

A detailed review of EGFR & HER2/NEU gene expression analysis in Gallbladder cancer

Inara Abeer ¹, Nazia Walvir ², Sabina Khan ³, Gazanfar Rasheed ⁴, Zafirah Zahir ⁵, Aiffa Aiman ⁶, Shadab Maqsood ⁷, Ibreez Rakshan ⁸

¹⁻⁶ Department of Pathology SKIMS, ⁷ Department of Radiology, SKIMS

⁸ Department of Orthopedics, GMC, Srinagar

BACKGROUND

Gallbladder cancer (GBC) is a highly malignant neoplasm characterized by delayed diagnosis, poor prognosis and ineffective treatment, especially in advanced stages. Despite the efforts of many researchers, GBC continues to represent a significant therapeutic challenge in oncology. Surgical resection remains the only curative treatment for this disease in its early stages. The roles of radiation, chemoradiation, and chemotherapy in neoadjuvant and adjuvant settings remain poorly defined.

The necessity to search for archetype markers of GBC will help to diagnose and predict the disease as well as help to choose the modality of treatment. This may allow making headway in a basic understanding of the biology of GBC and management paradigms. Furthermore, accurate molecular characterization of the tumours is necessary for enrolling the patients in clinical trials to define which patient groups will benefit from targeted therapy. Therefore, we have reviewed the available literature on GBC biomarkers and their potential clinical implications in managing GBC.

INTRODUCTION

Gallbladder cancer (GBC) is the most common biliary tract malignancy accounting for 80%95% of biliary tract cancers and is the sixth most common form of digestive tract malignancy worldwide.¹ GBC exhibits wide geographical and ethnic variations. The aetiology of the disease is influenced by several risk factors, some being increasing age, female gender, family history, cholelithiasis, and

exposure to certain chemicals and heavy metals being the predominant ones. The clinical presentation of GBC is like biliary colic or chronic cholecystitis; thus, it is not diagnosed in the early stages.¹

Despite the recent advancement in understanding cancer biology, the disease remains a therapeutic challenge with poor prognosis and low survival. The roles of radiation, chemoradiation, and chemotherapy in neoadjuvant and adjuvant settings remain to be defined in prospective studies.² To develop targeted therapy for GBC, it is essential to understand the various molecular alterations involved in the Pathogenesis Pathogenesisancer. We herein review the available literature on GBC biomarkers and the potential implications in the targeted therapy of GBC.

A literature review was performed utilizing MEDLINE/ PubMed in January 2022. The terms “gallbladder cancer”, “gallbladder carcinoma”, and “gallbladder tumour” in combination with the following terms: “molecular marker”, “biomarker”, “diagnosis”, “progression”, “survival”, “outcome”, “pathogenesis”, “signalling pathways” were searched in the title and abstract. The references of relevant articles were reviewed to identify additional eligible publications. Articles were assessed according to the above eligibility criteria. Data extracted included first author, year

Correspondence:

Dr. Inara Abeer, MD
Senior Resident, Department of Pathology, SKIMS
Email: drinaraabeer@gmail.com

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of publication, study type, size of the patient population, and type of molecular target/biomarker assessed.

Pathogenesis of Gallbladder Cancer

The pathogenesis of gallbladder cancer is likely multifactorial, with no single causative factor being identified. The development of gallbladder cancer is proposed to occur over 515 years, with tissue alterations including metaplasia, dysplasia, carcinoma in situ, and invasive cancer. In addition, the anatomy of the gallbladder is unique and predisposes cancer to direct invasion³ Pathogenesis of gallbladder cancer is shown in Figure 1.

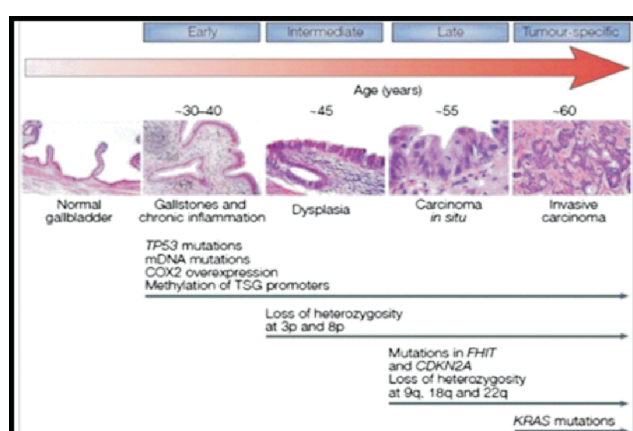


Fig. 1: Multistep pathogenesis of gallbladder cancer from gallstones.⁷³

Molecular Pathogenesis

Biological Pathways. Two distinct, independent biological pathways based on morphological, genetic, and molecular evidence leading to gallbladder cancer are hypothesized: (1) a dysplasia-carcinoma sequence arising from the metaplastic epithelium and (2) an adenoma-carcinoma sequence (Figure 2).^{4,5}

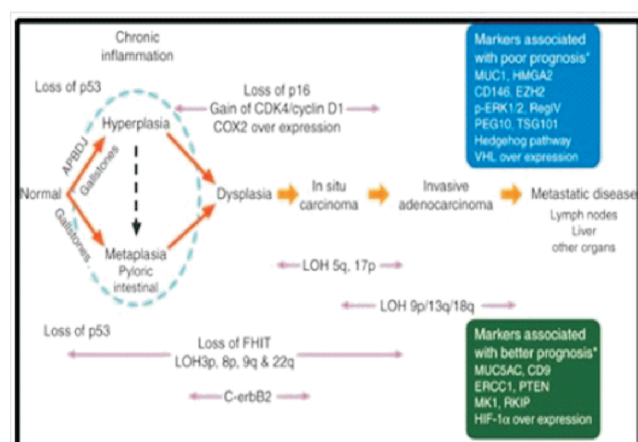


Fig. 2: The "Gallbladder carcinogenesis and dissemination model"⁷³

Metaplasia is expected in the chronically inflamed gallbladder, present in over 50% of cases. However, the precise relationship between metaplasia and dysplasia must still be established.⁶

Theory 2. Less than 3% of early carcinomas have adenomatous remnants, suggesting this mechanism has little importance in the carcinogenic pathway. There remains no way to predict which of these will undergo malignant transformation.⁷

Genetic Mutations: The precise genetic changes involved in developing gallbladder cancer are poorly understood. Various genetic alterations are likely implicated in gallbladder cancer, including oncogene activation, tumour suppressor gene inhibition, microsatellite instability, and methylation of gene promoter areas. Over 1281 genetic mutations have been identified in gallbladder cancer.

Carcinogenic pathways may include (1) inflammation secondary to gallstones leading to p53 mutations and eventual carcinoma, (2) point mutation of Kras contributing to hyperplasia then carcinoma as seen in patients with an anomalous junction of pancreaticobiliary duct, and (3) neoplastic foci in gallbladder polyps secondary to Kras mutation.³ Epigenetics may play a distinct role in gallbladder carcinogenesis.

The role of microsatellite instability (MSI) in the carcinogenesis of gallbladder cancer remains poorly described, with literature reporting MSI rates between 0 and 40% of cases; the most extensive and most recent study reported a prevalence of 7.8%.

Loss of heterozygosity (LOH) has been described in several tumour suppressor genes in gallbladder cancer. Expression of ADAM-17 is increased in GBC with a high histological grade and pT stage as well as shorter overall survival. A summary of the genes involved in GBC is in table 1.

Diagnostic Methods of Gallbladder Cancer

In addition to the following, Ultrasonography (USG); Endoscopic ultrasound (EUS), Computed Tomography Scan (CT Scan); Magnetic Resonance Imaging (MRI), Magnetic Resonance Angiography (MRA) and Magnetic Resonance Cholangiopancreatography (MRCP):

Fine Needle Aspiration Cytology (FNAC) is an essential diagnostic procedure. Ultrasound-guided FNAC of GB lesion is a very accurate method to diagnose and plan

Table 1 Summary of the major genes implicated in gallbladder carcinogenesis as available in the published literature (2000present).³⁰

+	Gene	Expression in GBC	Tissues of comparison	Additional information
Onco-gene	KRAS	Higher	Adenoma (0%)	Marker of GBC in PBM No correlation with stage, histology, and survival
	EGFR	Higher (63.4%)	Dysplasia (71.4%) Hyperplasia (15.4%) Normal (0%)	
	HER-neu (ERBB2)	Higher (16–64%)	Carcinoma in situ (0%), Gallstones (0%)	Marker of metastatic disease (70%) Marker of poor prognosis (10x mortality)
Tumor suppressor	TP53	Higher (58.3–100%)	Adenoma (10–20%) Normal (0%)	Unknown relation to prognosis More prominent with poor differentiation
	P16	Lower (48.8%)	Adenoma (100%) Chronic cholecystitis (100%)	Related to poorer prognosis Negative correlation with cyclin D1
	Fragile histidine triad (FHIT)	Lower	Normal	Early change in carcinogenesis
	Retinoblastoma	Lower (58.5%)	Adenoma (100%) Cholecystitis (100%)	Causes cell proliferation, apoptosis, and developmental defects
	VHL	Lower (48.1%)	Peritumoral tissue (80.4%) Polyps (80%) Chronic cholecystitis (88.6%)	Marker progression, biological behaviour, and prognosis
Adhesion molecules and mucins	Cadherins	Higher (N-cadherin 55%; P-cadherin 53%)	None	Associated with large tumor size, invasion, and node metastases
	MUC1	Higher (78%)	Normal tissue (absent)	Higher expression in more advanced tumours; poor survival
	Erythrocyte complement receptor 1 (CR1)	Lower	Chronic cholecystitis Cholelithiasis Normal	Role under investigation
Angiogenesis	Thrombospondin-1	Higher (74.5%)	Normal (0%) T1 cancer (0%)	Associated with venous involvement Predictor of vascular involvement and nodal metastases
	Cyclooxygenase-2	Higher (59.2–71.9%)	Normal (0–25%) Dysplasia (70.3%)	Associated with poor prognosis, mean survival, and tumor Progression
	VEGF-A	Higher (81%)	Chronic cholecystitis (5.1%)	Expression related to histologic grade, TNM stage, and prognosis
Cell cycle regulators	Cyclin E	Higher (33%)	Adenoma (12.5%)	
	Cyclin D1	Higher (41–68.3%)	Adenoma (57.1–67%) Chronic cholecystitis (7.1%) Normal (0%)	Marker of lymphatic/venous involvement and lymph node metastases
	P27Kip1	Lower (43–65%)	None	
Apoptosis	Caspases	Higher (95%; caspase 3; 77%; caspases 6 and 8)	None	Higher extent apoptosis in grade II/III GBC compared with grade I/dysplasia
	Bcl-2	Higher (34.7%)	None	

surgery because of its high sensitivity and specificity. Subtyping and grading the tumours on cytological material based on WHO classification helps decide further patient management and may prevent unnecessary burden on already stressed health in a developing country like ours.⁸

Image-guided FNA, including ultrasound-guided or CT-guided biopsy, has the potential for diagnostic accuracy of 80-90% with False-negative results of 11-41%, which may be attributable to incorrect sampling, necrosis, or fibrosis. In the latest study by Bhartiya R et al. (2017), the sensitivity of US-guided FNAC was 98.6% and specificity 97.3%. The authors concluded that US-guided FNAC is a safe, quick, and precise diagnostic procedure for early diagnosis and management of GB cancers and thus must be used in routine practice.⁹

Serological markers

Tumour markers such as CEA, cancer antigens (CA), CA125, CA242, and CA19-9 have been widely used to diagnose different types of cancer (e.g., liver, gastric, colorectal, and pancreatic). However, inconsistent results have been obtained when these markers are used individually for diagnosing GBC.

Morphology of Gallbladder Cancer

GBC can arise from either a pathway involving metaplasia or dysplasia or one in which there is a pre-existing adenoma. The former is the more common and, because it is often not associated with a macroscopically recognizable lesion, leads to the recommendation that all resected gallbladders need to be examined microscopically.¹⁰

Although it has been established that dysplasia and carcinoma in situ precede most gallbladder carcinomas, relatively little is known about the natural history of these precursor lesions. Most dysplasia and carcinomas in situ are diagnosed after cholecystectomy when the entire lesion is removed. However, there is indirect evidence that progression could occur from precursor lesions to infiltrating carcinoma. Dysplasia and carcinoma in situ are found in the mucosa adjacent to most gallbladder carcinomas, sometimes separated by the histologically normal epithelium.¹¹

If multiple sections of the gallbladder are removed for cholelithiasis are examined, dysplasia and carcinoma in situ are detected in 13.5% and 3.5% of the cases, respectively.

There is consensus that if dysplasia and carcinoma in situ are found, multiple additional sections of the gallbladder should be examined to rule out invasive cancer.¹²

On gross examination, approximately 10-37% of gallbladder carcinomas cannot be identified with certainty, and their macroscopic findings are similar to those of chronic cholecystitis. Gallstones are found in almost all cases of gallbladder cancer. Most carcinomas (60%) originate in the fundus of the gallbladder, 30% in the body, and 10% in the neck. The prevalence of gallbladder cancer associated with diffuse calcification of the gallbladder (so-called porcelain gallbladder) is 12-21%.

Accurate staging of invasive cancers is essential to determine prognosis and treatment, requiring extensive tumour sampling.¹⁰

The results show that combined detection of these markers could increase the sensitivity for diagnosis and prognosis of GBC. In terms of a single marker for the diagnosis of GBC, CA19-9 has the highest sensitivity with relatively low specificity but cannot be used alone as an effective tumour marker to identify GBC. CA242 has the highest specificity, probably because it is rarely affected by the serological level of liver inflammation and stone disease, nor is it affected by the low expression in the pancreatic duct system and pancreatic juice siltation. However, the expression of CA242 is high in almost all malignant tumours and, therefore, cannot be used to differentiate between GBC and pancreas cancer. On immunohistochemistry, hepatoid adenocarcinomas may express alpha-fetoprotein (AFP), albumin, transferrin, PIVKA, and alpha-1-antitrypsin. While AFP remains the most important marker of this lesion; not all hepatoid adenocarcinomas are positive for AFP. On immunohistochemistry, SpCC will usually demonstrate biphasic reactivity to cytokeratins (CK, EMA) and mesenchymal antibodies such as vimentin. Immunohistochemistry is helpful in accurately identifying carcinoids, with positivity for neuroendocrine markers.

Role of EGFR and her2/neu in carcinogenesis EGFR and HER2 are tyrosine kinase receptors encoded by proto-oncogenes. Growth factors such as the epidermal growth factor binding to these receptors in their extracellular binding domain and start cascades of intracellular signalling that lead to tumour cell proliferation, migration, invasion, resistance to apoptosis and angiogenesis.

EGFR: Epidermal growth factor (EGF) was discovered in 1962 by Dr Stanley Cohen during studying nerve growth factor (NGF). It is a single-chain polypeptide consisting of 53 amino acids that are derived from the cleavage of a large precursor, prepro-EGF. EGF is now known as the prototype of the group I EGF family, including transforming growth factor- α (TGF- α), heparin-binding EGF (HB-EGF), amphiregulin, betacellulin, epiregulin and epigene.¹³

Functionally, these growth factors share the ability to bind the same receptor, the EGF receptor (EGFR, ERBB1), activate its intrinsic tyrosine kinase activity, and couple the receptor to downstream signalling pathways controlling cell proliferation, differentiation, survival, or motility. It was identified that EGF is the prototypical member of a family of peptide growth factors that activate the EGF receptors. The EGF/EGF receptor signalling pathway plays essential roles in the proliferation, differentiation, and migration of various cell types, mainly in epithelial cells. After the crucial characterization of EGF function in the 1st decade or so after its discovery, the studies related to EGF and its signalling pathway have extended to a broad range of investigations concerning its biological and pathophysiological roles in disease development in human cancers.¹³

Since the discovery that a subgroup of patients with nonsmall cell lung cancer with specific mutations in the EGFR gene are responsive to the tyrosine kinase

Inhibitor gefitinib, many studies have focused on EGFR mutations as a potential target for growth inhibition in solid tumours.^{14,15, 16,17} Furthermore, the analysis of EGFR signalling in lung cancer revealed that responsiveness to gefitinib is related to the activation of these pathways.¹⁸

Yoon et al. reported that in cholangiocarcinoma cell lines, EGFR activation was prolonged upon EGF stimulation and that cell growth was significantly attenuated by EGFR kinase inhibitors.¹⁹ Leone F et al. (2005) showed that mutations in the EGFR gene are present and downstream pathways are activated in the bile duct and gallbladder carcinoma, suggesting that small-molecule kinase inhibitors, such as gefitinib or erlotinib, should be evaluated for clinical activity in these tumours.²⁰

The expression of EGFR is increased in the gallbladder, common bile duct, and ampullary carcinomas. Still, not in

non-malignant conditions of the gallbladder and biliary tract, and it seems related to some clinical and pathologic features, such as lymph node metastasis, aberrant p53 expression, proliferation activity, and differentiation.²¹ The discovery of acquired somatic mutations in the EGFR gene, which modify the movement of the tyrosine kinase domain, can be crucial in better understanding the molecular Pathogenesis Pathogenesis highly-aggressive tumour.²²

HER2/neu:

HER2/neu (c-ERBB2) stands for “Human Epidermal growth factor Receptor 2”. It was first identified in rodent neural tumour cell lines and named neu in the early 1980s. The neu oncogene (also known as HER2, ERBB2, or p185) was discovered by a group of scientists at the Massachusetts Institute of Technology, Rockefeller, and Harvard University. It is a normal cellular gene encoding a tyrosine kinase that is strongly related to the receptor for epidermal growth factor receptor (EGFR). The HER2 receptor is a 1255 amino acid, 185 kD transmembrane glycoprotein located at the long arm of human chromosome 17 (17q12). HER2 is expressed in many tissues, and its significant role in these tissues is to facilitate excessive/uncontrolled cell growth and tumorigenesis.²³

The human epidermal growth factor receptor (HER) family of receptors plays a central role in the Pathogenesis several human cancers. They regulate cell growth, survival, and differentiation via multiple signal transduction pathways and participate in cellular proliferation and differentiation. The family is made up of four principal members: HER-1, HER-2, HER-3, and HER-4, also called ERBB1, ERBB2, ERBB3, and ERBB4, respectively. All four HER receptors comprise a cysteine-rich extracellular ligand binding site, a transmembrane lipophilic segment, and an intracellular domain with tyrosine kinase catalytic activity. Its Amplification mainly translates into protein overexpression.^{23,24}

Most studies on HER2 have been carried out in breast cancer; Amplification or overexpression of the HER2 gene occurs in approximately 1530% of breast cancers and is associated with increased disease recurrence and worse prognosis.²⁵ With an increasing understanding of HER2 biology, it has now been recognized that HER2

overexpression occurs in other forms of cancers, such as stomach, ovary, uterine serous endometrial carcinoma, colon, bladder, lung, uterine cervix, head and neck, and oesophagus. Apart from its role in developing various cancers, it has also been intensely evaluated as a therapeutic target.²⁶ In patients with advanced breast and gastric cancer, which are known to overexpress or amplify the HER2/neu gene, HER2/neu receptor blockers have already been incorporated into conventional chemotherapy, using monoclonal antibodies, such as trastuzumab and pertuzumab, or tyrosine kinase inhibitors associated with EGFR or HER2/neu, such as lapatinib.²⁷

The role of HER2/neu in biliary carcinomas is a relatively recent concept, and only a few studies exist. A survey regarding HER2/neu and EGFR expression in transgenic mice showed that overexpression of wild-type ERBB-2 in adenocarcinomas of the GB, cystic duct, common bile duct, and intrahepatic bile duct carcinomas was observed to develop through the hyperplasiaadenoma sequence at high incidences of 100%, 100%, 87%, and 30%, respectively. These results highlighted that ERBB-2 overexpression plays a vital role in biliary carcinogenesis, especially early carcinogenesis of the extrahepatic biliary tract. The overexpression of HER2/neu has been described by using immunohistochemical scores. In breast and gastric cancers, the HER2/neu scoring system is relatively standardized; however, this is not the case with other HER2/neu-expressing cancers, including GBC (Figure 3).²⁸

Role of EGFR and HER2/neu in Gall bladder Cancer

The high incidence of GBC in our region and its poor prognosis necessitates a closer look at the molecular changes for evolving an effective early diagnostic and therapeutic strategy. Thomas MB et al. (2006), in a study on biological characteristics of cancers in the gallbladder, concluded that understanding carcinogenic molecular mechanisms of these malignancies may lead to improved therapeutic regimens for patients. While considerable progress has been made in the molecular Pathogenesis Pathogenesis human neoplasms, information about the genetic changes involved in gallbladder carcinogenesis is more limited.²⁹

The human EGFR family plays a crucial role in carcinogenesis and disease progression in several cancers. EGFR and HER2/neu are the ERBB2 receptor tyrosine

kinase family members. Preclinical studies suggest that EGFR and HER2/neu and their pathways have a crucial role in tumour growth.³⁰

The improved understanding of EGFR's role in oncogenesis has made it an attractive target for therapeutic intervention in several cancers. Clinical and preclinical data utilize this target in colon cancer, squamous cell carcinoma of the head and neck, non-small cell lung, and breast cancer.^{31,32} Several small studies, mainly from Asia, have complemented this work by examining the level of expression of EGFR in biliary tumours. These few studies demonstrated a significant and consistent over-expression of epithelial growth factor receptors in biliary tumours. The over-expression of EGFR in gallbladder carcinoma may have direct clinical implications with an alternative management strategy for managing this challenging disease.^{33,34} Further studies should be conducted to evaluate this association better. However, if this association were to be confirmed, it would justify routinely including EGFR in the prognostic assessment of gallbladder carcinoma and likely lead to an indication of the use of anti-EGFR therapy (targeted therapy).

Expression of HER2/neu has been intensively studied in different tumours entities and has led to targeted therapy in colorectal, breast, lung, and head and neck cancer.³⁵ Several recent studies have identified ERBB2 signalling as the most frequently mutated pathway in GBC (36.8%), giving rise to their oncogenic potential and underlining the significance of HER-2 in GBC development and progression. For biliary tract carcinoma, data for HER2/neu overexpression have been presented in mostly small patient cohorts. Its overexpression and Amplification have been found in a range between 5% and 76% in biliary tract cancers with varying rates of epidermal growth factor receptor (EGFR, HER-1) expression reported, ranging from 12.4100% in gallbladder cancer.³⁶

The study of HER2/neu expression in gallbladder carcinoma is limited. There are few but conflicting reports dealing with the clinical significance of the expression of HER2/neu in GBC. Overexpression of c-ERBB-2 indicates a worse prognosis.³⁷ However, small case numbers limited these studies, and the results are difficult to interpret because of the variety of criteria used to determine protein overexpression.³⁸

Previous Studies on EGFR & HER2/neu in Gall bladder Cancer EGFR Studies Zhou YM et al. (2003) investigated the relationship between the expression of EGF and EGFR in chronic cholecystitis and gallbladder carcinoma. The authors concluded that expression of EGF and EGFR is involved in gallbladder carcinogenesis and is related to high activity of cell proliferation.³⁹ Kalekou et al. (2004) reported that the c-ERBB2 expression was found in 30.4% of tumours studied but did not relate to other parameters. High rates of EGFR alterations with overexpression of HER2 were present in about a third of all cases of GBC.⁴⁰ Moon WS et al. (2005) examined the expression and cellular localization/co-localization of EGFR and c-ERBB2 in gallbladder cancer. c-ERBB2 and EGFR were over-expressed in 62% and 11% of invasive gallbladder cancers, respectively.⁴¹

Nakazawa K et al. (2005) assessed the potential for novel chemotherapies targeting these receptors. Overexpression of the tyrosine kinase receptor proteins was examined by immunohistochemistry in 221 biliary tract carcinomas. Overexpression of EGFR was found in 8.1% of tumours with no predominant location and was also associated with gene amplification with high frequency (77%). It is proposed that the new adjuvant chemotherapies could be directed to carcinomas of the biliary tract in which ERBB-2 and EGFR are overexpressed.⁴²

Leone F et al. (2006) analyzed EGFR mutations and related pathways in the gallbladder and bile duct carcinomas to consider the possible application of these alternative therapeutic strategies. Findings suggested that a subgroup of patients with cholangiocarcinoma or gallbladder carcinoma exhibits somatic mutations of EGFR in the tyrosine kinase domain that can elicit cell signals sustaining survival and proliferation. These tumours might be further evaluated for their susceptibility to small-molecule inhibitor treatment.²⁰

Philip A et al. (2006) found that HER1/EGFR expression by immunohistochemistry in tumour cells was detected in 29 (81%) of the 36 assessable patients. The authors suggested a therapeutic benefit of EGFR blockade with erlotinib.⁴³

Kaufman M et al. (2008) in their study evaluated the expression of EGFR in a series of 16 gallbladder cancer patients from North America. It was observed that there was

overexpression of EGFR in 93.75% of the patient population. It stated the need to evaluate agents targeting this pathway as single agents or combined with standard chemotherapy.⁴⁴

Ooi A et al. (2009) examined gene amplification of Myc, ERBB2 and EGFR on 97 gallbladder carcinomas. FISH revealed three tumours had Myc amplification. Authors concluded that genomic instability due to Myc amplification might cause specific Amplification of EGFR and/or ERBB2.⁴⁵

Harder J et al. (2009) analyzed the pathogenetic role and potential clinical the usefulness of the EGFR and the HER2 in patients with advanced BTC. EGFR expression was absent in 22/56 tumours (39.3%). Of the remaining samples, expression was scored as 1+ in 12 (21.5%), 2+ in 13 (23.2%) and 3+ in 9 (16%), respectively. HER2 expression was as follows: score 0 73/124 (58.8%), score 1+ 27/124 (21.8%), score 2+ 21/124 (17%) and score 3+ 4/124 (3.2%). [38] Akishi O et al. (2009) reported overexpression and activation of EGFR in 30-60% of biliary tract carcinoma (BTC) samples, thus suggesting that altered expression of ERBB2 and EGFR are central mechanisms underlying human BTC carcinogenesis.⁴⁶ Shafizadeh N et al (2010) examined the EGFR and HER2/neu expression in 51 cases of biliary tract adenocarcinomas. EGFR overexpression by immunohistochemistry correlated significantly with epidermal growth factor receptor copy number by fluorescence in situ hybridization ($P = .02$). HER2/neu expression is uncommon in these tumours.⁴⁷ Chang PY et al. (2010) retrospectively analyzed the outcomes of 5 biliary tract cancer patients receiving cetuximab-containing therapy. Three surgical specimens were available, and all revealed positive EGFR expression.⁴⁸

Pignochino Y et al. (2010), in a study, demonstrated that EGFR was expressed in 38.5% of GBCs while HER2 is overexpressed in 10% of GBCs. EGFR and HER2 pathways are suitable therapeutic targets for BTCs, and drugs targeting these pathways have given encouraging results, and further clinical studies could be warranted.⁴⁹

Martins SJ et al. (2012) studied the prognostic role of EGFR expression and its association with clinicopathologic features in 42 GBC patients surgically treated at their

centers. It was stated that tumour immunoreactivity for EGFR in nearly half of the cases was independently associated with poor survival.⁵⁰

Li M et al. (2014) reported that in their study, genes with a significant frequency [false discovery rate (FDR)<0.05] of non-silent mutations include TP53 (47.1%), KRAS (7.8%) and ERBB3 (11.8%). Somatic mutations were found in EGFR (4%), HER2 (10%), ERBB3 (12%), and ERBB4 (4%). Multivariate analyses showed that cases with ERBB2 pathway mutations have a worse outcome (P=0.001). These findings provide insight into the somatic mutational landscape in GBC and highlight the key role of the ERBB2 signaling pathway in GBC pathogenesis.⁵¹

In the Indian context, Kumar N et al. (2016) studied the EGFR expression in GBC. Fifty cases of GBC were evaluated histopathologically. The authors concluded that EGFR is expressed in most cases of GBC. In well-differentiated adenocarcinoma, the EGFR expression is less than EGFR expression in poorly differentiated tumours, leading to the conclusion that the differentiation of the tumour and EGFR expression is inversely related. Thus, the intensity of EGFR expression may correlate with the aggressiveness of the disease.⁵²

In an Indian study, Handi R et al. (2016) reported that EGFR was positive in 72.41% overall, 77.78% positive in cases and 63.64% positive in control. The authors concluded that with further studies based on molecular understanding and developing new targeted therapies, we will be in a better position to manage GBC and increase the survival rate.²

Viswanath S et al. (2017) assessed the incidence of EGFR and VEGF in biliary tract malignancies in the Indian population correlated the same with histological features and clinical staging and treatment outcomes. The authors concluded that advanced biliary tract malignancies show increased expression of EGFR.⁵³

Neyaz A et al. (2018) investigated targetable predictive and prognostic markers in Thirty-five cases of metastatic gallbladder carcinoma. Authors concluded that predictive targetable markers like HER2/neu, VEGF and EGFR are expressed in a high proportion of gallbladder carcinoma. Significant expression of RAS pathway molecules suggests that interactions take place among the different members of the ERBB2 family during tumour development.⁵⁴

HER2/neu studies

Suzuki T et al. (1993) investigated 43 cases of gallbladder cancer for c-ERBB2-2 gene amplification and c-ERBB2-2 protein over-expression. The authors concluded that while the c-ERBB2-2 gene and protein in gallbladder cancers might be related to invasiveness, they are not applicable as predictive factors for prognosis.⁵⁵

Kamel D et al. (1993) studied 30 cases of GBC and showed over-expression of C- ERBB22 in 10% of patients using IHC. C-ERBB22 has varied between 10% and 46.5% in gallbladder carcinomas, and its expression was correlated with increasing stage. The authors concluded that c-ERBB2-2 gene alterations play a role in the neoplastic transformation of gallbladder epithelial cells. Co-expression of p53 and c-ERBB2-2 suggests that modifications of these genes might act in concert with the neoplastic transformation.⁵⁶

Chow NH et al. (1995) evaluated overexpression of the gene product found in 27.8% of intrahepatic CC, 27.8% of carcinoma of the ampulla of Vater, and 63.6% of gallbladder cancer.⁵⁷

Kim YW et al. (2001) evaluated the expression of c-erb-B2 and p53 proteins in relationship with various prognostic factors in 71 cases of gallbladder carcinomas, two instances of gallbladder dysplasia and 20 cases of gallbladder adenoma. The authors concluded that c-erb-B2 expression is strongly associated with neoplastic progression in gallbladder carcinomas and that c-erb-B2 expression identifies patients with a worse prognosis.⁵⁸

In an Indian study, Chaube et al. (2006) investigated the role of HER2/neu in the carcinogenesis of the gallbladder by IHC and found 25% of cases positive for HER2/neu expression. They have concluded that decreasing over-expression of c- ERBB2-2 with increasing grade suggests that c-ERBB2-2 will likely play a lesser role in tumour progression.³⁷

Puhalla et al. (2007) investigated the expression of p21, p57 and HER2/neu in an unselected GBC patient population. They assessed the association of these markers with p27(Kip1) expression, p53 gene mutation status and clinical parameters of the patients. Expression of HER2/neu was seen in 13% of patients. HER2/neu had no impact on the survival of the patients.⁵⁹

Kawamoto et al. (2007) examined tumour specimens from patients with gallbladder carcinoma for expression of HER2/neu. The authors concluded HER2/neu amplification was identified in over 20% of GB. There was a strong correlation between HER2/neu IHC and FISH positivity.⁶⁰

In an Indian study by Kumari N et al. (2012), 104 cases of GBC were retrospectively studied concerning clinical features, histological type, grade and stage of the tumour. C-ERBB2 overexpression was seen in 9.4% of patients with complete staining and both complete and incomplete staining (2+ and 3+) was seen in 13.4% cases. C-ERBB2 over-expression may be considered a target for therapy in an advanced stage of GBC.⁶¹

Toledo C et al. (2012) found that HER2 receptor protein was absent in adenomas and normal gallbladder epithelium. Protein up-regulation of HER2 in the epithelium with metaplasia or carcinoma in situ was not accompanied by HER2 gene amplification. It was concluded that there is upregulation of HER2/neu in precursor lesions of gallbladder carcinoma and suggested that these receptors may be necessary for the modulation of cell proliferation in gallbladder carcinoma.⁶²

Roa I et al. (2014) conducted a study to determine the frequency of HER2/neu overexpression and identified a subgroup of patients with gallbladder cancer who would benefit from targeted therapy. The authors concluded that the results of their study indicated that HER2/neu overexpression occurred in 14% of the advanced gallbladder cancer cases. This subgroup may benefit from inhibitors of the HER2/neu pathway.²⁷

Yan M et al. (2015) examined more than 37,000 tumour specimens across different histology for HER2 overexpression and found it to be present in 9.8% of 194 GBC specimens in the study. GBC was the fourth highest regarding frequencies of HER2 overexpression after bladder cancer, oesophageal and gastroesophageal junction cancers and breast cancer.⁶³

Ashai F et al. (2015) correlated HER2/neu positivity with the histopathological features in carcinoma gallbladder. It was found that there was a lot of heterogeneity in the expression of HER2/neu showing 1+ and 2+ positivity in different areas. The authors concluded that HER2/neu expression occurs in gallbladder cancer, especially in

advanced-stage disease, and its therapeutic targeting seems promising.⁶⁴

In the Indian context, Singh A et al. (2016), in a study on sixty resectable tumour specimens, observed that 36.67% (22/60) tumour cases showed strong HER2/neu overexpression. Multivariate analysis showed HER2/neu expression to be an independent prognostic factor and suggested the alleged role of HER2/neu expression in the worse prognosis of GBC in a north Indian population.⁶⁵

Yoshida et al. (2016) have also observed HER2/neu overexpression heterogeneity. There was no significant association between HER2 status and clinicopathological variables or survival.⁶⁶

Pujani M et al. (2016) reported that a trend of decreasing HER2/neu expression with increasing grade of tumour was observed. The authors concluded that future studies with more patients would be required to precisely define the correlation of HER2/neu expression with clinicopathological parameters.⁶⁷

I.Abeer et al. (2020) studied EGFR and HER-2 neu correlation in cases of GBC. They concluded that two markers, HER2/neu and EGFR, can be used as predictive and prognostic markers, respectively, with a rationale to explore further the use of anti-HER2 and anti-EGFR therapy in gall bladder cancer and warranted more extensive studies to narrow down the population that would benefit with the same.⁷⁰

Chhanda Das et al. (2021) concluded EGFR is greater in poorly differentiated carcinomas than in well-differentiated ones. On the contrary, HER-2/NEU expression is more in well-differentiated carcinomas. These two genes serve as independent prognostic factors and target therapy in GBCs.⁷¹

Lingli Chen et al. (2022) stated that the predictive value of HER2 varies by clinical stage, with no prediction in the early stages, better in stage III, and worse in stage IV.⁷²

Though, to date, there are various studies on the importance of biomarkers such as HER2/neu and EGFR for targeted therapy in Gallbladder Cancer, different parameters correlated with such markers need additional data to address the specified treatment profoundly. There are no extensive sample-size studies on the expression of these molecular markers in Gallbladder carcinoma, especially in an Indian

setting; and thus, our study acts as a stepping stone in the ladder to reach the molecular targeting of GBC.

Targeted Therapies in Gallbladder Cancer

Common mutations reported in gallbladder cancer are KRAS (10%67%), EGFR (63%), BRAF (0% to 33%), and ERBB2/HER2 (16%64%). Early data suggest possible benefits from the epidermal growth factor receptor blockade by the oral tyrosine kinase inhibitor erlotinib or anti-EGFR monoclonal antibody cetuximab. Vascular endothelial growth factor (VEGF) is overexpressed in biliary tract cancers and has been proposed as a therapeutic target.⁶⁹

CONCLUSION

Gallbladder cancer (GBC) is a highly malignant neoplasm characterized by delayed diagnosis, poor prognosis and ineffective treatment, especially in advanced stages. Despite the efforts of many researchers, GBC continues to represent a significant therapeutic challenge in oncology. Surgical resection remains the only curative treatment for this disease. The roles of radiation, chemoradiation, and chemotherapy in neoadjuvant and adjuvant settings remain poorly defined.

This systematic review took into account the significance of gallbladder cancer in North India, and only a few common and specific gene studies on gallbladder carcinoma are available in the literature from this high-propensity region.

To conclude, limited studies have been conducted in the Indian subcontinent on EGFR and Her2neu expression in gallbladder carcinoma. Thus, more studies can act as a stepping zone for further large-scale studies to find out EGFR and HER2/neu expression patterns in Indians with GBC to identify the existence of patients who could benefit from targeted therapy, particularly in advanced GBC.

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