%	No.	%
1.	Malignant (85)	59	69.4	8	9.4	11	12.9	7	8.2
2.	Premalignant (47)	45	95.7	0	0	2	4.3	0	0
3.	Control (22)	21	95.5	0	0	1	4.5	0	0

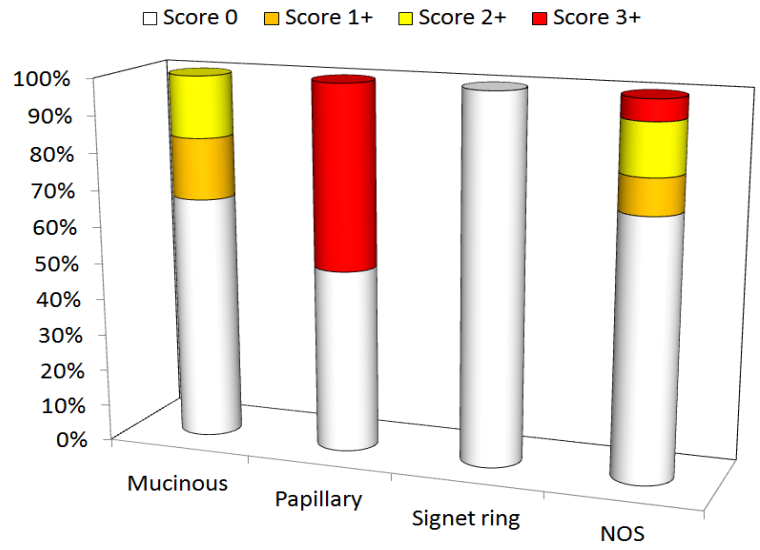
H=16.77; p<0.001 (Kruskall-Wallis test)

Majority of cases in all the groups did not show Her2-neu expression [**Table 3,**]. In malignant group, expression was seen in 30.6% cases – 8 (9.4%) had expression score 1+, 11 (12.9%) had score 2+ and remaining 7 (8.2%) had expression level 3+. In premalignant group, only 2 (4.3%) cases showed expression with 2+ score. In control group only 1 (4.5%) case showed expression with 2+ score. Statistically, there was a significant difference among groups with respect to IHC expression of Her2-neu.

Table 4: Her2-neu Expression in different variants of Adenocarcinoma

SN	Variant	N	0		1+		2+		3+	
			No.	%	No.	%	No.	%	No.	%
1.	Mucinous	6	4	66.7	1	16.7	1	16.7	0	0
2.	Papillary	6	3	50.0	0	0.0	0	0.0	3	50.0
3.	Signet ring	1	1	100.0	0	0.0	0	0.0	0	0
4.	NOS	72	51	70.8	7	9.7	10	13.9	4	5.6

χ^2=16.42 (df=9); p=0.059.77; p<0.001 (Kruskall-Wallis test)



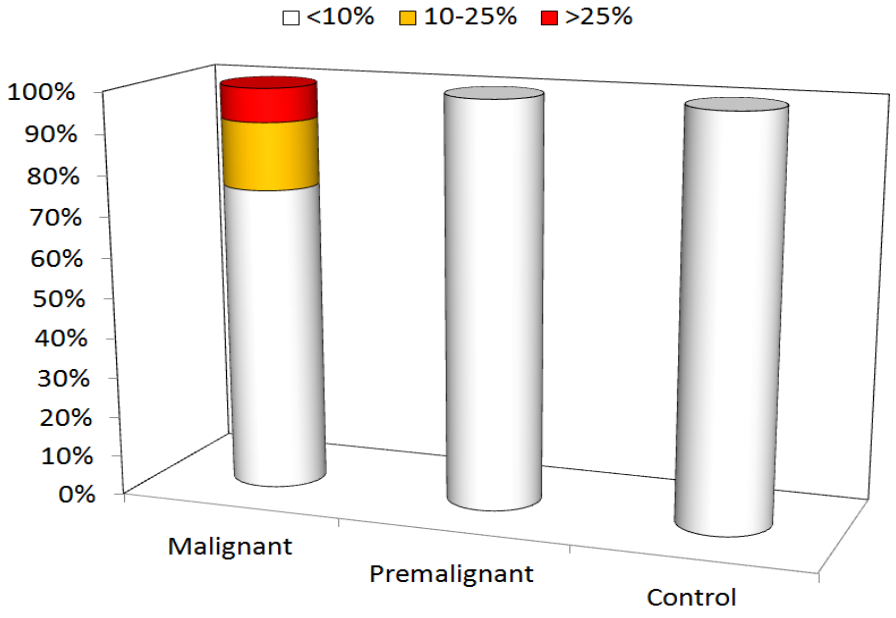
[Figure 1]

In all the variants except papillary type, majority of cases had no expression **[Table 4, Figure 1].** In Papillary type 50% had no expression and remaining 50% had expression score 3+. [**Figure 2**] In mucinous type [**Figure 3**], 66.7% had no expression and 16.7% each had score 1+ and 2+ respectively. Among NOS cases 70.8% [**Figure 4**] had no expression, 9.7% had score 1+, 13.9% had score 2+ and remaining 5.6% had score 3+.Statistically, there was no significant association between Her2-neu expression score and type of variant (p=0.059).

Table 5: Ki-67 Li Expression level in different groups

SN	Group	Expression					
		<10%		10-25%		>25%	
		No.	%	No.	%	No.	%
1.	Malignant (85)	64	75.3	14	16.5	7	8.2
2.	Premalignant (47)	47	100	0	0	0	0
3.	Control (22)	22	100	0	0	0	0

H=19.52; p<0.001 (Kruskall-Wallis test)



[Figure :6]

Ki-67 Li expression was observed [**Table 5, Figure-6**] to be <10% in 75.3% of malignant and all the cases in premalignant and control groups. In malignant group, a total of 14 (16.5%) patients had Ki-67 Li expression in range 10-25% and remaining 7 (8.2%) had Ki-67 Li expression >25% [**Figure 5**]. On evaluating the differences among groups statistically, the difference was found to be significant (p<0.001).

Table 6: Association between Her2-neu positivity and survival (n=49)

SN	Status	Total No.	Alive		Died	
			No.	%	No.	%
1.	Positive	9	5	55.6	4	44.4
2.	Negative	40	19	47.5	21	52.5
Total		49	24	49.0	25	51.0

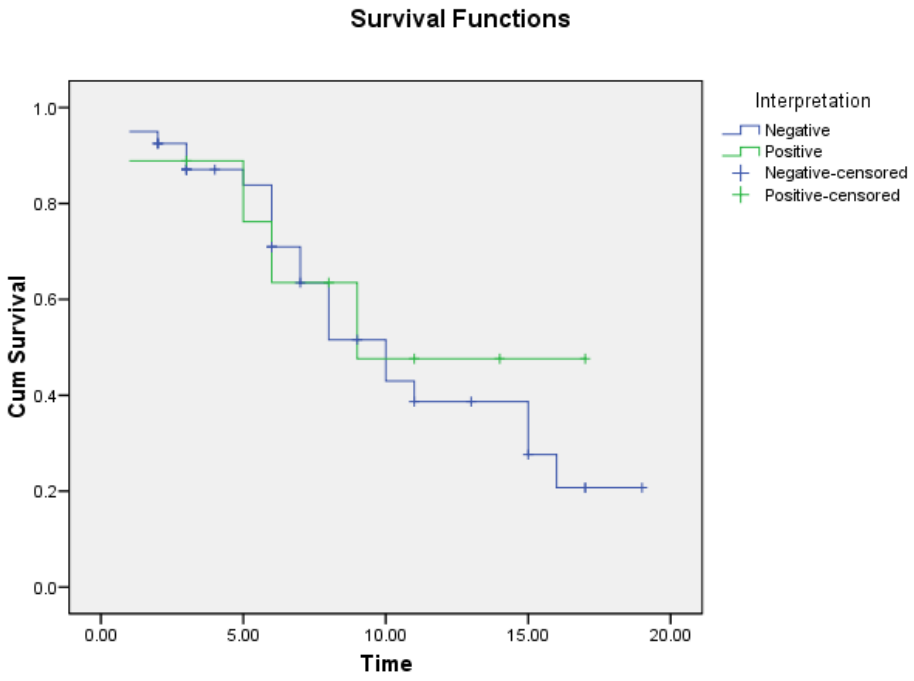
χ^2=0.191; p=0.662 (NS)

Mortality rate up to a maximum follow up period of 19 months was **[Table 6]** lower in Her2-neu positive (44.4%) as compared to Her2-neu negative (52.5%) patients. However, this difference was not significant statistically.

Table 7: Comparison of Survival Time between Her2-neu positive and Her2-neu negative patients (n=49)

Her2/neu Expression Status	Mean(a)			
	Estimate	Std. Error	95% Confidence Interval	
Negative (n=40)	10.705	1.080	8.587	12.822
Positive (n=9)	11.032	2.167	6.785	15.278
Overall	10.957	.999	8.999	12.915

Log rank (Mantel-Cox) χ^2=0.222; p=0.637 (NS)



[Figure: 3]

Mean survival time **[Table 7, Figure 3]** was 10.705±1.080 months among HER2-neu negative as compared to 11.032±2.167 months among Her2-neu positive patients. Though mean survival time among HER2-neu positive patients was slightly higher as compared to that in HER2-neu negative patients yet this difference was not significant statistically (p=0.637).

Table 8: Association between Ki-67 positivity and survival (n=49)

SN	Status	Total No.	Alive		Died	
			No.	%	No.	%
1.	Positive	11	4	36.7	7	63.6
2.	Negative	38	20	52.6	18	47.4
Total		49	24	49.0	25	51.0

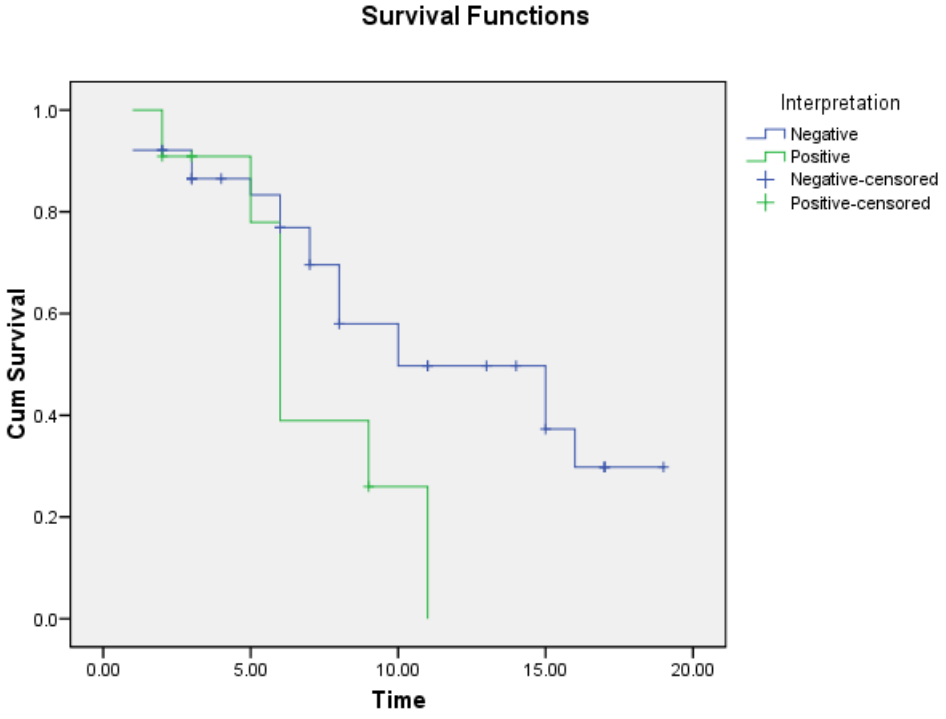
χ^2=0.903; p=0.342 (NS)

Mortality rate up to a maximum follow up period of 19 months was 63.6% among Ki-67/Li positive and 47.4% among Ki-67/Li negative patients **[Table 8]**. Although survival rate was higher among Ki-67/Li negative as compared to Ki-67/Li positive patients yet this difference was not significant statistically (p=0.342).

Table 9: Comparison of Survival Duration between Ki-67/Li positive and Ki-67/Li negative patients

Ki-67/Li Expression Status	Mean(a)			
	Estimate	Std. Error	95% Confidence Interval	
Negative	11.783	1.150	9.528	14.038
Positive	7.195	1.031	5.175	9.215
Overall	10.957	.999	8.999	12.915

Log rank (Mantel-Cox) χ^2=3.676; p=0.055 (NS)



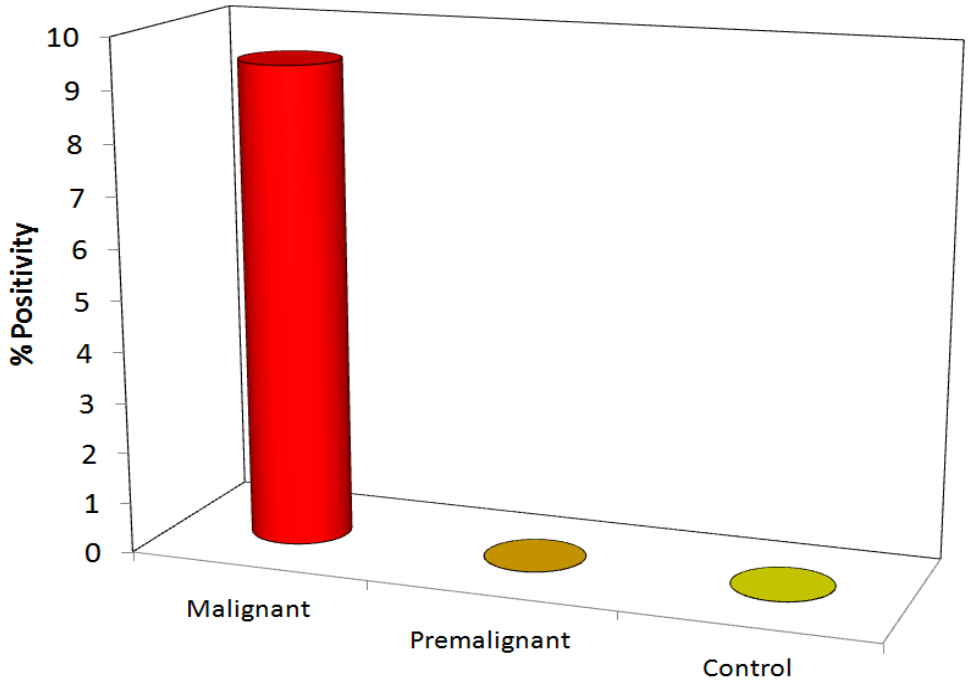
[Figure 4]

Mean survival duration was 11.783±1.150 months in Ki-67/Li expression negative and 7.195±1.031 months in Ki-67/Li expression positive patients **[Table 9, Figure 4].** Although mean survival duration was longer in Ki-67/Li negative as compared to positive patients yet this difference was not significant statistically (p=0.055).

Table 10: Co-expression of HER2-neu and Ki-67 Li in different groups

SN	Group	Total No.	No. with Co-expression	% Co-expression
1.	Malignant	85	8	9.4
2.	Premalignant	47	0	0
3.	Control	22	0	0

χ^2=6.850; p=0.033



[Figure 5]

Co-expression was seen in 8/85 (9.4%) of malignant cases **[Table 10, Figure 5]**. None of the cases in premalignant and control group had co-expression. Statistically, this association was significant.

Table 11: Association between Co-expression and survival (n=49)

SN	Status	Total No.	Alive		Died	
			No.	%	No.	%
1.	Positive	4	1	25.0	3	75.0
2.	Negative	45	23	51.1	22	48.9
Total		49	24	49.0	25	51.0

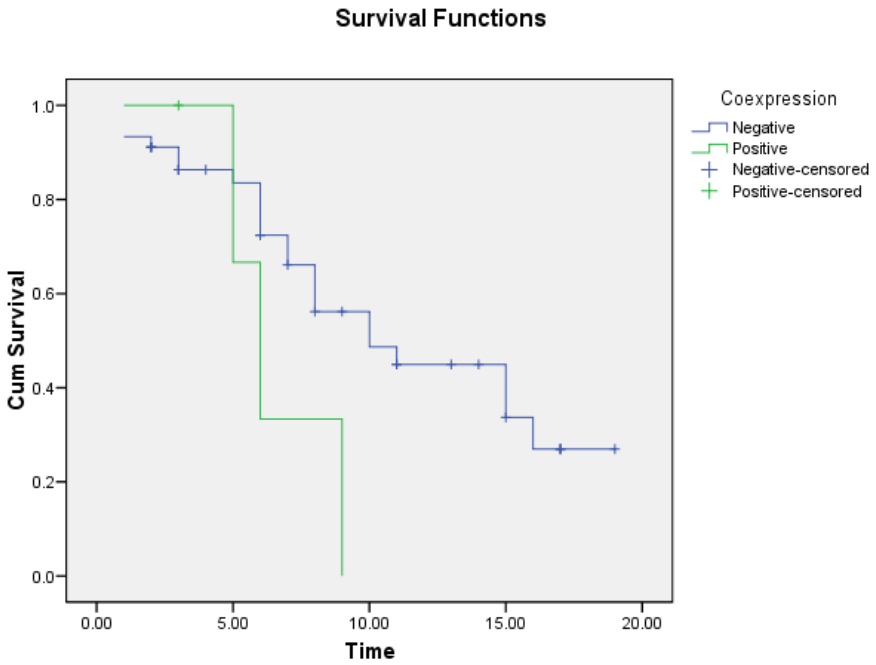
χ^2=1.002; p=0.317 (NS)

Mortality rate up to a maximum follow up period of 19 months was 75% among patients with co-expression and 48.9% among coexpresion negative patients **[Table 11]**. Although survival rate was higher among co-expresion negative as compared to co-expression positive patients yet this difference was not significant statistically (p=0.317).

Table 12: Comparison of Survival Duration between Co-expression positive and Co-expression negative patients

Co-Expression Status	Mean(a)			
	Estimate	Std. Error	95% Confidence Interval	
Negative	11.35	1.06	9.27	13.42
Positive	6.67	1.20	4.31	9.02
Overall	10.96	1.00	9.00	12.91

Log rank (Mantel-Cox) χ^2=2.572; p=0.109 (NS)



[Figure 6]

Mean survival duration was 11.35±1.06 months in co-expression negative and 6.67±1.20 months in co-expression positive patients **[Table 12, Figure 6]**. Although mean survival duration was longer in co-expression negative as compared to positive patients yet this difference was not significant statistically (p=0.109).

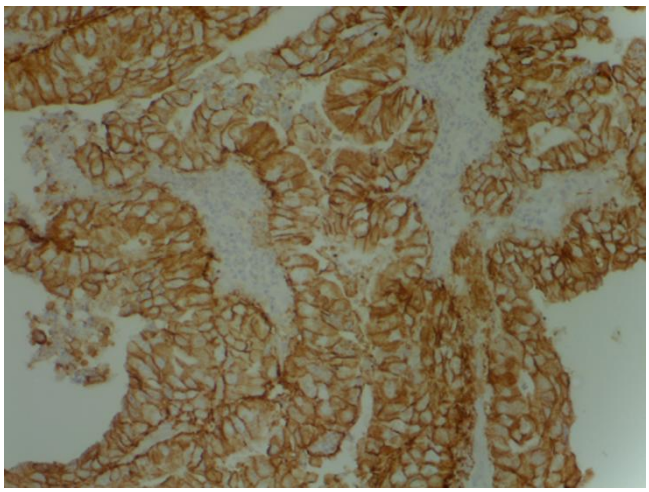


Figure 2 Expression of Her-2 neu 3+ staining in Papillary Adenocarcinoma gall bladder

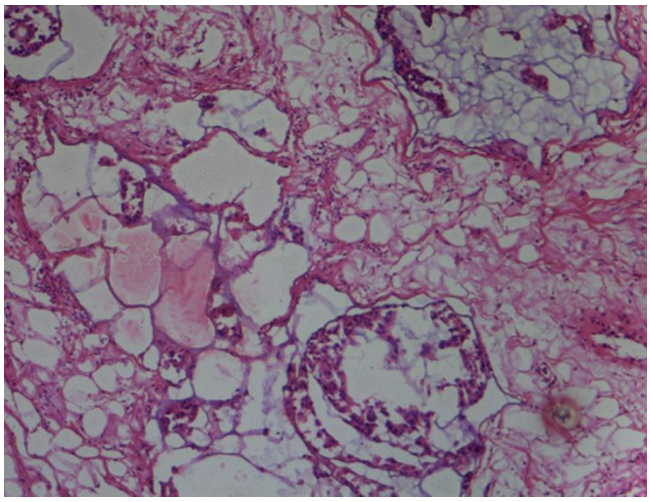


Figure 3 Mucinous Adenocarcinoma gall bladder (Hematoxylin & Eosin, 20X)

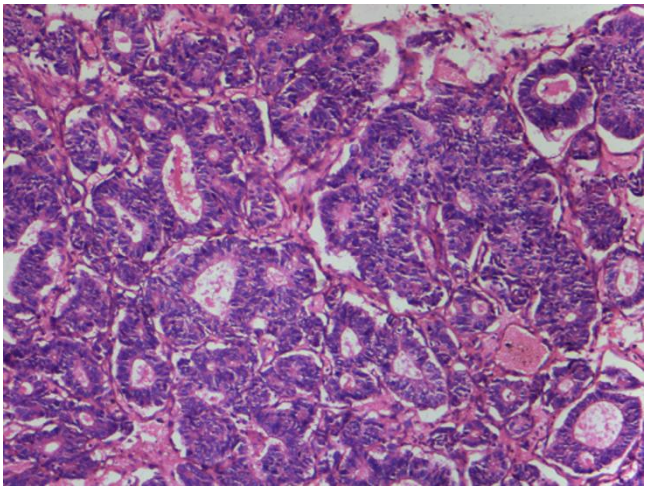


Figure 4 well differentiated Adenocarcinoma of gall bladder (Hematoxylin & Eosin, 20X)

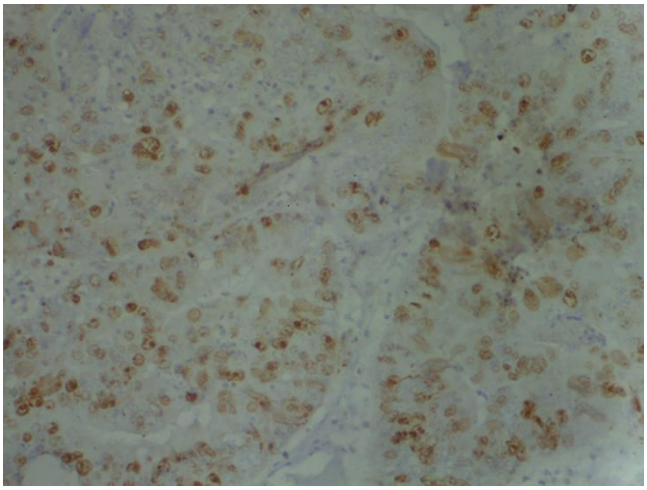


Figure 5 Ki-67 expression >25% in moderate differentiated Adenocarcinoma gall bladder

Discussion

Carcinoma of gall bladder, an aggressive disease with poor prognosis. The poor prognosis, increasing incidence besides ineffective therapy of GBC make its management challenging. Various genetic and molecular markers have been studied which include P53, CEA, CDNK1, MUC1, FHIT, growth factor and their receptor etc. However till now, there is no clear understanding of gallbladder carcinoma pathogenesis and no candidate molecules has been explored for targeted therapy [17] Therefore, there is a need of effective therapeutic agents for proper targeted therapy. The present study was planned to investigate the immunohistochemical expression of HER2-neu, and Ki-67 in GBC, comparing with dysplastic and metaplastic lesions, beside the correlation with survival time.

Expression of HER2-neu has been intensively studied in different tumour entities and has led to the use of targeted therapy with specific inhibitors or antibodies of these receptors in colorectal, breast, lung, as well as head and neck cancer[18,19]. For gallbladder cancer data for HER2-neu overexpression have been presented in mostly small patient cohorts. Targeted therapy with anti-HER2-neu in breast cancer is effective when HER2-neu receptor over- expressed[20]. However, its study in gall bladder carcinoma is limited.

In the present case control study 154 cases were included; 85 gall bladder cancer, 47 premalignant and malignant like lesion and 22 controls i.e. chronic cholecystitis.

Among the three groups i.e. malignant, premalignant and control, each showed a frequency of 55.2%, 30.5% and 14.3% respectively. Majority of patients irrespective of groups (malignant and benign), were females i.e. 119 (77.3%). Our study is in concordance with previous study done by Rao I et al.[21] which enrolled 187 cases (165 female, 22 male) out of which 88.2% cases were females of gall bladder carcinoma along with 75 control (chronic cholecystitis). Our study is also in concordance with the study conducted by Doval D.C. et al [22]

which enrolled fifty consecutive sample of cholecystectomy/ biopsy from gall bladder in which 80% were females. Similarly in the study done by Yoshida H el al.[22] which enrolled large number of gallbladder cases i.e. 211 gall bladder carcinoma and they did not include chronic cholecystitis (control) and premalignant lesion. Their study was largest case study in the literature for HER2-neu and gall bladder carcinoma.

In our study among malignant cases, maximum were moderately differentiated adenocarcinoma (n=40; 47.1%) followed by well differentiated adenocarcinoma (n=34; 40%) along with 5 (5.9%) cases of each with poorly differentiated adenocarcinoma and adenocarcinoma with xanthogranulomatous cholecystitis. There was also 01 (1.2%) case of adenocarcinoma with sarcomatoid differentiation. In the study conducted by Doval D.C. et al [9] 86% cases were of adenocarcinoma and 14% cases adenosquamous carcinoma. Most of the tumors were moderately differentiated (68%).

In our study premalignant and premalignant like lesion were included -14 cases of antral metaplasia (29.8%), one case (2.1%) each of dysplasia and intestinal metaplasia and 31 cases of xanthogranulomatous cholecystitis (66.6%). This goes in concordance with study conducted by Kim YW et al.[23] who also studied premalignant lesion including two cases of gall bladder dysplasia and 20 cases of gall bladder adenoma.

Considering the variants of adenocarcinoma, we found 6 cases of mucinous adenocarcinoma, 6 cases of papillary adenocarcinoma and 1 case of signet ring adenocarcinoma in which is concordance with the study of Kumari N et al.[17] who taken total 97 cases of adenocarcinoma gallbladder. Out of these 97 cases, 81 cases were of conventional adenocarcinoma, 8 cases of papillary adenocarcinoma, 1 case of adenocarcinoma with signet ring cells, 3 cases of mucinous adenocarcinoma, 1 case of mucinous adenocarcinoma with signet ring cells, 3 cases of signet ring cell carcinoma, 6 cases of adenosquamous cell carcinoma and 1 case of squamous cell carcinoma.

In our study, majority of cases in all the groups did not show HER-2 neu expression. In malignant group, its expression was seen in 30.6% cases in which 8 (9.4%) had expression score 1+, 11(12.9%) had score 2+ and remaining 7 (8.2%) had expression level 3+. In premalignant group, only 2 cases (4.3%) showed expression with 2+ score and control group only one case (4.5%) showed expression 2+ score. We concluded 2+ score and 3+ score as over-expression and thus, % of overexpression in malignant group is 21.2%, in premalignant and control group it was 4.3% and 4.5% respectively. This finding is in concordance with the study of Kim YW et al.[23] in which thirty-three gallbladder carcinomas (46.5%) showed positive staining for c-erbB-2, but none of the dysplasia and adenoma were positive ($p<0.05$). In the study conducted by Kumari et al.[17] 10 (9.8%) cases of GBC showed complete membranous (3+ score) for Her-2 neu, 8 (80%) of these cases were well differentiated carcinoma and 2 (20%) were moderately differentiated carcinoma. 4 cases had incomplete membranous expression (2+ score). Considering both 3+ and 2+ staining as overexpression, Her-2 neu (c-erbB2) overexpression was seen in 13.4% cases of GBC. None of these with c-erbB2 expression were associated with xanthogranulomatous inflammation. Chaube et al.[8] studied 40 cases of GBC and showed over-expression of c-erbB2 in 25% cases. They also studied premalignant lesions and observed 4 out of 10 (40%) papillary adenomas of the gall bladder showing over expression of c-erbB2. Nakazawa et al.[13] showed over-expression of c-erbB2 in 16% of their 89 cases by combining both immunohistochemistry and FISH. Considering 3+ complete membranous staining as positive they had only 8% c-erbB2 expression in their study. Kamel D et al.[24] studied 30 cases of GBC and showed over-expression of c-erbB2 in 10% cases on immunohistochemistry. Expression of c-erbB2 has varied between 10% and 46.5% in gall bladder

carcinoma and its expression was correlated with increasing stage. Kim HZ et al.[25] investigated 55 cases of extrahepatic cholangiocarcinoma and found 36 cases (65.5%) with score 0, 3 (5.4%) cases with score 1+, 14 (25.5%) case with score 2+ and 2 (3.6%) cases with score 3+. They observed as positive immunostaining (2+ or 3+) for HER-2 neu protein in 16 (29.1%) out of 55 cases of extrahepatic cholangiocarcinoma, which is in concordance with our study revealing positive immunostaining (2+ or 3+) for HER-2 neu in 18 cases (21.2%).

Rao et al. [21] in their study undertook 187 cases of gall bladder carcinoma along with 75 control cases. In control group no cases expressed 3+ expression. In gall bladder carcinoma specimen, 90 (48.1%) stained negative, 35(18.7%) were 1+, 38(20.3%) were 2+, and 24 (12.8%) were considered positive 3+ for overexpression of HER-2 neu which is concordance to our study showing overexpression (HER-2 neu 2+ and 3+) in 21.1% cases.

HER-2 neu expression in variants of adenocarcinoma: In our study expression of mucinous adenocarcinoma was 0 in 4 (66.7) cases, 1+ in 1case (16.7%), 2+ in one case (16.7%) and 3+ in none of the case. Papillary adenocarcinoma expressed zero staining in 3 (50.0%) cases, none of the case with 1+ or 2+ expression and 3+ expression was found in 3 cases (50%). There was only 1 case of Signet ring adenocarcinoma which did not express positivity. Our study is in concordance with the study conducted by Doval DC et al.[9] who also found higher expression of HER-2 neu in papillary adenocarcinoma.

In our study Ki-67 Li expression was observed to be <10% in 75.3 % of malignant and all the cases in premalignant and control groups. In malignant group, a total of 14 (16.5%) patients had Ki-67 Li expression in range of 10-25% and remaining 7 (8.2%) had Ki-67 Li expression of >25%. Our study is in concordance with the study of Wang X. et al.[26] who studied 30 cases of cholangiocarcinoma out of which 29 cases ie 96.7% were positive for Ki-67. analysed Ki-67 index in 5 cut offs (10%, 15%,17% (median), 20%, 25%) and other proliferative markers and showed that the optimal Ki-67 cut off was 25% and that the mitotic index was the proliferative variable that best discriminated between low and high MIB-1 (Ki-67) samples. Lee CS[27] observed that MIB-1 (Ki-67) indices in chronic cholecystitis were significantly lower than those obtained in both in moderately and poorly differentiated adenocarcinoma of gall bladder which is in concordance with our study in which there was no positive expression of Ki-67 (ie >10%) index in control group (chronic cholecystits) was seen. Hidalgo Grau LA et al.[28] calculated Ki-67 expression by MIB-1 index >20% of stained cells was considered positive. They observed that out of 29 gall bladder carcinomas, 24 cases (58.5%) were positive for MIB-1 index.

SURVIVAL Time: In our study the association between HER-2 neu positivity and survival could be studied only in 49 cases because follow up could be maintained only in 49 patients. Among these 49 cases, mortality rate, up to maximum follow up period of 19 months, was lower in HER-2 neu positive (44.4%) as compared to HER-2 neu negative (52.5%) patients. In the study conducted by Kim YW et al[23], total of 71 cases of gall bladder carcinoma were enrolled and observed that the mean survival periods of HER-2 neu positive and negative groups were 26 months and 52 months respectively. Kumar N et al.[17] studied 104 cases of gall bladder carcinoma and observed median survival was 30 months in cases with HER-2 neu over-expression and 12 months in HER-2 neu negative cases. Yoshida H et al.[22] studied 211 cases of gall bladder carcinoma and observed that there was no significant association between HER-2 neu status and survival of patients. Kim HJ et al.[25] observed that the status of HER-2 neu protein over-expression did not have impact on the total patients survival. Rao I et al.[21] observed that the patient with over-expression of HER-2 neu had a worse overall survival, when compared with those who had no expression at 5year (34% vs. 41%).

In our study the association between Ki-67 positivity and survival could be studied only in 49 cases because follow up could be maintained in 49 patients only. In 49 cases, mortality rate up to maximum follow up period of 19 months, was 63.6% among Ki-67 Li positive and 47.4% among Ki-67 Li negative. Although survival rate was higher among Ki-67 Li negative as compared to Ki-67 Li positive patients. Hidalgo Grau LA.et al.[28] observed that five year survival of patient with a MIB-1 positive index was 9.2% as opposed to 27.7% for those with a negative index.

In our study co-expression of HER-2 neu and Ki-67 was seen in 8/85 (9.4%) of malignant cases. None of the cases in premalignant and control group had co-expression. Statistically this association was significant in our study. Mortality rate up to a maximum follow up period of 19 month was 75% among patients with co-expression and 48.9% among co-expression negative patients. Although survival rate was higher among co-expression negative patients as compared to co-expression positive patients. These data of co-expression and co-expression of survival was not in concordance with other studies. Harder J et al.[29] observed that there was no statistical association between grade, stage, overall survival and treatment response.

Conclusion

HER2-neu, and Ki-67 are overexpressed in GBS cases compared with control and premalignant group with no significant correlation survival time. Based on that, HER2/neu can be considered as a candidate for targeted therapy of GBC treatment strategy.

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