Scholars Journal of Applied Medical Sciences
Abbreviated Key Title: Sch J App Med Sci
ISSN 2347-954X (Print) | ISSN 2320-6691 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Pathology

Immunohistochemical Expression of Her2neu in Premalignant and Malignant Lesion of Gall Bladder

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DOI: 10.36347/sjams.2022.v10i05.007 | **Received:** 30.03.2022 | **Accepted:** 08.05.2022 | **Published:** 12.05.2022

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Abstract **Original Research Article**

Purpose*:** To evaluate the expression of Her2-neu in benign and malignant gallbladder lesions, and to establish correlations with clinico-pathologic parameters. ***Materials and Methods*:** Retrospective and prospective analysis was conducted on formalin fixed paraffin embedded (FFPE) 154 tissue sample included Premaligant and premalignant like lesions (including dysplasia, metaplasia and xanthogranulomatous cholecystitis)(n=47) and malignant gallbladder (n=85) with 22 cases of chronic cholecystitis as control. 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). Immunohistochemistry for Her 2 neu was performed and data analysis was conducted using SPSS 15.0 software. P value of ≤0.05 was considered significant. ***Results: The difference of Her 2 neu expression between benign and malignant groups was found to be statistically significant. Her2/neu positivity did not have any significant correlation with various clinicopathological parameters. ***Conclusions:*** The present study demonstrated overexpression of Her2-neu in gallbladder cancer. A trend of decreasing Her2/neu expression with increasing grade of tumor was observed. The results however are encouraging and suggest evaluation of Her2/neu as a candidate for targeted therapy.

Keywords: Gall bladder cancer, Her2neu expression, chronic cholecystitis.

INTRODUCTION

Gall bladder cancer (GBC) is the most prevalent malignancy in biliary tract and sixth in gastrointestinal (GI) tract [1, 2]. However, the global rates for gallbladder cancer exhibit striking variability, reaching epidemic levels for some regions and ethnicities. The basis for this variance likely resides in differences in environmental exposure and intrinsic genetic predisposition to carcinogenesis. The main associated risk factors for gallbladder carcinoma includes cholelithiasis, obesity, reproductive factors, chronic infection of the gallbladder and environmental exposure to specific chemicals like heavy metals, nickel, cadmium, free radicals, lipid peroxidation products and inflammatory bowel disease [2, 3]. Congenital malformations of biliary tract (more common in Japan, China) are also risk factor for GBC [4, 5]. In most instances, gallbladder cancer develops over 5 to 15 years, when metaplasia progresses to dysplasia, carcinoma in situ, and then, invasive cancer [6]. GBC has poor prognosis and the survival rate in 5 years is less than 10% [7-9]. It affects predominantly women, four times more as compared to men [9, 10]. GBC is asymptomatic in nature which makes it difficult in diagnosis and treatment. Adenocarcinoma is most prevalent histo-pathological type of GBC [11].

A satisfactory outcome depends on an early diagnosis and surgical resection. 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 [12, 13]. However, in Chile and India, gallbladder cancer remains a major problem [14-16]. North India is one of the region having highest incidence of GBC. According to Indian council of medical Research, cancer registry 2001 in north India, the incidence of gallbladder cancer was 2.5-8.9/100,000 female population [17]. However, despite the suggested declining trend of this cancer in the world, it

is imperative that a better understanding of the disease and the factors influencing its course is needed to develop treatment strategies aimed at improving its overall outcome.

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 [18, 19]. At present, three major putative pathways of gallbladder carcinogenesis seem to be important: dysplasia, adenoma and anomalous pancreaticobiliary ductal junction.

Receptor tyrosine-protein kinase erbB-2, also known as CD340, proto-oncogene Neu, ErbB2 (rodent), or ErbB2 (human) is a protein that in humans is encoded by the ErbB2 gene, which is also frequently called HER 2 (from human epidermal growth factor receptor 2) or HER2/neu. ErbB2 located at the long arm of chromosome17 (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 increasing evidence that over-expression of tyrosine kinase growth factor receptor such as c-erbB2 (HER2-neu) may play an important role in the development of biliary tract carcinomas [20, 21].

AIMS AND OBJECTIVE

To evaluate expression of Her-2neu in pre-malignant and malignant lesions of gall bladder and correlated with clinicopathological features.

MATERIAL AND METHODS

Prospective and Retrospective case control study

STUDY DURATION

Two year (One year retrospective and one year prospective)

STUDY SAMPLE

Tissue samples of primary gall bladder carcinomas and premalignant lesions of gall bladder 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 are studied which included 85 cases of malignant lesions (adenocarcinomas) 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 CRITERIA

Included Cases are-All gall bladder carcinoma and premalignant lesions along with chronic cholecystitis as controls

EXCLUSION CRITERIA

Secondary gall bladder carcinomas (Metastatic). Post chemotherapy or post radiotherapy gallbladder malignancies.

H & E SECTION HISTOLOGICAL TYPING & HISTOLOGICAL 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).

IMMUNOHISTOCHEMISTRY CONTROL

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

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, and within > 10% of tumor cells.

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.

RESULTS

The present study was carried out with an aim to evaluate the HER2/neu expression in carcinoma gallbladder, premalignant and chronic cholecystitis and to carry out a clinicopathological correlation of the same. 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 and Figure1.] 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

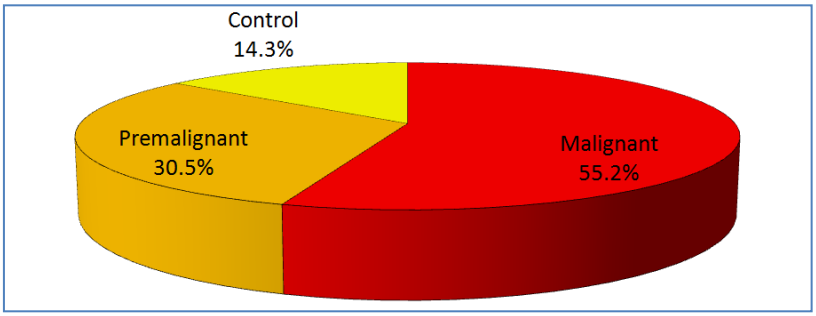

Fig-1

Out of 154 subjects enrolled in the study, a total of 85 (55.8%) were cases of malignant lesions of gallbladder and comprised the malignant group of study. 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)

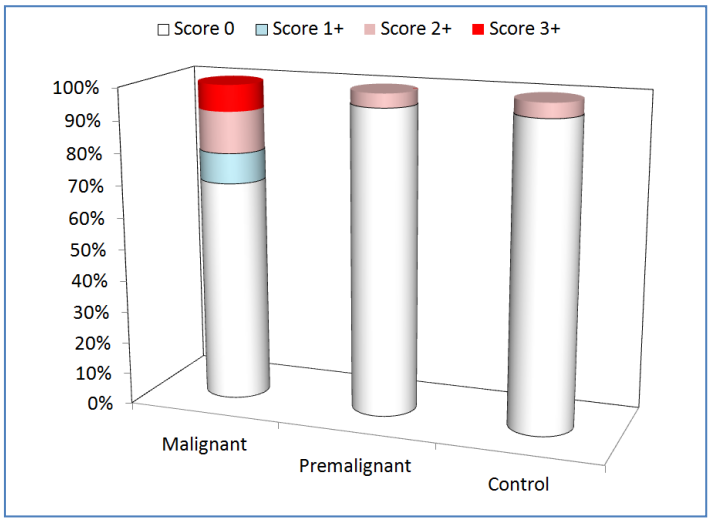

Fig-2

Majority of cases in all the groups did not show Her2-neu expression [Table 3, Figure2]. 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: IHC 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)

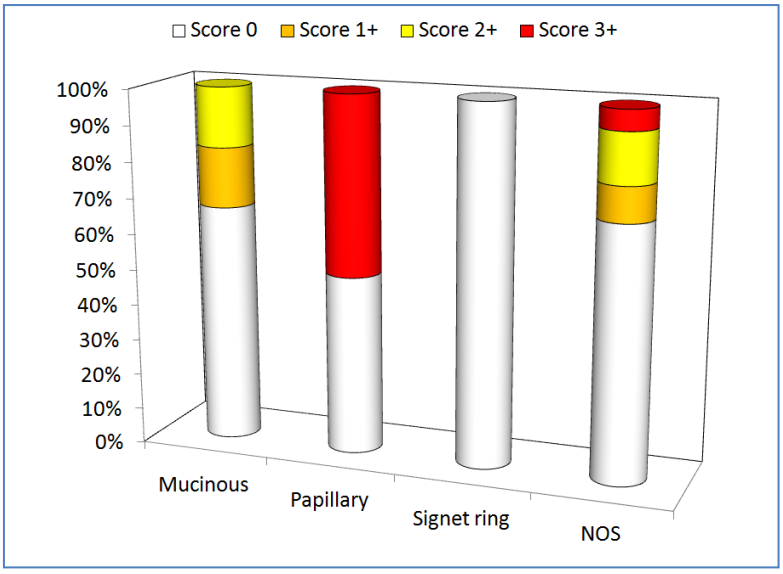

Fig-3

In all the variants except papillary type, majority of cases had no expression [Table 4, Figure 3]. In Papillary type 50% had no expression and remaining 50% had expression score 3+ [Figure 4, Figure 5]. In mucinous type, 66.7% had no expression and 16.7% each had score 1+ and 2+ respectively [Figure6, Figure7]. The single case of signet ring did not show expression. Among NOS cases 70.8% 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: Comparison of Her2-neu Positivity rate in different groups

SN	Group	Total No.	No. Positive	% Positive
1.	Malignant	85	18	21.2
2.	Premalignant	47	2	4.3
3.	Control	22	1	4.5

χ^2=9.159; p=0.010

Positivity rate was 21.2% in malignant, 4.3% in premalignant and 4.5% in control. Statistically, this difference was significant (p=0.010) [Table 5].

Table-6: Association of HER2-neu Expression with different clinicopathological parameters

SN	Parameter	Total	No.	%	Statistical significance	
1.	Age				OR (95% CI)	'p'
	<40 Years	49	3	6.1	Ref.	0.063
	>40 Years	105	18	17.1	3.17 (0.89-11.34)	
2.	Gender					
	Male	35	2	5.7	Ref.	0.120
	Female	119	19	16.0	3.48 (0.77-15.78)	
3.	Adenocarcinoma type					
	Well/Moderately differentiated	67	11	16.4	N/A	0.065
	Other types	18	0	0		
4.	Adenocarcinoma subtypes					
	NOS	72	14	19.4	0.24 (0.04-1.33)	0.326
	Mucinous	6	1	16.7	0.20 (0.01-2.91)	
	Papillary	6	3	50.0	Ref.	
	Signet ring	1	0	0	-	
5.	Tumor size					
	<2 cm	16	3	18.8	Ref.	

SN	Parameter	Total	No.	%	Statistical significance	
	>2 cm	45	10	22.2	1.24 (0.29-5.22)	0.771
6.	Nodal involvement					
	No	43	7	16.3	Ref.	0.822
	Yes	16	3	18.8	1.19 (0.27-5.29)	
7.	Surrounding tissue involvement					
	No	41	8	19.5	Ref.	0.428
	Yes	18	2	11.1	0.52 (0.10-2.71)	
8.	Gall stones					
	No	42	9	21.4	Ref.	**0.007**
	Yes	87	5	5.7	0.22 (0.07-0.72)	
9.	Chemotherapy					
	No	17	4	23.5	Ref.	0.496
	Yes	32	5	15.6	0.60 (0.14-2.62)	
10.	Clinical condition					
	Improved	32	4	12.5	Ref.	0.146
	Deteriorated	17	5	29.4	2.92 (0.66-12.79)	
11.	Chief Complaint					
	Abdominal pain only	67	9	13.4	Ref.	0.857
	Others	83	12	14.5	1.09 (0.43-2.76)	

On evaluating the association of HER2-neu expression with different clinicopathological variables [Table 6], no significant association was found with any of the other variables except for gallstones although the odds of HER2-neu expression were higher among those aged >40 years, females, papillary subtype, nodal involvement, those not having surrounding tissue involvement, having gall stones, those not undergoing chemotherapy, having deteriorated clinical condition and having chief complaint other than abdominal pain alone. Among cases with gallstones, the expression was significantly lower as compared to that in patients without gallstones.

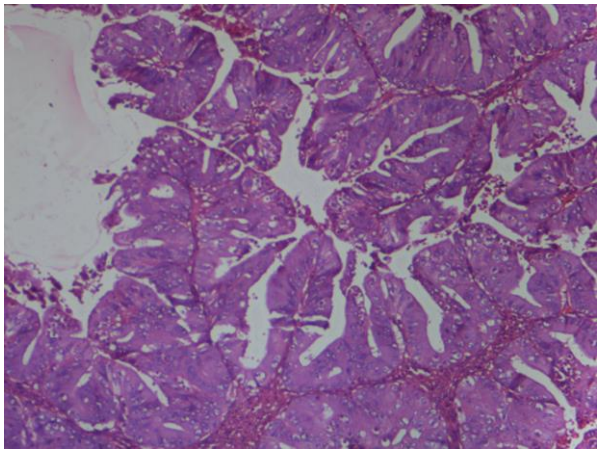

Fig-4: Papillary Adenocarcinoma of gall bladder (Hematoxylin & Eosin, 400X)

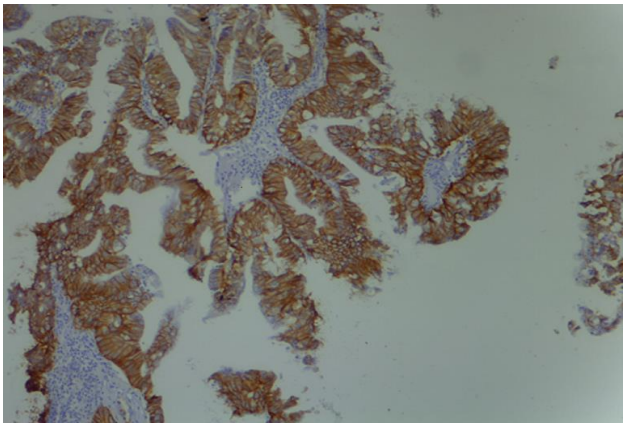

Fig-5: Expression of Her-2 neu 3+ staining in Papillary Adenocarcinoma gall bladder

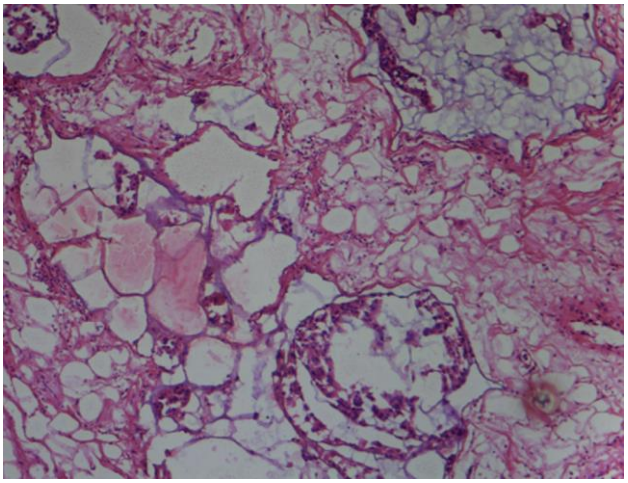

Fig-6: Mucinous Adenocarcinoma gall bladder (Hematoxylin & Eosin, 20X)

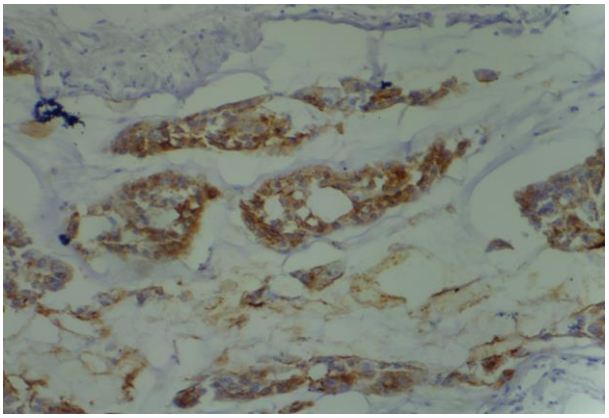

Fig-7: Her-2 neu expression 2+ staining, in mucinous Adenocarcinoma gall bladder

DISCUSSION

Gallbladder cancer is an aggressive disease and its pathogenesis is not well known. However till now, there is no clear understanding of gallbladder carcinoma pathogenesis and no candidate molecules has been explored for targeted therapy [22]. 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 [23-25]. For gallbladder cancer data for HER2-neu overexpression have been presented in mostly small patient cohorts [31, 32]. Targeted therapy anti-Her2neu for breast carcinoma is well known However, its study in gall bladder carcinoma is limited [33].

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*. in 2014, [30] 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*. [26] 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*. [22] 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.* [26] 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*. [22] 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*. [27] 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*. [20] showed over-expression of c-erbB2 in 16% of their 89 cases by combining both immunohistochemistry and FISH. Their study also showed over-expression of c-erbB2 in 21% of cases by combining both 2+ (cytoplasmic and incomplete membranous staining) and 3+ staining (complete membranous staining) on IHC. However, after gene amplification by FISH, they found only 16% cases to show over-expression, as 33% of their 2+ positive cases on IHC did not show gene amplification. Considering 3+ complete membranous staining as positive they had only 8% c-erbB2 expression in their study. Kamel D *et al.* [28] 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*. [29] 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%).

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+ expressions and 3+ expressions 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.* [30,] Hadi who also found higher expression of HER-2 neu in papillary adenocarcinoma.

On studying the association of HER-2 neu expression with different clinicopathological parameters in our study, among 154 patients we found that among patients with age >40 years, 18 (17.1%) cases showed positive expression, and in patients with age <40 years 3 (6.1%) cases showed positive expression of HER-2 neu. On studying the association with gender, we found that there were 19 (16%) females and 2 (5.7%) males who were for positive Her-2neu expression. Among the well and moderately differentiated adenocarcinoma 11 cases (16.4%) were positive for HER-2neu expression while other type had no expression. In our study the records of tumor size was available in only for 61 cases. Out of 16 cases with tumor size of <2cm, 3 cases (18.8%) were positive for HER-2 neu expression while in remaining 45 cases with tumor size of >2cm, 10 cases were positive for the same (22.2%).

CONCLUSION

The present study was carried out with the aim to evaluate the HER2- neu expression in carcinoma of gall bladder and to carry out a clinicopathological correlation of the same. For this purpose a case control study was carried out in which a total of 154 subject were enrolled ,in which were divided in three groups i.e. malignant group with 85 cases (55.2%), premalignant and premalignant like lesion with 47 cases (30.5%) and control group with 22 cases (14.3%). Among malignant group, majority of the cases were with moderately differentiated adenocarcinoma. Majority of cases were females in the malignant as well as in premalignant/ control group. HER2-neu showed 3+ positivity in 50% of total cases (n=6) of papillary adenocarcinoma. HER2-neu expression was higher among those aged >40 year, females, papillary subtype, nodal involvement and having gall stones.

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