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ORIGINAL RESEARCH

Comparative Observational and Analytical Study in Patients with Adenocarcinoma Gall bladder for Amplification of *ERBB2/Her-2neu*_Receptor

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Abstract

Background: Gall Bladder carcinoma (GBC) being an aggressive disease with a very poor 5year survival rate needs an early diagnosis. Though in recent times surgical resection with neoadjuvant therapy the survival rate has improved but still it is very difficult to treat patients presenting with large tumour. we conducted this study as very limited data is available regarding the molecular changes of gall bladder carcinoma which are very much essential to find out an early therapeutic approach. We tried to reveal the degree of *ERBB2/Her2-Neu* amplification in GBC and looked for as if any correlation existed between adenocarcinoma of gall bladder and *ERBB2/Her2-neu* receptor amplification.

Methods: A hospital based observational, analytical study conducted in a tertiary hospital in Kolkata. A non-probability purposive sampling was done and included 31 patients conforming to our guideline from Jan. 2018 to May 2019. Patients were admitted followed by a thorough history taking, clinical examination and radiological imaging. After taking 5ml of blood as sample they were posted for elective operation. Intra-operatively four containers of gall bladder tissue (two of tumour tissue and two of normal tissue) were collected for tumour analysis. Thirty-one such tissue of Adenocarcinoma Gall Bladder collected for studying the *ERBB2/Her2Neu* amplification. Tissue genetic analysis was done by ERBB2 amplification study, DNA isolation and quantification, *ERBB2/Her2-neu* amplification.

Result: Mean age of patients was 59.84±10.26 yrs. 87% were in 40-70 yrs. age group with 74% being female. 97% had gall bladder stone.. In staging and resection of the disease we found the origin of lesion from the body of gall bladder (47%) >Neck (34%)> fundus (19%) with patients being detected at fairly advanced stage i.e. 52% in stage III and 42% in stage II. The Grade of differentiation was 42% poorly, 35% moderately and 23% well differentiated cancer. Fifty two percent (n=16) of the samples showed above 2-fold amplification in the tumor tissue when compared with corresponding normal tissue. The p-values for Ct values

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distribution of *RNASEP* and *ERBB2* for both tumor and normal group were <0.05. 2-tailed Peerson correlation for the binary variables revealed no significant correlation between *HER2/ERBB2* amplification with any other variables. Negative correlation (p= 0.001, r= -0.558) between diabetes and gall stone disease existed in our patient group while positive correlation (p=0.002; r = 0.559) was observed between lymph node status and tumor histopathological grade. No significant difference was seen for survival analysis between *HER-2/ERBB2* amplified and not amplified group. (p=0.48).

Conclusion: Despite efforts gall bladder carcinoma remains a major challenge. Till now surgical excision remains the definitive treatment. Our study revealed a significant association between ERBB2/Her2-neu amplification and gall bladder carcinoma. So this gene can be targeted using monoclonal antibody which can help in early management of GBC.

Key words: Gall bladder carcinoma (GBC), *EGFR* mutations,*ERBB2/Her2-Neu* amplification.

Background

Gall Bladder carcinoma (GBC) is often a rapidly progressing disease with often detection at a fairly advanced stage. Though it is a rare malignancy, but it is the most common malignancy of Hepatobiliary tract and 3rd most common Gastrointestinal Malignancy.

The Worldwide incidence of this neoplasm is 2 in 100,000 however it has relatively higher incidence in Countries of South America and Asia like India, Pakistan, Korea and Japan. The need for an early detection is proven from the fact that 5-year survival rate is less than 5% and average survival rate for a patient at the time of detection is 6 months. Detection of cancer in Early Stage of disease has huge impact on the survival of the patient.[1]

Long-term survivals have now been reported following radical resection of large tumors invading the liver. But, overall its still difficult to treat, especially when the patient presents with a large tumor. Surgery still remains the mainstay of treatment, the role of neoadjuvant or adjuvant therapy is limited and high rate of recurrence still remains a stumbling block for satisfactory treatment of the disease. Surgical resection of the tumor with or without adjuvant therapy is the current treatment offering promising survival benefits.

Though, gall bladder carcinoma is not uncommon in this part of India, there is paucity of published data about the disease. Its poor prognosis necessitates a closer look at the molecular changes for evolving an effective early diagnostic and therapeutic strategy. Studies have shown molecular features of gall bladder carcinoma as mutation in *KRAS, INK4a, p53* as well as human epidermal growth factor *HER2/neu* amplification with rare mutation in *PIK3* and *BRAF*. Activating Epidermal growth factor receptor (*EGFR*) mutation have also been identified in a subset of biliary tract cancer, to the tune of 13.6- 15%, which gives an opportunity for a subset of this individuals to be benefitted from the known Inhibitors available.[2,3]

The current study was done to find out the strength and degree of *ERBB2/Her2-Neu* amplification among Adenocarcinoma Gall Bladder and its Clinical staging and to identify whether *ERBB2/Her2-neu* receptor amplification significantly correlated with Adenocarcinoma Gall Bladder with respect to their normal tissue.

Materials and Method

This Hospital based observational, analytical study was conducted jointly between IPGME & R and SSKM Hospital, Dept. of General Surgery and Indian Statistical Institute, Kolkata Dept. of Human Genome Unit.

This prospective observational study was done in Department of General Surgery, IPGME & R and SSKM Hospital, Kolkata after obtaining necessary permission from the institutional ethics committee. A non-probability purposive sampling was done and all patients attending

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the OPD with diagnosed / highly suspicious adenocarcinoma Gall Bladder who were planned for an operative management during the period Jan. 2018 to May 2019 and conforming with the inclusion criteria (mentioned below) were included in our study.

Patient inclusion criteria

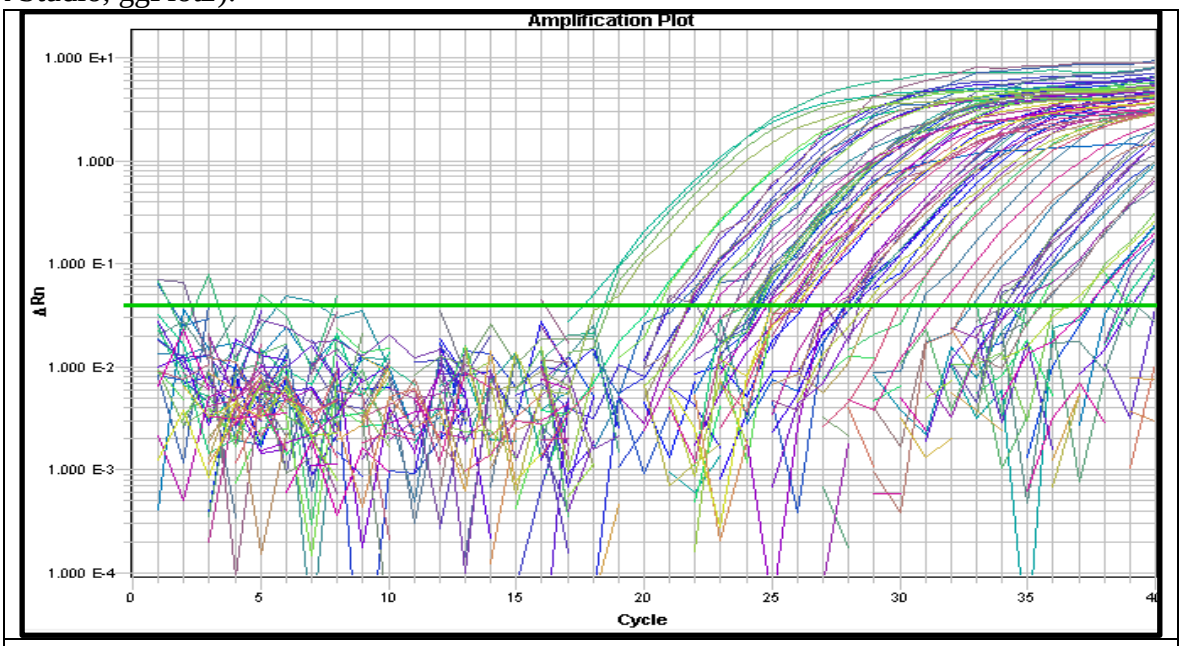
1. Highly suspected or Tissue diagnosis proven Adenocarcinoma Gall Bladder
2. Patient planned for an operative intervention, and willing to take part in study.

Patient Exclusion criteria

1. Patient unwilling to take part in this study.
2. Suspected case of Carcinoma latter proven Non-Adenocarcinoma gall bladder
3. Suspected case of Carcinoma latter proven Benign disease on Histopathological examination
4. Gall Bladder Polyp, Porcelain Gall Bladder and other Pre-Cancerous Lesions, which are not proven Carcinoma.

Thus we have selected 31 patients for our study. Informed consent was taken from all of them after they got admitted in SSKM Hospital and was thoroughly evaluated by history, clinical examination and radiological imaging. A sample of blood (5ml approximately) is withdrawn in case there is no prior recent blood transfusion. Patient is then worked up and planned for an elective operation. Intra-operatively gall bladder tissues are collected: In 4 (four) containers, 2 (two) tumor tissue for analysis, 2 (two) Normal tissue of gall bladder used as control, each one in 10% formalin and another in RNA LATER (Invitrogen, USA) fluid container. Thirty-one such tissue of Adenocarcinoma Gall Bladder collected for studying the *ERBB2/Her2Neu* amplification. AJCC (American Joint Committee on Cancer)-TNM staging and Grading used.

Tissue genetic analysis was done by ERBB2 amplification study, DNA isolation and quantification, *ERBB2/Her2-neu* amplification. ERBB2 amplification was assessed by real time PCR on all 93 samples using TaqMan probe comparing tumor vs. normal tissue/blood DNA samples. Above 2 fold change was identified as amplified samples. In addition $2^{-\Delta ct}$ values of tumor and normal group were compared by Wilcoxon signed rank test to identify significant difference of fold change in *ERBB2* copy number in this two groups (R Packages, R Studio, ggPlot*2*).



Amplification plot of *ERBB2* gene in Real time PCR in carcinoma gall bladder (7900 HT)

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Statistical Analysis

All data documented in binary variables and data were analyzed by SPSS software (PWAS statistics version 18). Correlation between clinic-pathological data and mutation were also analyzed by this software. Survival analysis for *ERBB2/HER2* amplified and non-amplified group were done using Kaplan-Meier estimator by SPSS.

Results

The mean age of study population was 59.84±10.26 yrs. median age being 60 yrs. 87% of our study sample was in the age group 40-70 yrs. The study population included majority of female (74%) with a M:F ratio of 2.8:1. Most of the patients had a positive risk factor of gall stone disease (97%) and only a handful of patients had positive history of smoking (23%) and alcoholism (10%) as a risk factor. In staging and resection of the disease we found the origin of lesion from the body of gall bladder (47%) >Neck (34%)>fundus (19%) with patients being detected at fairly advanced stage i.e. 52% in stage III and 42% in stage II. The Grade of differentiation was 42% poorly, 35% moderately and 23% well differentiated cancer. Hence, we can say that a large percentage of disease is detected late in stage.

We assessed *ERBB2* amplification in 31 tumor normal paired tissue. Fifty two percent (n=16) of the samples showed above 2-fold amplification in the tumor tissue when compared with corresponding normal tissue. The p-values for Ct values distribution of *RNASEP* and *ERBB2* for both tumor and normal group were <0.05, thus the hypothesis of an underlying normal distribution was rejected at 0.05 significance level. The fold change between tumor and normal group were compared and significant difference were observed (Wilcoxon signed rank test, $p<0.05$). Ideally the *ERBB2* amplification results needs to be validated by other methods like, in situ hybridization and immunohistochemistry, but it is limited by relevant tissue availability for this work. No correlation of *ERBB2* amplification with any other demography or clinicopathological factors was observed.

In our study, we performed 2-tailed Peerson correlation for the binary variables. There was no significant correlation observed for *HER2/ERBB2* amplification with any other variables. However, we observed significant negative correlation (p= 0.001, r= -0.558) between diabetes and gall stone disease in our patient group. In addition, we also observed a positive correlation (p=0.002;r = 0.559) between lymph node status and tumor histopathological grade.

The survival analysis between *HER-2/ERBB2* amplified and not amplified group showed no significant difference (p= 0.48) between them.

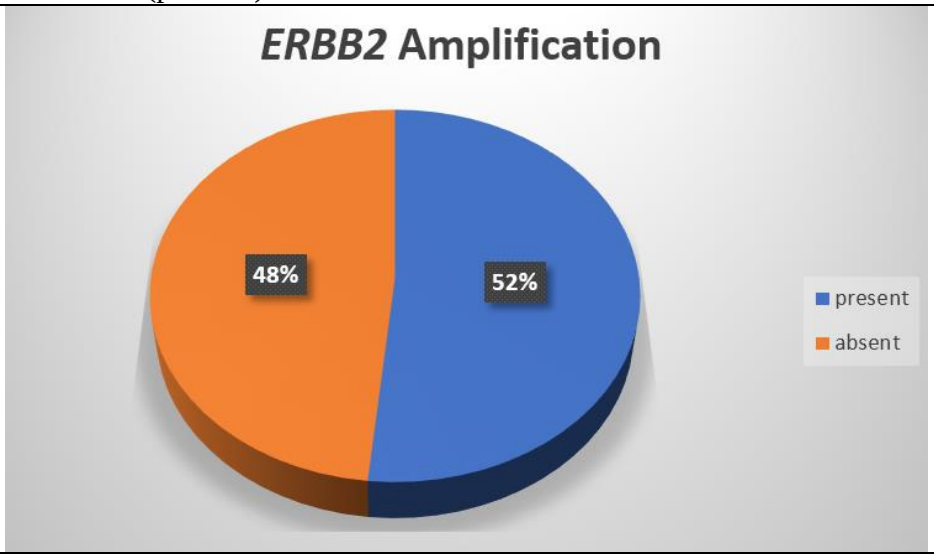

Figure 1: Amplification Present in Cases of Carcinoma Gall Bladder

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Among the 31 patients, 16 of them showed positive mutation for *HER-2/ERBB2* receptor which has been more as compared with 6-15 % of positive results found in the previous studies.

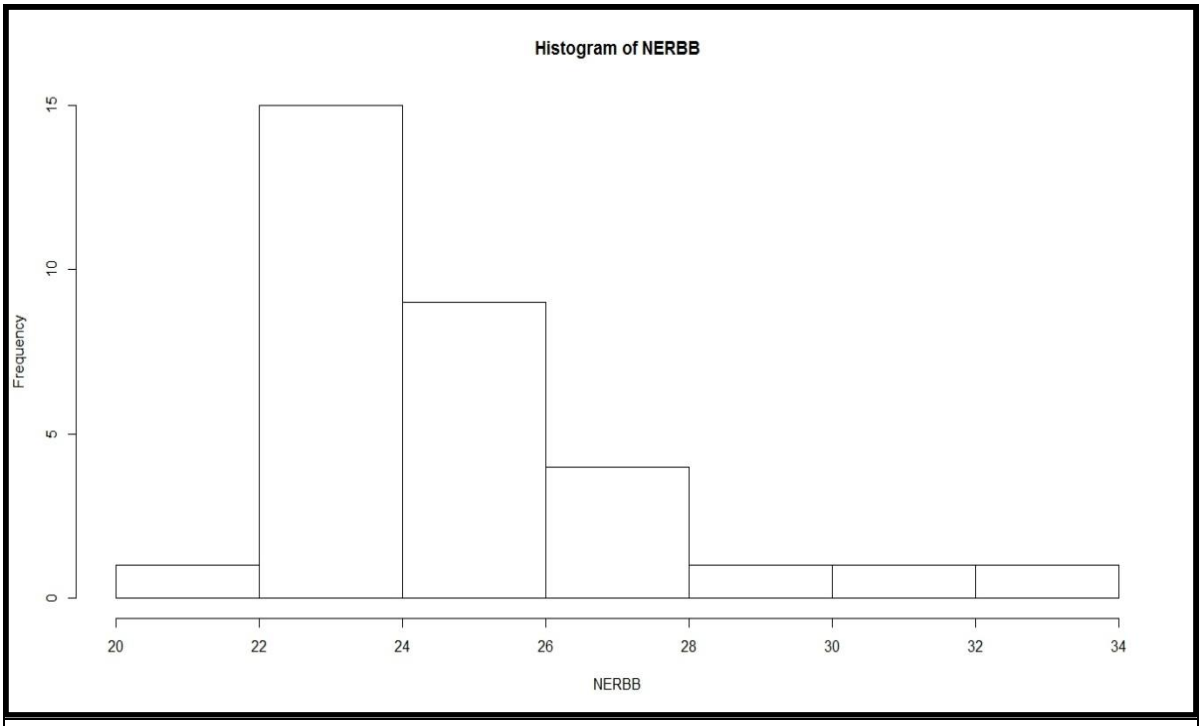

Figure 2: Histogram of CT values for ERBB2 in 31 Normal Gall bladder tissues

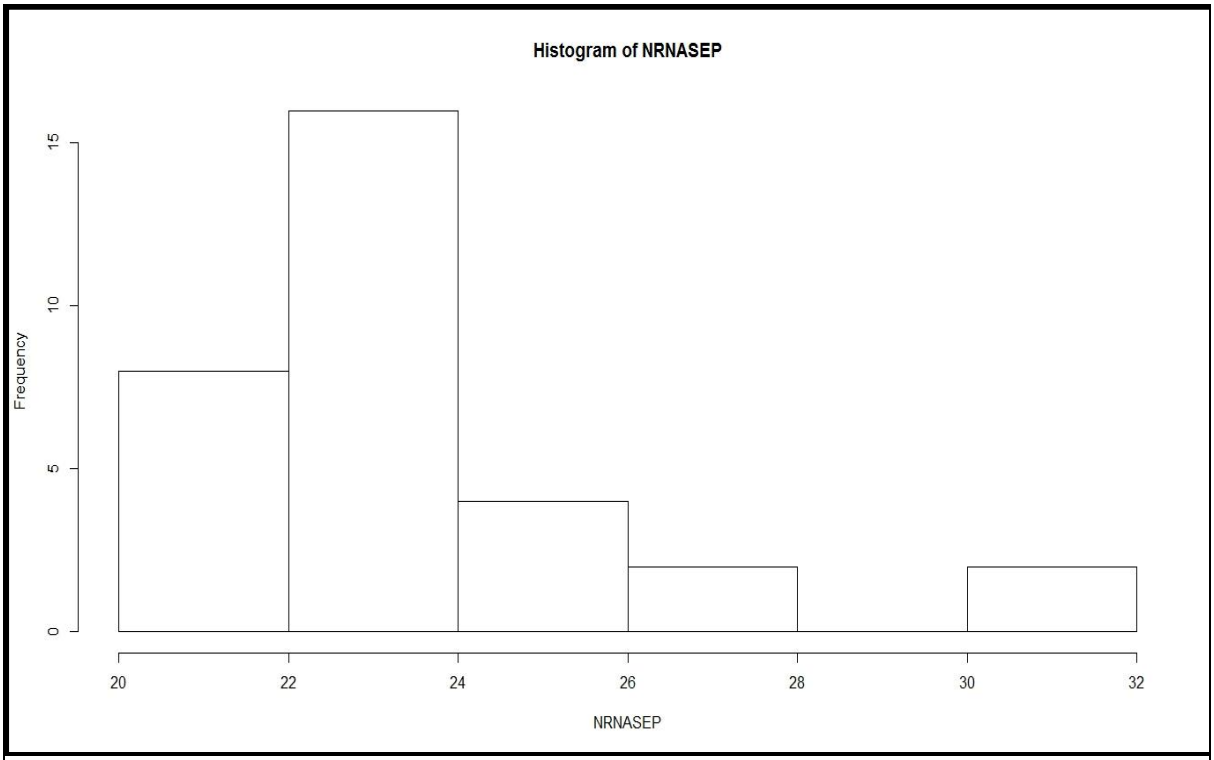

Figure 3: Histogram of CT value of RNASEP in normal gall bladder tissue

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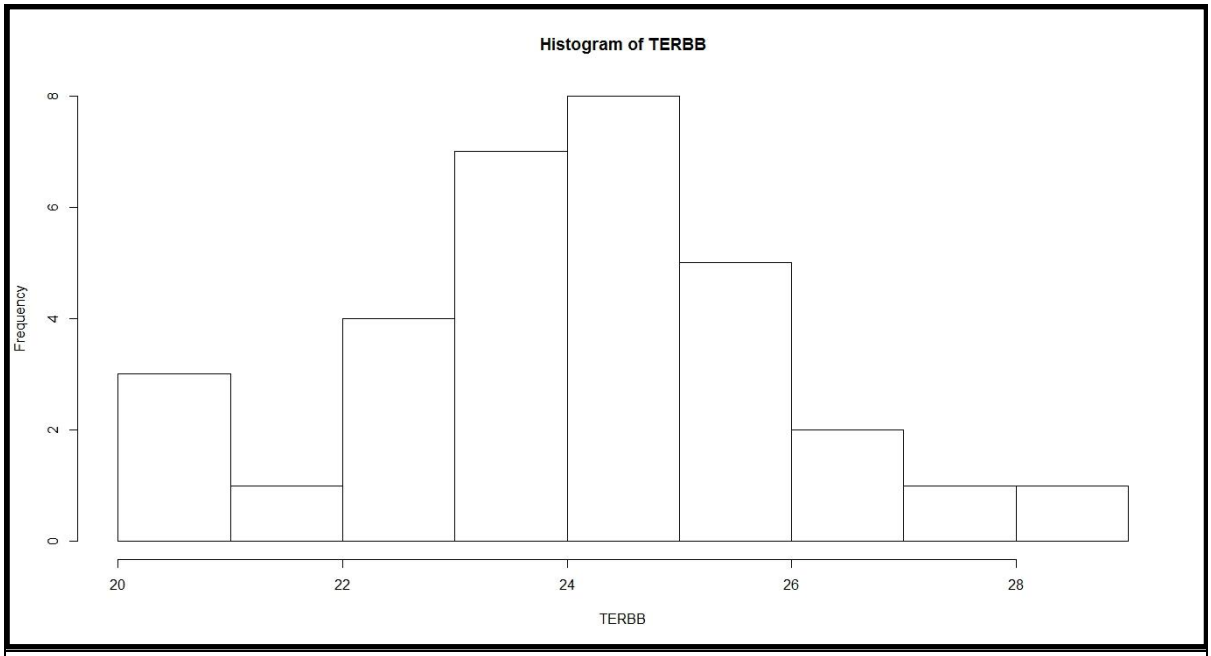

Figure 4: Histogram of CT value of ERBB2 receptor in tumor gall bladder tissue

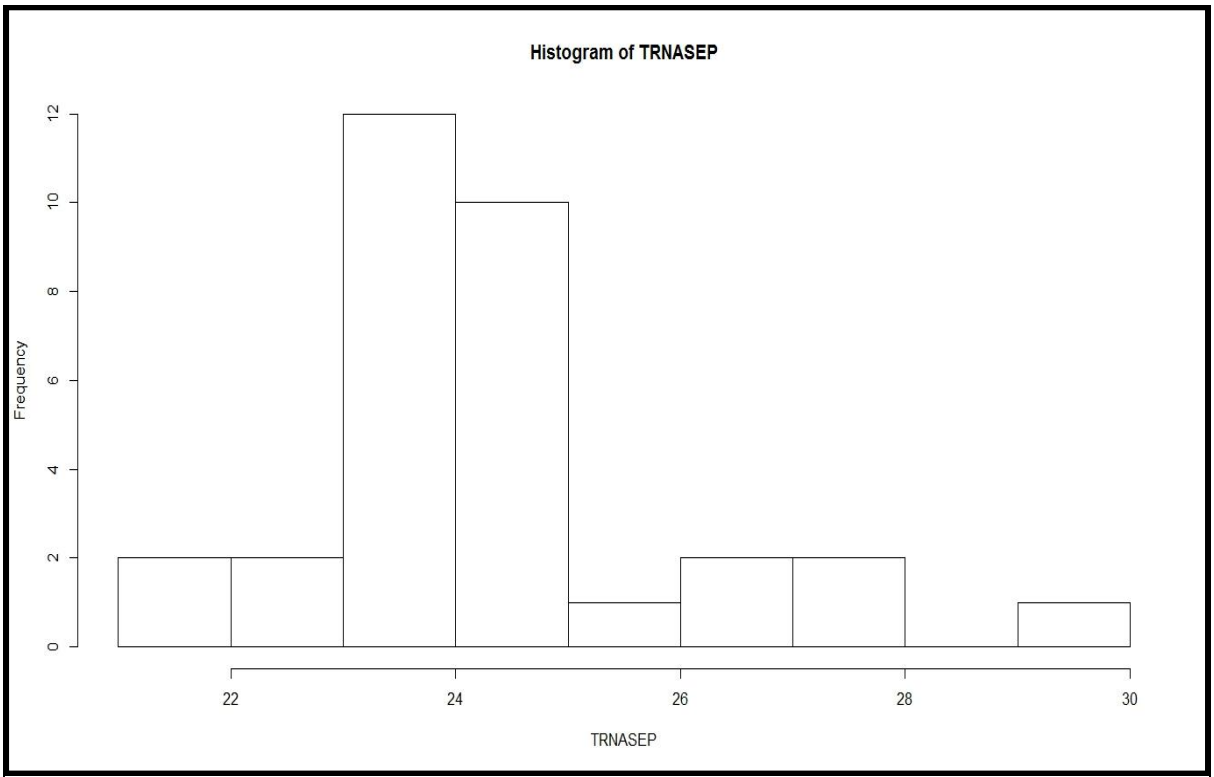

Figure 5: Histogram of CT value of RNASEP in tumor gall bladder tissues

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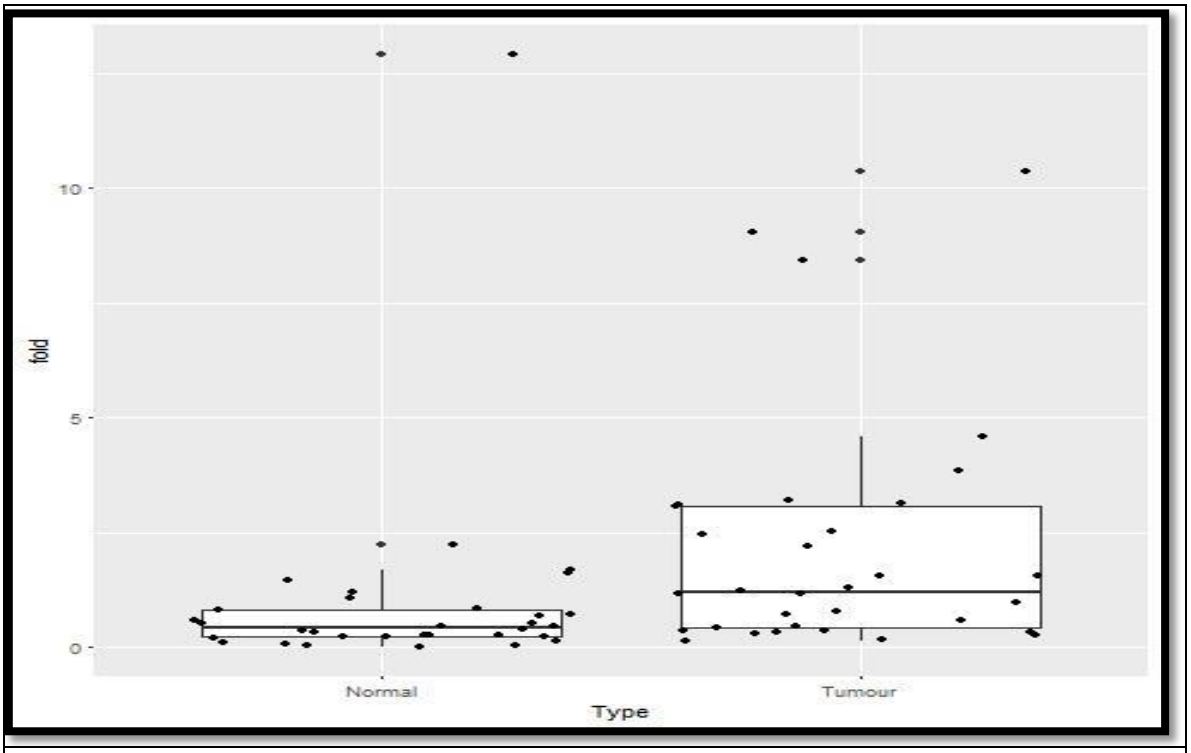

Figure 6: Comparative dot plots of ERBB2/Her2-Neu between normal and tumor tissue

We have compared the *ERBB2/Her2-Neu* amplification among the all gall bladder tumors with respect to their normal adjacent tissues. Every tumor tissues were compared individually with their respective normal adjacent tissue. We have found that *ERBB2/Her2-Neu* amplifications in tumor group was statistically significantly higher than their normal counter parts (*p=0.03*)

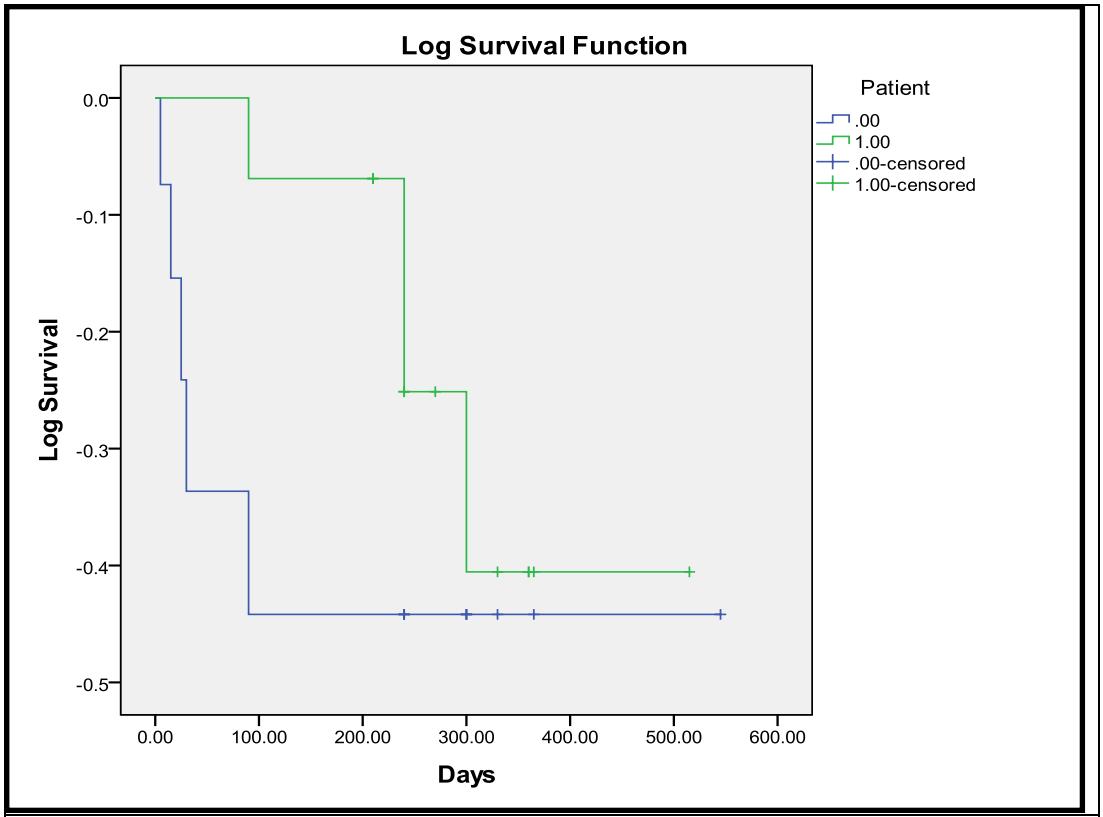

Figure 7: Survival analysis by Kaplan- Meier

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Discussions

Gall Bladder cancer remains the iceberg disease being detected in later stage of the course. There is no significant improvement in the survival rate of gall bladder cancer patient over decades. Since surgery is the only promising treatment, early detection is key to reduce disease burden. In our study we included 31 patients of carcinoma gall bladder who underwent resection of gall bladder mass i.e. simple/extended/ radical cholecystectomies as per the evaluation and guidelines. These patients who were enrolled in the study were analyzed on the basis of various parameters for their personal history and habits and the resected specimen was subjected to analyses for *ERBB2/HER2-neu* amplification.

We found that the mean age of our study population was 59.84±10.26 yrs., median age being 60yrs which is very close to the median age in a study by Duffy A et al where it was 67 yrs.[4] Cholelithiasis is considered as most consistent and time tested risk factor for development of gall bladder carcinoma. Majority of our sample belonged to female gender. We found 97% of our study population had gall stone, similarly in a study by Shaffer EA it was seen that 85% patients had cholelithiasis.[5] Goldin RD et al also found gall stone as an important risk factor for gall bladder CA. In various other studies advanced age, female gender, cholelithiasis, porcelain gall bladder, gall bladder polyps, chronic infections, smoking were found as risk factors for gall bladder carcinoma.[6,7,8]

The *ERBB2*gene is located on Chromosome on 17q21 and encodes a 185kDatransmembrane receptor belonging to the EGFR family, this gene encodes a member of EGFR family of receptor tyrosine kinase. It has no ligand binding domain of its own and therefore cannot bind growth factor. However, it binds to other Ligand bound EGFR family members to form a heterodimer, stabilizing ligand binding and enhancing kinases mediating activation of down streaming signaling kinase such as *PIK3* kinase. The uniqueness of *ERBB 2* among its family members is that it not only binds with any of its family ligand but also get permanently fixed in an active conformation. Two key signaling pathways activated by the *ERBB* family dimers are the *MAPK* pathway which stimulates proliferation and the *PI3K- Akt* pathway which promotes tumor cell survival. *MAPK* leads to transcription of genes driving cellular proliferation, migration, differentiation and angiogenesis. Signaling through the *PI3K- Akt* pathway leads to several cellular endpoint and the anti-apoptosis signaling. Of 3 classes of *PI3K*, Class 1a member is primary responsible for mediating the signal.[9]

Three principal mechanism of oncogenic mechanism of *ERBB2* have been identified.

1. Amplification and overexpression
2. Molecular alteration of receptor
3. Inhibition of phosphatase activity

Here in this study we assessed *ERBB2/HER2* amplification status in resected gall bladder cancer tumors. We identified above 2-folds amplification of *ERBB2* gene in 52% of the patients. The amplification was observed statistically significantly associated with GBC (*p= 0.03*).

There are several studies that revealed an overexpresson of HER2/Neu in gall bladder carcinoma and biliary malignancy. Study by Roa I et al revealed overexpression of HER2/neu in 12.8% of the patients with gall bladder carcinoma and 13.8% of patients with the deep infiltrating cancers.[10] Su WC et al also revealed similar finding.[11]

The EGFR family is mostly targeted as drug able oncogene in many cancers. *EGFR* mutations already been targeted in breast, gastric and few other cancer treatments. *ERBB2*, one of the elite member of the EGFR family is a alternative target in treatment of many cancers. *ERBB2/HER2* amplification status already used in breast cancer targeted therapy and it has shown a very positive outcome in patient's overall survival. Kumari N. et al in her study found complete membranous staining of C-erB2 in 9.6% of GBC cases and also inferred that it may become a candidate for therapy in GBC as being used in breast cancer

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and being explored in gastric cancer.[12] In another study by Abeer I. et al it was seen that among gall bladder carcinoma patients 72.5% had EGFR expression and 32.5% had HER2/neu expression and concluded that those two markers can be used as predictive and prognostic markers respectively with rationale to futher explore the use of anti-HER2 and anti-EGRF therapy in gall bladder carcinoma.[13] In another study by Hadi et al high positivity of both EGRF and HER2/neu was noted in GBC.[14]

In our study population which belonged to eastern part of India (West Bengal), we observed a higher *ERBB2/HER2* amplification rate i.e. 52%. Thus, *ERBB2/HER2* is a promising target for treatment of a sub-group of patients. *ERBB2/HER2* is also an attractive target because we now have several drugs which could inhibit *ERBB2/HER2* for anti-cancer effect with success seen in the treatment of breast gastric cancer.

Conclusion

From our study we can conclude that ERBB2/Her2-neu amplification is significantly associated with GBC. The ErbB2 signaling pathway is composed of *EGFR*, *ERBB2*, *ERBB3*, and *ERBB4* receptors and their downstream genes. In between those genes *ERBB2* is the most frequently amplified and overexpressed gene in GBC. This condition has a predictive value for ERBB2-targeted therapy using the monoclonal antibody trastuzumab (Herceptin) and newer drugs such as lapatinib, pertuzumab and ertumaxomab.

Strength and Limitation

The study is an observational study. For this type of study, we need to have more patients to be included in the study. During the resection, tumor tissue collection all samples were not collected as frozen section collection. So, there might be the possibility of tumor heterogeneity and mixed normal tissue. We need to follow up for the patient and measured the survival for at least 5 years. We need to do overexpression of *ERBB2*/Her2-neu for validation of the amplification result. We did not recruit more patient as the patients were collected only for two years. To confirm the amplification, we need to perform the immunohistochemistry and FISH study that would give better confirmation of the result.

Last but not the least patient can be followed up to check the *ERBB2/Her2-neu* amplification after chemotherapy treatment and monitoring the survival.

Reference

1. Shaffer EA. Gallbladder cancer: the basics. *GastroenterolHepatol (N Y)*. 2008;4(10):737-741.
2. Andrén-Sandberg A. Molecular biology of gallbladder cancer: potential clinical implications. N Am J Med Sci. 2012 Oct;4(10):435-41.
3. Yanagisawa N, Yamashita K, Kuba T, Okayasu I. Sporadic TP53 transition mutations in chronic cholecystitis are possibly linked to gallbladder carcinogenesis. Anticancer Res 2010;30:4443-9.
4. Duffy A, Capanu M, Abou-Alfa GK, Huitzil D, Jarnagin W, Fong Y, et al. Gallbladder cancer (GBC): 10-year experience at Memorial Sloan-Kettering Cancer Centre (MSKCC). *J Surg Oncol.* 2008;98(7):485-9
5. Shaffer EA. Gallbladder cancer: the basics. *Gastroenterol Hepatol (N Y).* 2008;4(10):737-41.
6. Goldin RD, and Roa JC. Gallbladder cancer: a morphological and molecular update. *Histopathology.* 2009;55(2):218-29.
7. Rawla P, Sunkara T, Thandra KC, Barsouk A. Epidemiology of gallbladder cancer. ClinExpHepatol. 2019 May;5(2):93-102.

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8. Goetze TO. Gallbladder carcinoma: Prognostic factors and therapeutic options. World J Gastroenterol. 2015 Nov 21;21(43):12211-7.
9. Pignochino Y, Sarotto I, Peraldo-Neia C, Penachioni JY, Cavalloni G, Migliardi G, et al. Targeting EGFR/HER2 pathways enhances the antiproliferative effect of gemcitabine in biliary tract and gallbladder carcinomas. *BMC Cancer*. 2010;10:631.
10. Roa I, de Toro G, Schalper K, de Aretxabala X, Churi C, Javle M. Overexpression of the HER2/neu Gene: A New Therapeutic Possibility for Patients With Advanced Gallbladder Cancer. Gastrointest Cancer Res. 2014 Mar;7(2):42-8.
11. Su WC, Shiesh SC, Liu HS, Chen CY, Chow NH, Lin XZ. Expression of oncogene products HER2/Neu and Ras and fibrosis-related growth factors bFGF, TGF-beta, and PDGF in bile from biliary malignancies and inflammatory disorders. Dig Dis Sci. 2001 Jul;46(7):1387-92.
12. Kumari N, Kapoor VK, Krishnani N, Kumar K, Baitha DK. Role of C-erbB2 expression in gallbladder cancer. Indian J PatholMicrobiol. 2012 Jan-Mar;55(1):75-9.
13. Abeer, I., Khan, S., Hasan, M. and Hussain, M., 2021. *EGFR and HER2neu Expression in Gall Bladder Carcinoma and Its Association with Clinicopathological Parameters - An Institutional Experience from North India*. [online] WAOCP.org. Available at: <http://waocp.com/journal/index.php/apjcb/article/view/543> [Accessed 18 September 2021].
14. Hadi R, Pant M, Husain N, Singhal A, Khurana R, Agarwal G, et al. EGFR and HER-2/neu Expression in Gallbladder Carcinoma: An Institutional Experience. Gulf J Oncolog. 2016;1:12-9