TITLE: Role of Her2/neu in gall bladder carcinomas: An Immunohistochemical and Flouorescent-in-situ Hybridization study

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INTRODUCTION

Gallbladder carcinoma (GBC) is a relatively rare neoplasm world over with rising trends in the recent past with poor prognosis and less treatment options[1]. It is the sixth most common cancer related to gastrointestinal tract and 80-95% of biliary tract carcinomas are GBC. The incidence of cases of gallbladder cancer at the global level were 2.2/100,000 and mortality rate of 1.7/100, Incidence varies geographically with higher rates in Chile, Peru, Nepal, India, Bolivia, Bangladesh, Japan, Korea and the Czech Republic. [2]

In India, during 2001, the estimated number of gallbladder cancer was 14,986 and is likely to increase to 23,750 by 2016 as a result of aging and increase in size of the population.[3] The present trend analysis has revealed a steady incidence rate of gallbladder cancer from almost all the registries, though at a higher rate in North and Eastern India among both genders.[3] Among women, it is the 4th common cancer in Delhi, 5th in Bhopal, and 10th in Mumbai.[4] The Indian Council of Medical Research Registry has reported that rate of GBC in north India is 4.5% and 10.1% per 100,000 in males and females respectively.[5]GBC has a high mortality with 5 year overall survival rate of less than 5 %, overall survival rate is 6 months. The wide geographical variation is attributable to various risk factors which include Cholelithiasis[6] (especially untreated chronic symptomatic gallstones), obesity, reproductive factors, chronic infections of the gallbladder, and environmental exposure to specific chemicals. These suspected factors likely represent promoters of carcinogenesis. The molecular biology and pathogenesis of GBC is poorly understood and markedly limited by the sample size in various studies. Most of these studies have focused on *ras,TP53*, and *p16Ink4/CDKN2* gene abnormalities and deletions ("loss of heterozygosity") at several chromosomal regions harboring known or putative tumor suppressor genes. These loci include 3p (20 to 52 percent); 5q21 (*APCMCC*genes, 6 to 66 percent); 8p22-24 (22 to 44 percent); 13q14 (*RB* gene, 20 to 30percent); and 18q22 (*DCC* gene, 18 to 31percent).[7, 8, 9, 10]

Recently tyrosine kinase (TK) has been implicated in pathogenesis of various neoplasms. Furthermore, several oncogenes, which encode for growth factor receptors, have TK activity. The epidermal growth factor receptor (EGFR,HER-1) and c-erbB-2 (HER-2) are members of the EGFR family with TK activity. The over expression of these

oncoproteins is often a result of gene amplification, and is widely observed in various solid malignancies. These genes are of particular interest in the recent past due to availability of specific chemotherapeutic targets against them with very promising results. A very limited subset of studies focusing on overexpression of EGFR and HER-2 genes in GBC exist.[11-22]

Symptoms and signs of GBC are not specific and often appear late in the clinical course of the disease. Due to this, diagnosis is generally made when the cancer is already in advanced stages, and thus the prognosis is usually very dismal with stage wise 2-year survival as follows: stage I disease: survival is 45%, stage II :15% , stage III : 4% , and stage IV: 2% .[23]

The present treatment modalities include surgical resection, chemotherapy and radiotherapy. Used individually or in combination, these modalities are largely ineffective leading to an overall poor patient outcome. Therefore there is a need for specific targeted therapies in these patients.

It has been shown that a constitutive expression of HER2/neu in gallbladder epithelium results in the development of adenocarcinomas of the gallbladder. HER2/neu overexpression in GBC has a broad range between 9% to 65% in different studies. [11-21]

HYPOTHESIS

1. Trastuzumab based chemotherapy may be useful in improving outcome of Her2/neu over expressing gall bladder carcinomas.
2. Unlike the western world Her2/neu overexpression in gall bladder carcinomas is expected be higher in Indian patients as seen in breast carcinomas.

LACUNAE IN EXISTING KNOWLEDGE:

1. Very few studies exist regarding IHC and fluorescent in-situ hybridization of genetic markers in GB in world and Indian literature.

AIMS AND OBJECTIVES:

1. To study the presence of Her2/neuoverexpression by Immunohistochemistry and Flourescent in situ Hybridization (FISH) in gallbladder carcinomas.
2. Comparison of two modalities in order to estimate concordance rate.
3. Correlation of overexpression with clinical outcome (survival) and tumour characteristics

MATERIALS AND METHODS

The study was conducted in the department of Pathology and Gastrointestinal Surgery GIPMER, NewDelhi, over a period of three years.

STUDY POPULATION

CASES

Number of cases: Total 100 cases of gall bladder adenocarcinoma

Inclusion Criteria:

1. Cases of resected gall bladder adenocarcinomas received in the department of Pathology

Exclusion criteria:

1. Any other histological variant of gall bladder carcinoma other than adenocarcinomas eg. Squamous or neuroendocrine carcinomas

CONTROLS: Total 100

InclusionCriteria:

1. Cholecystectomy specimens with areas of normal appearing epithelium and with minimal inflammation

Exclusion Criteria:

1. Any other neoplastic and non-neoplastic lesion.
2. Any degree of dysplasia.

Methodology:

1. Relevant clinical details such as age, sex, presenting features and duration of disease, relevant family histories etc. were obtained.
2. Follow up pertaining to disease free survival, overall survival/death due to disease was obtained. This was done in keeping with the follow-up protocol followed in department of GIS routinely for GBCA patients.
3. Clinical examination and radiological details were obtained in relation to liver metastasis, lymph node status and distant metastasis.
4. All radical cholecystectomy specimens received in the pathology department and tissue sections were obtained from tumour, non-tumor areas and resection margins as per standard grossing protocol followed in the department. Tissues were processed as routine histopathology methodology (Annexure-1).
5. Then relevant sections from the cases (CaGB Adenocarcinoma positive samples) and controls (Chronic Cholecystitis) were selected for IHC. IHC was performed using antibody against Her2/neu antigen(clone Sp3, Rabbit monoclonal antibody by Thermo Scientific). Detection system used was Avidin-biotin method with Di-amino-benzidine (DAB) as chromogen. (Methodology details as shown in Annexure-2)
6. Positive (Breast carcinoma her2/neu positive case) and negative controls were put up for Immunohistochemistry with each batch.
7. Expression of HER2/neu was scored[24,25] 0 to 3 as per gastric carcinoma scoring system followed in Annexure-3:

   Her2/neu scoring - IHC

   The IHC test gives a score of 0 to 3+ that measures the amount of HER2 receptor protein on the surface of cells in a cancer tissue sample. If the score is 0 to 1+, it's called "HER2 negative." If the score is 2+, it's called "equivocal." A score of 3+ is called "HER2 positive."(We also made note of the cytoplasmic staning, if present).
8. Fluorescent in-situ Hybridization(FISH) was performed on 10 cases each from IHC 1+, 2+ and 3+ groups, i.e. a total of 30 patients . we were select the tissue block with lowest grade tumor morphology in tissue biopsy.

Her2/neu detection – FISH

For FISH the ZytoLight SPEC HER-2/CEN 17 Dual Color probe kit was use which uses two different probes. One is a locus specific identifier (LSI) HER-2/neu labeled in Spectrum Green and another is a chromosome enumerator probe (CEP) 17 labeled in Spectrum Orange. The methodology used is given in Annexure-4:

To evaluate[26] the sample material by fluorescence microscopy filter sets for the following wavelength ranges are required:

ZyBlue – excitation: 418 nm emission: 467 nm

ZyGreen – excitation: 503 nm emission: 528 nm

ZyOrange – excitation: 547 nm emission: 572 nm

Her2/neu scoring – FISH

Signal enumeration was conducted at 1000X magnification with the appropriate filter. At least 20 cells (non overlapped tumor nuclei) were evaluvated for her2 prob and CEP17 signal elumination and the results were averaged. In equivocal cases additional 20 were counted.[26]

- HER-2/neu gene amplification was defined as **HER-2/CEP 17**
- ratio of ≥2.2 was taken as Positive/Amplified
- ratio of 1.8-2.2 was considered equivocal
- ratio of <1.8 was considered negative

9. Follow-up of patients of GBCA wherever possible
10. Correlation of IHC and FISH findings with histological type, stage of disease and response to therapy was done.
11. Correlation and comparisons between IHC and FISH was studied.

STATISTICAL ANALYSIS

To analyze the relationship between dependent and independent categorical variables, chi-square test was used. For the inter-group comparisons (independent variables) with dependent, one-way ANOVA or Kruskal-Wallis tests was used.

For the correlation between IHC and FISH spearman Rho test was used. Statistical analyses were using SPSS v.16 program, and results were considered as statistically significant with the p value = 0.05.

REVIEW OF LITERATURE

Her2/neu proto-oncogene, also called c-erbB2, was first identified in rodent neural tumor cell lines and therefore named neu in the early 1980s.[27] It is a normal cellular gene located on chromosome 17q12q21.[28] It encodes a tyrosine kinase that is strongly related to receptor for epidermal growth factor receptor (EGFR).[29] This family has four homologous receptor proteins following activation of erbB-1, 2, 3, 4 receptor proteins and are ubiquitously expressed in epithelial, mesenchymal and neuronal normal cells and their progenitors.[30,31] Each receptor contains an intra cellular protein kinase domain which has a carboxyl terminal segment containing a phosphorylated or tyrosine residual site, a trans membrane protein and an extra cellular ligand binding domain.[30,31,32] Erb-2 receptor undergoes homo or hetero dimerization and then transphosphorylation of their intracellular domain and then activates the various intra cellular signaling molecules and adaptor proteins.[33]

Erb-2 has a stronger catalytic kinase activity. Subsequently a cascade of downstream pathways is activated which results in gene transcription. It is believed that Her2/neu is critical for control of growth, grading and mobility of many normal and transformed epithelial cells; it increases the signal transduction and activates the Ras, Raf, MAPK and PI3K/Akt, m-TOR pathways.[31,34,35] Except erb2 all member of erb family show active receptors in basal condition but erb2 does not bind with growth factor unless over expressed.[36] At the different steps of carcinogenesis like initiation, promotion and progression structural as well as functional alteration of Her-2.[37]

Biliary tract carcinomas have poor prognosis and limited therapeutic options. Therefore, it is essential to combine standard therapies with molecular targeting. Evidence that the c-erbB-2 proto-oncogene is important in prognosis and oncogenesis in a number of human malignancies is increasing.[38-41] DNA hybridization and immunoblotting techniques are most commonly utilized to determine the amplification and protein expression of this

proto-oncogene, respectively. However, little is known about the starting point of amplification of Her2/neu and how it progresses from benign to malignant lesions.

The role of Her2/neu over expression has been studied in number of human malignancies though the maximum literature still exists regarding breast carcinomas in which over expression is in approx 20-30% of cases.[42,43] Her2/neu is over-expressed in various epithelial malignancies like 13-22% gastric,[44-46] 30% of salivary duct carcinomas,[47] 0-50% Pancreatic,[48-50] 2-11% colorectal,[51-55] oral, and adenocarcinoma of lung, cervix, 4-22% esophageal[56-59] and ovarian carcinomas, aggressive forms of uterine cancer, such as uterine serous endometrial carcinomas have also been extensively studied.

With the approval of United States Food and Drug Administration (FDA), for use in Her2/neu over expressing breast carcinoma and in gastric cancer, anti Her-2 antibodies e.g. trastuzumab, pertuzumab, a new conjugate ado-trastuzumab emtansine, and Her-2 inhibitor lapatinib is widely used for as a treatment for advanced metastatic disease and is also being studied as an adjuvant treatment for earlier stage disease.[60] In breast carcinomas, Her2/neu expression has shown potential value in predicting response to cytotoxic chemotherapy and hormonal therapy. Using recombinant technologies, trastuzumab, a monoclonal IgG1 class humanized murine antibody has been developed to specifically bind the extracellular portion of Her2/neu.

Role of Her2/neu in biliary carcinomas is a relatively recent concept and only few studies exist. In a study regarding Her2/neu and EGFR expression in transgenic mice showed that on overexpression of wild-type ErbB-2 in adenocarcinomas of the GB, cystic duct, common bile duct, and intrahepatic bile duct carcinomas were observed to develop through the hyperplasia–adenoma sequence at high incidences of 100%, 100%, 87%, and 30%, respectively . These results clearly highlighted that ErbB-2 overexpression plays an important role in biliary carcinogenesis, especially early carcinogenesis of the extrahepatic biliary tract.[61] It has been suggested that later in the process of cancer evolution, these ErbB-2-positive clones might be replaced by negative clones that have some growth advantage.[61]

However, there are conflicting opinions regarding the actual role of studying this marker in GBCs. A study demonstrated that only 13% of the patients showed overexpression of Her2/neu and did not have impact on survival. Thus these authors opined that therapeutic

options of HER2/neu in GBC will rarely ever be used and may not improve treatment of GBC.[19] However, articles based on IHC and molecular studies have suggested that immunohistochemical expression correlates with gene amplification thus can be used as potential therapeutic target in a subset of patients. Moreover, the frequency of expression and gene amplification in GB cancers is comparable to those observed in breast carcinomas where the therapeutic efficacy of trastuzumab is already proven.

Previous immunohistochemical and molecular studies reporting expression of Her2/neu in Billiary carcinomas (BC especially GBC)have been summarized in Annexure-5.

In a study Her-2 is overexpressed in 10% of caces[65] while in another, reported none of the six cases included in the study showed immunohistochemical or molecular expression positivity of Her2/neu[22]. However the study is markedly confounded by the sample size therefore may not included in calculating the range. Roaet. al., in 2014 reported 14% of advanced gall bladder carcinoma cases overexpressed Her2/neu and these kind of cases may get an advantage by inhibiting the Her-2/neu pathway.[68]

Those patients who had low staining for HER-2 had higher rate of survival as compared to others with a borderline statistical significance (ρ=0.052).[75]

Though Her2/neu has a role in early carcinogenesis of biliary tract neoplasms, its exact correlation with histological grade, clinical stage, locoregional/distant metastasis, prognosis and response to conventional therapy is yet to be understood and statistically proven. Thus, since there is a need for larger studies and especially considering such a high prevalence of GBC in the part of the world, such studies are required.

Since in breast carcinoma most centers all over the world use trastuzumab based treatment modalities[46], it is absolutely imperative to establish standardization in Her2/neu detection and interpretation. Currently, only immunohistochemistry (IHC) and fluorescence in situ hybridisation (FISH) are approved by the US Food and Drug Administration(FDA) for HER2/neu testing in various other malignancies also. Other methods such as chromogeninsituhybridisation (CISH) and silver enhanced in situ hybridisation (SISH), are gaining popularity as alternatives to FISH because of lower cost, shorter turnaround time and ease of analysis by routine microscopy with minimal training.[76]

According to American Society of Clinical Oncology and College of American Pathologists (ASCO/CAP) Her2/neu score for gastric carcinoma IHC (0 to 3) is as follows: 0 (negative): no membranous staining ; 1 (negative): faint staining involving a portion of the circumference of the cytoplasmic membrane of at least 30% of neoplastic cells; 2 (positive): weak but definitive staining of the membrane over 100% of the cytoplasmic circumference in at least 30% of neoplastic cells; 3 (positive): strong positive staining of the membrane over 100% of the cytoplasmic circumference in at least 30% of neoplastic cells different from previously suggested 10% by FDA.[24,25]

Similarly, a modification for FISH analysis was carried out, a HER2/CEP17 signal ratio of ≥2.2 was established for positive (modified from the previously recommended FDA ratio of ≥2.0 for positive HER2/neu gene expression), a ratio of 1.8-2.2 for equivocal, and a ratio of<1.8 for negative.[76,77]

As far as effectiveness of both these procedures is concerned, it is still unclear as which method is ideal for Her2/neu detection. Most of the studies on breast carcinomas have showed an overall high concordance between IHC 3+/FISH amplified and IHC negative/FISH non-amplified groups. The discordant results between IHC and FISH have been mostly attributed to 2+ scores on IHC indicating that IHC 2+ connotes uncertainty.[78] Previous studies reported 6-25 per cent incidence of IHC 2+/FISH amplified cases.[79-82,26] The ASCO/CAP guide lines report an incidence of 23.9 per cent.[26]

Most authors have accepted FISH to be more reliable than IHC for determining the HER2 status; FISH is an expensive, time consuming and labour intensive procedure, which requires training for interpretation. These limitations especially in an Indian scenario make IHC the most common method used for testing HER2. Moreover, IHC is a good ‘first-step’ to screen tissue samples and determine suitability for the technically demanding FISH test, which should be used as a confirmatory test especially in IHC equivocal cases.[78] Every lab should standardize the IHC protocol by establishing concordance with FISH results. Subsequently FISH may be used only in equivocal cases which are 6-25%.

Results:

Case demographics:

In this study population we have incuded 100 cases of gall bladder adenocarcinoma(GBCA) samples along with 100 chronic cholecystitis controls.

Age and sex distribution:

Cases: n=100

20 male and 80 female patients, sex ratio being 1:4. Mean age was 51.7±5 (Range 25-80yrs).

Control: n=100

30 male and 70 female patients, sex ratio being 3:7. Mean age was 50.2±5(Range 13-65yrs).

Table 1 : Study Demographics

	Cases	Controls
Male	20	30
Female	80	70
Ratio	1:4	3:7
Mean age	51.7±5	50.2±5

Histopathological demographics: (Table 2)

Of the 100 cases 84 were reported as adenocarcinoma while 16 were reported as Intra-cholecystic papillary neoplasm (ICPN)

- **Tumor stage – Adenocarcinoma vs ICPN**

Adenocarcinomas more frequently had higher stage (≥ stage II tumors) (92.8%) as well as higher T stage-3 and then T Stage-2 (43 and 31 cases respectively), as compared to ICPN (56.25% having (≥ stage II tumors). The differences in T-stage of tumors in adenocarcinooup was also significant. There was no significant difference between the two with respect to N stage. None of the ICPN showed metatstasis while 18 cases of adenocarcinoma had metastasis ($p<0.05$).

Table-2

Clinical findings		**Adenocarcinoma n=84**	**Intracystic-papiliary carcinoma n=16**	**Total No. of cases**	**Pearson's Chi-Square**	**P-value**
Grade	WDAC	8	15	23	53.86	<0.001
	MDAC	61	1	62		
	PDAC	15	0	15		
Stage	0	1	1	2	17.28	0.002
	I	5	6	11		
	II	21	3	24		
	III	43	6	49		
	IV	14	0	14		
T-Stage	1	6	8	14	21.22	<0.001
	2	31	5	36		
	3	43	3	46		
	4	3	0	3		
N-Stage	0	54	13	67	1.749	0.186
	1	30	3	33		
M-Stage	0	66	16	82	4.18	0.041
	1	18	0	18		

Her2neu scoring: Table 3

In all cases and controls membranous staining patterns were evaluated according to gastric carcinoma scoring system for HER2/neu as described in the section on methods. In our study 65% cases were negative (in which 42 have IHC score 0 and 23 have IHC score 1+), 21% cases are equivocal with IHC score 2+ and 14% cases are positive with IHC score 3+. 10% of the control cases also show 0/1+ membranous staning (with the background staning) and were thus considered negative.

Table-3 : Her2neu scoring		
Her-2/neu Scoring	**Total cases**	**Results**
Cases		
0	42	65%cases negative
1+	23	
2+	21	21%cases equivocal
3+	14	14%cases positive
Controls		
0	20	All are negative
1+	10	

Correlation of tumor characteristics with IHC score: Table 4

None of the independent parameters like Grade, Stage, Adeno/ICPN, or T, N, M Status showed any statistical significance with the dependent parameter which is IHC-score.

However N-Stage vs IHC mann-Whitney 'U' test is significant (ρ-value = >0.04).

Ordinal logistic Regression test does not show any effect of Tumor Grade, Stage or ICPN on IHC Status.

Table-4 : Correlation of tumor characteristics with IHC score			
S. No.	**Parameters**	**Pearson's Chi-Square (p-value)**	**Kuskal Wallis Annova(1, 2, 3) / Mann Whitney 'U' test(4, 5, 6) (p- value)**
1.	**Grade**	0.387	0.494
2.	**Stage**	0.87	0.79
3.	**T**	0.6	0.16
4.	**N**	0.15	0.04
5.	**M**	0.08	0.7
6.	**Adeno/ICPN**	1.009	0.799

FISH Score: validating the IHC results Table 5

FISH scoring done for the correlation with IHC in 30 cases shows a positive correlation with the Spearman Rao correlation ρ-Value is <0.01 which is significant.

Our study shows highly correlation betweenIHC results and FISH results, thus validating the IHC results.

1+ IHC negative cases also showing FISH negativity (0.52-1.2) and

2+ IHC cases are equivocal(0.9-2.22) in FISH also and

3+ IHC cases are positive(1.25- 3.42) in FISH.

Table-5		
Her-2/neu Scoring	**Total cases (10 cases from each catogry)**	**Fish results**
1+	23	0.52-1.2
2+	21	0.9-2.22
3+	14	1.25-3.42

Correlation with Survival:

In the current study we correlated the survival data with histopathological data (Grade, Stage, T,N and M stage) and with IHC and FISH results.

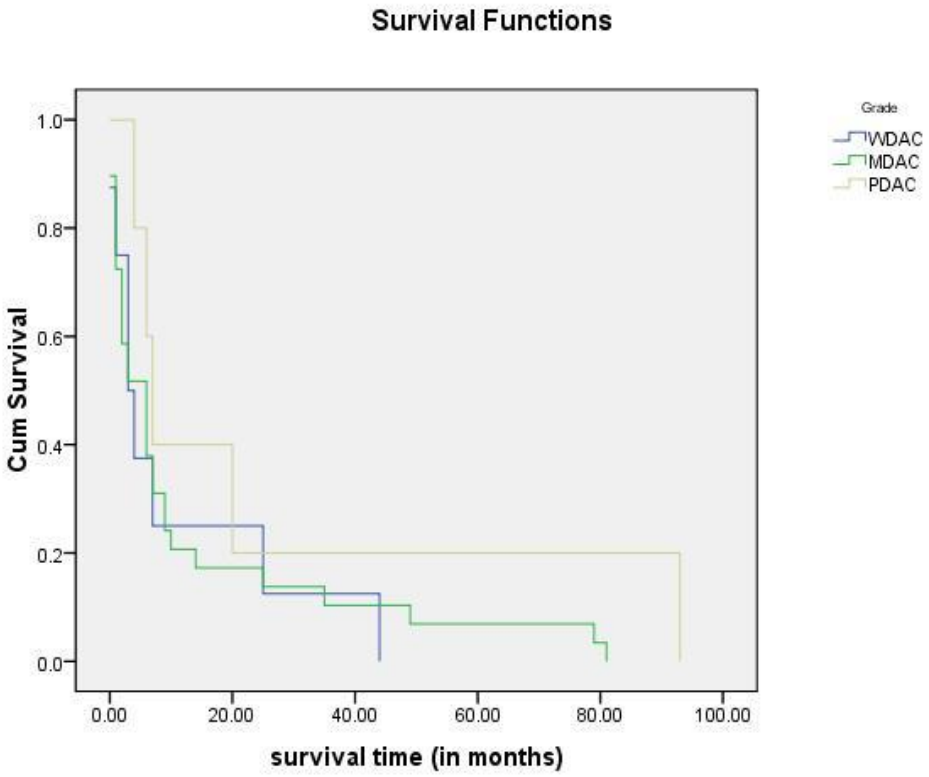
Survival data was available in 42 cases. Of these 18 are alive and 24 died due to disease. 24 months was the minimum follow-up time included and as some prospective cases alive but survival time recorded was less then 24 months, they were excluded from this analysis.

No significant correlation between histologic parameters and follow-up data was found, however the numbers were small.

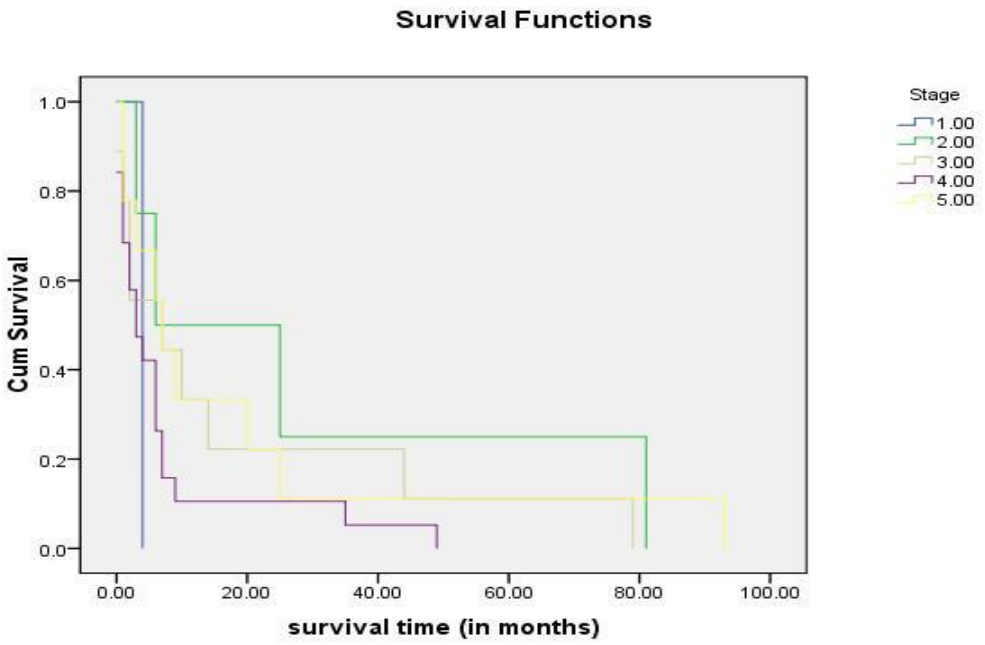
Table-6	
Parameters	**Sig.**
Adeno/ICPN	0.611
Grade	0.359
Stage	0.306
IHC	0.897
FISH	0.407

Kaplan-Meire survival curves given below:

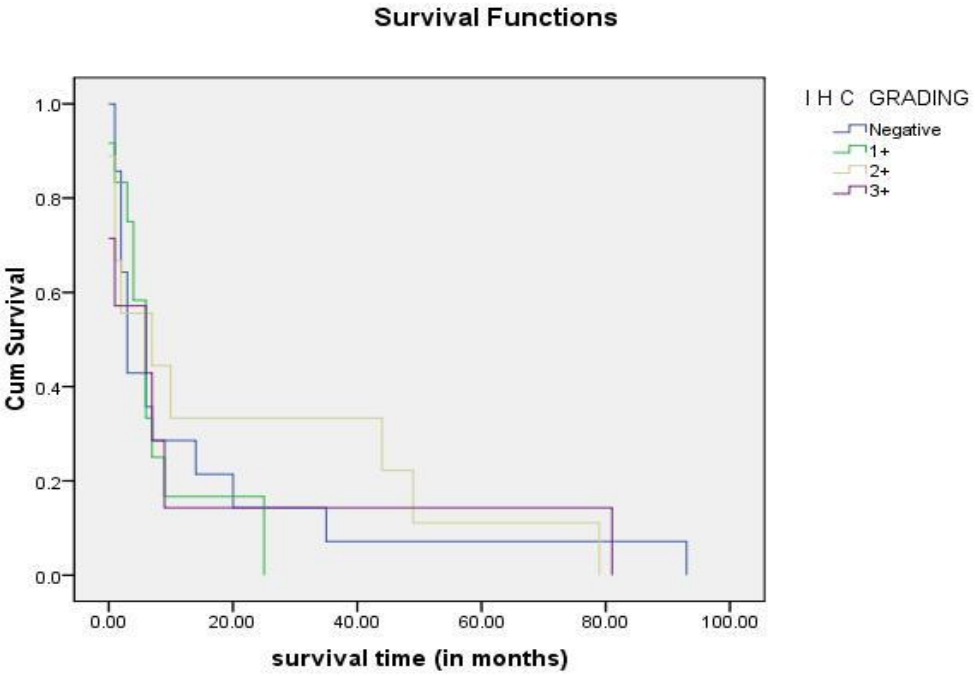
Grad vs survival time :



Stage vs survival time :



IHC grading vs Survival time:



Discussion:

The increasing global incidence, poor prognosis and lack of effective therapy make the management of Gall bladder carcinomas challenging. This calls to attention the need of effective therapeutic agents and an attempt to assess possible targeted therapy. Thus in this study we aimed to look for a subgroup of carcinoma gallbladder that may have expression of Her2/neu and thus be a possible target for Her2neu inhibitor therapy.
Gene status and protein expression of HER2/neu and their pathways may be potential biomarkers for predicting the response to HER2/neu inhibitors.

HER2/neu gene is a key driver of tumorigenesis and its overexpression as a result of gene amplification is a critical target for therapy in breast cancer. Other solid tumors with reported overexpression of this gene include gastric adenocarcinoma (11 %), pulmonary adenocarcinoma (28 %), colorectal adenocarcinomas (17 %), pulmonary squamous (11 %), and pancreatic adenocarcinoma (7 %). The expression of HER2 followed a pattern of distribution in invasive carcinoma and in its putative precursor lesions. Her2/neu receptors were absent in the normal epithelium but were strongly expressed in carcinoma *in situ* and the epithelia with intestinal metaplasia. Absence of HER2 staining in normal gallbladder epithelium is in agreement with previous studies performed using normal breast tissue [20]. Two previous reports failed to demonstrate expression of HER2 protein in gallbladder dysplasia [13,21]. However the significance of looking for the expression in dysplasia is not much. This was not attempted in this study. But there are several existing reports of HER2/neu overexpression in gallbladder carcinomas and the incidence has varied widely. Data with regard to the prognostic value of HER2/neu overexpression in gall bladder cancers is mixed, with some studies suggesting a worse prognosis[23, 24], while others suggest the contrary [25, 26]. In our study we did not find it to correlate with disease free survival, however a limitation of the study was small numbers.

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 . In our study 14% cases were positive for

Her2/neu. The discrepancy between these results may be explained by the use of different immunostaining procedures (e.g., monoclonal *vs* polyclonal antibodies and sensitivity of the technique), time of fixation, preservation of antigenic sites and number of samples analyzed or this marked variation could be due to the different scoring systems adopted by different authors. Moreover, some authors have considered 2+ as well as 3+ score (cytoplasmic as well as membranous) as positive while others and in this study we considered only 3+ (strong membranous staining) to be Her2/neu positive. This highlights the need to evolve a uniform consensus on immunostaining procedures and scoring for Her2/neu staining in gall bladder cancers on similar lines as breast cancer. In our study, HER2 receptor protein stained intensely along the basolateral plasma membrane of the metaplastic and carcinoma *in situ* cells.

In the present study we have validated our IHC results with the florescent in situ hybridization for confirmation of Her2/neu gene amplification in 1+, 2+ and 3+ cases (on IHC) and we got a significant correlation in IHC and FISH results.

We did not find any statistical correlation 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).

Our experience demonstrates that HER2/neu-directed monoclonal antibodies, such as trastuzumab and pertuzumab, or tyrosine kinase inhibitors such as lapatinib therapy appears to be beneficial for gallbladder cancer cases with HER2/neu amplification and should be further explored in a clinical trial. An estimated 80–100,000 cases of gallbladder cancer are diagnosed worldwide annually, and the benefit of targeted therapy for those with HER2/neu amplification would be substantial.

Conclusion: Our study has shown that 14% of cases are Her2/neu positive for Ner2/neu using IHC and the results are validated using FISH. As the prognosis of GBC is poor , any therapeutic advantage available a subset of patients is worthy of consideration. and thus on the basis of experience from breast cancer and gastric cancer we should evaluate this group for a therapeutic advantage using targeted therapy.

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