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Review Article

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Immune-Markers in GallBladder Lesions and their Clinico-Diagnostic and Prognostic Significance - An Overview

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ABSTRACT

Gallbladder benign diseases are common and usually cured without further consequences. Some benign illnesses increase the chance of cancer development significantly, whereas others resemble malignant disorders. The biomarkers discussed thus far are among the most extensively researched concerning the different diseases of GBC. Patients with gallbladder cancer are usually diagnosed in later stages when conventional treatments are ineffective. The lack of responsiveness of advanced instances of GBC to existing therapies necessitates the identification of novel prognostic and therapeutic approaches. Novel prognostic biomarkers may provide a crucial breakthrough in this area. Despite the available data and years of research, a prognostic marker that is 100% specific and sensitive to GBC is not yet available. A diverse number of molecular markers have been studied for their potential to be prognostic markers in GBC. p53 and HER2 have been studied very extensively and have shown promise. The deregulation and accumulation of the molecular markers we have discussed so far impact carcinogenesis of the gall bladder. Further analytical studies on the concentration levels of these markers in normal vs precancerous vs cancerous tissues should be carried out. Highly specific prognostic markers can help individualize treatment options to bring down the mortality rate in GBC.

Key words: *Gallbladder, Gallbladder cancer, Immune-markers, Clinico-diagnostic significance, Prognostic significance*

INTRODUCTION

Gallbladder cancer (GBC) is the most prevalent malignant tumor of the biliary system worldwide, with broad regional variation [1-4]. Although the overall incidence is low, it is the most aggressive biliary tract cancer, having a short median survival from the time of diagnosis. The aggressive biologic nature of the carcinoma, as well as the lack of sensitive screening methods for early diagnosis, may be to blame for GBC's dismal prognosis. Owing to the delayed diagnosis at an advanced stage, only 10% of the patients are found to be suitable for surgical resection. The anatomically complicated Porto-biliary-hepatic system further increases the mortality and morbidity following surgical intervention. Moreover, the chances of tumor spread after tumor manipulation and increased risk of tumor recurrence adds to the disease load [5].

The pathogenesis of GBC is multifactorial and the risk factors include female gender, advanced age, obesity, genetic predisposition, and gallbladder abnormalities. Gallstone size is likely to be a risk factor as well, with individuals with stones bigger than 3 cm having a higher chance of developing cancer [5].

GBC aetiology is reported to take 5-15 years and includes phases of metaplasia, dysplasia, carcinoma in situ (CIN), and invasive malignancy. Anatomically, the fundus is most commonly affected, followed by the body and

neck. The submucosa is conspicuous by its absence in the gallbladder and this unique feature predisposes cancer to direct local invasion. Two pathways had been suggested for the development of GBC namely, the dysplasia-carcinoma sequence developing from metaplastic epithelium, and an adenoma-carcinoma sequence. GBCs exhibit epigenetics in the form of methylation patterns of tumor suppressor genes (p16, APC, MGMT, hMLH1, RARbeta2, and p73) [6].

Dysplastic changes of the gallbladder epithelium often precede invasive GBC. Gallstones and chronic cholecystitis, characterized by repeated attacks of acute cholecystitis are frequently seen to be associated with GBC. Xantho-granulomatous cholecystitis may be misdiagnosed as GBC both pre and intra-operatively, due to severe fibrosis and xantho-granulomatous appearance. Epithelial cancers are associated with several genetic alterations, including the activation of oncogenes and the inactivation of tumor suppressor genes. E-cadherin (CDH1) is a tumor suppressor gene found on chromosome 16q22.1, Performs a crucial role in the establishment and maintenance of Ca2+-dependent cell adhesion, polarity, and epithelial cell differentiation. It has been reported that there are notable differences in the expression of E-cadherin in normal, inflammatory, and malignant gall bladder tissues. Loss of P53 function leads to accelerated mutagenesis and genomic instability, thus forming the genetic basis of carcinogenesis [6].

Reduced expression of E-cadherin was often observed in advanced GBC, and its reduced expression correlated with decreased apoptosis [7]. Amongst the various high biomarkers being studied in GBC, the preneutrophil–lymphocyte ratio and carcinoembryonic antigen (CEA) are known to be independent prognostic factors for worse overall survival [8].

The depiction of the natural course of gallbladder illness will aid in the allocation of additional resources for the early treatment of symptomatic gallbladder disease in high-risk groups. Prophylactic cholecystectomy in individuals with asymptomatic gallstones in high-risk locations can further support the concept of secondary prevention of GBC. The recent increase in the number of laparoscopic cholecystectomies for the treatment of gallstone disease may be to blame for the increasing prevalence of GBC [9].

Considering the high incidence of GBC, it is imperative to identify reliable molecular prognostic markers which can aid in early diagnosis and improved prognosis [6]. Despite all developments, there is woeful improvement in the survival of GBC patients [10]. This review article shall serve as a basis for arming surgeons regarding better therapeutic possibilities owing to early detection of GBC and serve as a guide to pathologists to improve histopathological diagnosis of GBC, to prevent mortality due to late detection.

Review of literature

The incidence of cancer in developing countries is low compared to Western countries. However, factors like drastic changes in lifestyle patterns and economic status have led to the transition of cancers in these countries also over the past decade. Alcohol, consumption of a high-calorific diet, obesity, and physical inactivity are the main demographic and epidemiological factors responsible for the transition. Countrywide, the five common cancers are as follows:

In men

1. Lung
2. Oral cavity
3. Stomach
4. Colorectal and
5. Pharynx (excluding nasopharynx)

In women

1. Breast
2. Cervix uteri
3. Colorectal
4. Ovary and
5. Oral cavity

Gallbladder cancer is one of the leading cancer sites in various cancer registries in females. Changes in diagnostics, screening of cancers, and better access to care may also justify the increased disease burden in recent times.

Structural anatomy, morphology, function of gallbladder

Lately, there has been an increase in the frequency of laparoscopic cholecystectomies for the treatment of gallstone disease. The gallbladder is a thin-walled, distensible, pear-shaped sac located in the cystic fossa on the liver's visceral surface. The cystic fossa is located to the right of the liver's quadrate lobe. accountable for the rising prevalence of GBC [9]. It has a capacity of approximately 50ml. The peritoneum that covers the liver extends over the inferior surface of the gallbladder, whereas the superior surface of the gallbladder is in direct touch with the liver, and the intervening peritoneum is conspicuously absent. Occasionally, a peritoneal anomaly may predispose the gallbladder for torsion, wherein the entire gallbladder is covered with the peritoneum and is suspended by a mesentery.

The gallbladder's major role is to store bile and concentrate it by active reabsorption of water, sodium chloride, and bicarbonate. The gallbladder also secretes mucous which makes the bile viscous.

Histologically, the gallbladder and the sphincter of Oddi contain involuntary muscles. The remaining parts of the biliary tract have few muscle fibers in the structure. The gallbladder and biliary tract mucosa have a columnar epithelium lining and there are abundant mucus-secreting goblet cells in the gallbladder.

The cystic artery provides blood flow to the gallbladder. Additionally, the gallbladder receives blood supply from numerous arterial twigs that arise directly from the right hepatic artery from the gallbladder bed. The surgical importance of a thorough understanding of the Calot’s triangle, epiploic foramen, and structures in the hepatoduodenal ligament cannot be stressed enough. Various aberrations in the origin and course of the cystic artery are known e.g. origin from the left hepatic artery or the gastroduodenal artery which has to be carefully considered for surgical procedures on the gallbladder and biliary system outside the liver.

Lymphatics from the gallbladder flow into lymph nodes in the porta hepatis, also known as hilar nodes, as well as the cystic lymph node of Lund, which is located in Calot's triangle. The superior pancreaticoduodenal and retroduodenal lymph nodes are the other set of lymph nodes involved in the draining of the common bile duct. All of the lymph nodes in the gallbladder and extrahepatic biliary train eventually drain into the celiac group.

Several variations in anatomy have been observed in the embryological development of the gallbladder, such as a long cystic duct joining the existence of supplementary hepatic ducts; a short or nonexistent cystic duct; the cystic duct flowing into the left side of the common hepatic duct; and the presence of the common hepatic duct below the duodenum. Agenesis of the bile duct, multiple gallbladders, and bilobed gallbladder are some of the well-known developmental abnormalities of the gallbladder.

Non-neoplastic and neoplastic lesions of the gallbladder

Gall bladder polyps (GBPs) are the most common disease of the biliary system, which are benign lesions originating from the mucosa and the rate of conversion of polyps into cancer has been reported as 0-27%. The clinical significance of polyps is worth highlighting as often gallbladder cancer presents as polypoidal lesions, hence may lead to diagnostic confusion. The majority of polypoid lesions include triglycerides, cholesterol esters, and their precursors accumulated in the gallbladder's lamina propria.

In a pediatric population, GBP can be characterized as primary (adenoma, hyperplasia) or secondary (Petz-Jegher syndrome, leukodystrophy, pancreato-biliary malunion). Benign gallbladder polypoidal lesions are classified into two types: neoplastic (real polyp) and non-neoplastic, as shown in **Table 1**:

Table 1. Gallbladder polypoid lesions [11]

Non-neoplastic (Pseudotumor) – Benign	Pseudopolyps	• Cholesterol, cholesterolosis • Granulomatous • Inflammatory • Hamartomas
	Hyperplasia	• Adenomatous • Adenomyomas • Lymphoid
	Heterotopia	• Ectopic tissue • Gastric mucosa • Intestinal mucosa • Pancreas tissue • Liver

	Miscellaneous	• Granulomatous inflammations • Parasitic infections • Other
	Adenomas	• Adenoma • (Papillary) • Adenoma (Non-papillary)
Neoplastic (Tumor)	Mesenchymatous tumors	• Hemangioma • Lipoma • Leiomyoma • Fibroma • Neurofibroma • Granular cell tumor
Malign ant		• Adenocarcinoma • Melanoma • Clear cell carcinoma • Metastasis

(Reference: [11])

Neoplastic lesions are true polyps and include adenomas and mesenchymatous tumors. They are pre-neoplastic, and the malignant formation originates from the flat and dysplastic epithelium Cholesterol deposits and hyperplasia of inflammatory, granulomatous, ectopic, and heterotopic tissues are examples of non-neoplastic polyps or pseudopolyps. The most common clinically encountered polypoid lesions are cholesterol polyps and do not have neoplastic potential. The majority of polypoidal lesions do not cause clinical symptoms and are discovered by chance on ultrasonography.

The relationship between cholelithiasis and GBC is well known. The high risk of GBC in females may be attributed to exposure to estrogen and progesterone; and because cholesterol cytolysis is higher in men than women. Xanthogranulomatous cholecystitis (XGC) is a rare, purulent gallbladder disease that can invade the neighboring structures and can be associated with cancer.

The first description of GBC dates to about two centuries, however, attempts at early detection and a potential cure have been evaded ever since. A retrospective study conducted [10] concluded that GBC, due to its vague symptomatology is often difficult to diagnose early. The main presenting clinical features observed were abdominal pain and abdominal mass. The primary diagnostic tools in the study were ultrasonography (USG), which was further confirmed by fine-needle aspiration cytology (FNAC). The only available aids for early detection and thus improving overall survival (OS) is a high index of suspicion and health education.

The average 5-year survival rate for gallbladder cancer in the USA is reported to be 18%. The 5-year survival rate for Stage I cancer is 60%. However, it is worth noting that these statistics apply to just one out of every five instances that are diagnosed early on when the disease is confined to the gallbladder before metastasis. With regional lymph node metastasis, the survival rate drops to 25%, while those with distant metastases had a 5-year survival rate of less than 2%, indicating a very poor prognosis [7-9].

The pathogenesis of GBC is multifactorial, and no single cause can be delineated. The molecular pathogenesis of GBC includes biological pathways and genetic mutations. Two theories explaining the role of biological pathways have been described based on morphological, genetic, and molecular evidence [5]:

- Dysplasia-carcinoma sequence arising from metaplastic epithelium – Progression of dysplastic lesions towards carcinoma-in-situ (CIS) is evidenced by molecular and genetic mechanisms. It has been proved that the progression of gallbladder dysplasia progresses invasive cancer characteristically occurs over 15 to 19 years.
- Adenoma-carcinoma sequence – As opposed to the dysplasia-carcinoma sequence, Adenomatous leftovers are found in fewer than 3% of early carcinomas. Thus, because this mechanism has a limited role in the carcinogenic pathway, it is being investigated less carefully.

Although sufficient evidence back these theories, it is still impossible to determine which of these may develop into cancer.

Clinical experience and research have identified the following risk factors [5]:

- Demographic factors

- Old age, feminine gender, overweight and obesity, geographical distribution: South American, Indian, Pakistani, Japanese, and Korean; ethnicity: Caucasians, Southwestern Native American, Mexican, and American; hereditary predisposition.

- Gallbladder disorders
  - Porcelain gallbladder, cholelithiasis, gallbladder polyps, congenital biliary cysts, and pancreaticobiliary maljunction defects.

- Patient exposures
  - Heavy metals, drugs such as methyldopa, oral contraceptives, isoniazid, and oestrogen, as well as smoking.

- Infections
  - Salmonella and Helicobacter.

The following **Table 2** depicts the relative risk for various risk factors of gallbladder cancer.

Table 2. Risk factors for gallbladder cancer [5]

Risk Factor	Relative risk
1. Gall stones	
a. Size of gallstones (cm)	
2.0-2.9	2.4
> 3.0	9.2-10.1
b. Duration of gallstones (years)	
5-19	4.9
> 20	6.2
2. Body mass index	
30.0-34.9	1.8 2.1
3. Infections	
Chronic typhoid and paratyphoid carriers	12.7-167
Helicobacter bilis	2.6-6.5

(Reference: [5])

The presence, size, and the number of gallstones were explored to serve as red flags for patients with gallstone disease requiring surgical treatment. Females are 8.48 times more likely than males to develop gallstones if they have GBCA. A significant association was found between gender and cholelithiasis, analyzed using the Chi-square test. The majority of the patients had multiple gallstones. The stone size analysis revealed that numerous stones were generally 3 cm in diameter and single stones were 3 cm in size [5, 9-11].

Gallstone disease and GBC have several similar risk factors e.g. female gender, advanced age, fertility, and obesity. It has also been reported that long-standing gallstone disease is a significant risk factor for GBC development [5, 10].

The extent of the tumor and its histologic type dictate the prognosis of carcinoma of the gallbladder. The most important prognostic markers are histopathological characteristics such as tumor invasion depth and the existence of regional or distant metastases [12]. GBC might manifest as a bulk, localized wall thickening with wall induration, or polypoidal development. Quite often an hourglass deformity with obstruction of the neck along with the cystic duct may be observed. Cytopathologically, gallbladder cancers may be classified as follows [5].

- Adenocarcinoma papillary
- Adenocarcinoma mucinous
- Ring cell signet cancer
- Adenosquamous cancer
- Squamous cell cancer
- Neuroendocrine cancer
- Squamous cell carcinoma

- Undifferentiated cancer

Adenocarcinomas account for 98% of all gallbladder carcinomas, with two-thirds being moderately/poorly differentiated. Papillary, mucinous, squamous, and adenosquamous subtypes are also prevalent histological variations. all the other variants are rare. Tumors may contain more than one histological variant. Invasive papillary carcinomas have a less severe clinical history than traditional adenocarcinomas, as well as mucinous and adenosquamous carcinomas. Small cell gallbladder carcinoma has a poor prognosis, with 5- and 10-year rates of relative survival of 8% and 0%, respectively [12].

Several staging methods for gallbladder cancer have been reported, including Nevin's staging system **(Table 3)**, the Japanese Biliary Surgical Society staging system **(Table 4)**, and the American Joint Committee on Cancer TNM staging system **(Table 5)** [13-15].

Table 3. Nevins's staging [13, 14]

Stage	Definition
I	The tumor invades the mucosa
II	The tumor has invaded the mucosa and the muscularis.
III	The tumor invades the mucosa, muscularis, and subserosa.
IV	The tumor has infiltrated all three layers of the gallbladder and a cystic lymph node.
V	The tumor spreads into the liver bed or metastasizes

(Reference: [14])

Japanese Biliary Surgical Society staging method [15, 16] is shown in **Table 4**.

Table 4. Japanese Biliary Surgical Society staging method

Stage	I	II	III	IV
Invasion of the capsule	No capsular invasion (**S 0**)	Capsular invasion is suspected (S 1)	Capsular invasion is seen (S 2)	Direct invasion of neighboring viscera (S 3)
Invasion of the liver	No hepatic invasion (**Hinf 0**)	Hepatic invasion is suspected (Hinf 1)	Hepatic invasion surrounding the gallbladder is noticeable (Hinf 2)	Hepatic invasion is extensive (Hinf 3)
Invasion of the bile duct	No involvement of extrahepatic bile duct (**Binf 0**)	Bile duct involvement is suspected (Binf 1)	significant biliary involvement (Binf 2)	Bile duct involvement is extensive (Binf 3)
Metastases to lymph nodes	No lymph node metastasis (**N 0**)	Metastases to lymph nodes around extrahepatic bile duct (primary group, **N 1**)	Metastases in hepatoduodenal lymph nodes (secondary group, N 2) OR adjacent area (tertiary group, N 3)	Metastases are farther apart than in stage III (fourth group, N 4)
Liver metastasis	No liver metastases (**H 0**)	No liver metastases (**H 0**)	No liver metastases (**H 0**)	One lobe of the liver has metastases (H 1) OR Small liver metastases in both lobes (H 2) OR Multiple liver metastases in both lobes (H 2) (H 3)
Peritoneal dissemination	No peritoneal dissemination (**P 0**)	No peritoneal dissemination (**P 0**)	No peritoneal dissemination (**P 0**)	Peritoneal spread towards the tumor (P 1) OR A few peritoneal disseminations away from a tumor (P 2) OR Multiple peritoneal disseminations away from a tumor (P 3)

(Reference: [16])

Table 5. TNM staging [17, 18]

Stage	T-stage	N-stage	M-stage
0	Tis	N0	M0

I	T1	N0	M0
II	T2	N0	M0
IIIA	T3	N0	M0
IIIB	T1, T2, T3	N1	M0
IVA	T4	N0, N1	M0
IVB	Any T	Any N	M1

(Reference: [18])

Primary tumour (T)

T1 Tumour invades lamina propria; Tis Carcinoma in situ (a) or T2 Tumour invades perimuscular connective tissue (b); muscular layer; T3 tumor perforates the skin and/or invades the liver and/or other organs. (stomach, duodenum, colon, pancreas, and bile ducts extrahepatic); T4 tumor invades the major porta vein or hepatic artery, as well as many extrahepatic organs.

Regional lymph nodes (N)

N0 No metastasis to regional lymph nodes; N1 metastasis to nodes along the cystic duct, common bile duct, hepatic artery, and/or portal vein; N2 metastasis to nodes along the periaortic, pericaval, superior mesenteric artery, and/or celiac artery.

Distant metastasis (M)

M0 There is no distant metastasis; M1 There is distant metastasis.

Diagnosis of gallbladder cancer

Clinical presentation

The clinical presentation of GBC is varied and imprecise and may mimic symptoms of Biliary colic also known as chronic cholecystitis. Epigastric or right upper quadrant discomfort, jaundice, nausea and vomiting, weight loss, and anorexia are some of the common presenting. Typically, GBC may be suspected preoperatively based on the clinical presentation, it may be discovered by chance during cholecystectomy for a benign condition, or it may be detected incidentally during a normal cholecystectomy.

Diagnostic imaging

Ultrasound

USG is the first modality of diagnosis used for gallbladder diseases. The sensitivity and specificity of USG for diagnosis of advanced-stage GBC are reported to be approximately 85%, however, in the early stages, it may not be able to detect tumors especially if associated with cholelithiasis. High-resolution contrast-enhanced ultrasonography detects 70-90% of polypoidal growths of the gallbladder. GBC may appear as a mass invading the gallbladder, intraluminal growth, or gallbladder wall thickening with asymmetry. Apart from its diagnostic utility, ultrasonography also provides information for disease staging. Endoscopic ultrasonography (EUS) is the gold standard in the staging of gallbladder cancer. It provides accurate imaging for fine needle aspiration (FNA) biopsies. Recent advances include contrast-enhanced harmonic EUS (CEH-EUS).

CT scan

CT scan is an important imaging modality for the diagnosis, and staging of GBC and to evaluate the presence of Invasion of the liver, lymphadenopathy, and metastasis to other organs are all symptoms of porta hepatis. Multidetector row CT (MDCT) has been instrumental in the differentiation between malignant and benign gallbladder thickening. The appearance of GBC may present as luminal polypoidal growth, diffuse or localized gallbladder thickening, and mass replacing the gallbladder.

Endoscopic retrograde cholangiopancreatography (ERCP)

ERCP is reported to be a poor tool for the diagnosis of GBC. Although it is a good modality for delineating filling defects, polypoidal growths cannot be detected by ERCP.

MRI, MRA, and MRCP

Invasion of the vascular system, biliary tract, liver, and lymph nodes can be accurately diagnosed using a combination of imaging with magnetic resonance (MRI) with MRA (magnetic resonance angiography) and MRA

cholangiopancreatography (MRCP). The addition of diffusion-weighted imaging (DWI) provides greater sensitivity and aids in the differentiation of malignant from benign gallbladder disease.

FDG-PET scan

Fluorodeoxyglucose-PET Scan combines metabolic and anatomical localizations of gallbladder lesions requiring imaging investigation. This modality can be used both pre-operatively for determining operability and post-operatively for restaging the disease.

Percutaneous approaches

Fine needle percutaneous transhepatic percutaneous aspiration and transhepatic percutaneous cholecystostomy, although time-consuming, invasive, and poorly tolerated by patients, give an accurate diagnosis of gallbladder polyps. The diagnostic accuracy of image-guided fine needle aspiration (FNA), which may include ultrasound-guided or CT-guided biopsies, is 80-90%.

Cytopathological/ histopathological diagnosis

The cytopathological examination aids in the classification of gallbladder malignancies, such as papillary and mucinous adenocarcinoma, signet ring cell, adenosquamous, squamous, neuroendocrine, small cell, and undifferentiated carcinoma.

Gallbladder cancer typically may be diagnosed as a preoperatively predicted malignancy, discovered accidentally during cholecystectomy for benign illness, or cancer detected incidentally following regular cholecystectomy during the cytopathological examination. Patients diagnosed during surgery or postoperatively are over two-thirds of the total cases diagnosed. It is worth reiterating that in the past 85 years, the fact that symptomatic patients present with advanced disease have not changed. The only curative therapy for GBC is complete surgical tumor excision [5]. Much debate exists about the ideal surgical intervention for GBC, ranging from cholecystectomy to ultra-aggressive resections e.g. major hepatic resection and pancreaticoduodenectomy. In a study conducted by Fong *et al.* [19] for a comparison of patients presenting for definitive surgery for the first time with those presenting after prior non-curative intervention, Even with big tumors with substantial liver invasion, aggressive excision provides long-term survival. Long-term survival is also achievable for individuals who have had an earlier non-curative surgical investigation.

The only curative treatment for GBC is complete surgical resection of the gallbladder. However, this approach is not feasible as the majority of the cases are diagnosed late. The approach includes “radical cholecystectomy”, first proposed by Glenn and Hays in 1954, and “extended radical cholecystectomy”. Owing to the anatomical proximity of vital structures e.g. porta hepatica and cancer's proclivity to infect the liver and spread through the lymphatic system total gallbladder resection is often challenging. Surgical management varies according to the stage of the illness and might range from a basic cholecystectomy to a simple cholecystectomy with partial hepatectomy and lymph node dissection as needed.

Chemotherapy is advised in the following scenarios for the curative and palliative treatment of gallbladder cancer:

- as adjuvant treatment on its own or in conjunction with radiation (post-surgical resection)
- in patients with locally advanced unresectable cancer, either alone or in conjunction with radiation treatment,
- in advanced-stage metastatic disease

The incidence of incidental gallbladder cancer (iGBC) following laparoscopic cholecystectomy is reported to be 0.7%-2.1%. Although there is a unique opportunity for cure in such cases, a poor prognosis must be expected if biliary spillage occurs. It is worth mentioning that, the high rate of iGBC is evidence that the detection of iGBC preoperatively is indeed difficult. Warning signs such as irregular gallbladder wall thickening, non-visualization of the gallbladder, large polyps, and lymphadenopathy may serve as an alert for surgeons regarding iGBC.

Although there has been considerable understanding concerning the pathogenesis of cancer at the molecular level, the genetic know-how is still not fully tacit. Multiple mutations consisting of both passenger and driver mutations have been implicated in the pathogenesis of GBC. The clinical implementation of targeted therapies is thus dependent on these hotspot driver mutations [20]. The specific genetic variations involved in the development of gallbladder cancer are still not understood clearly. Some of the genetic abnormalities involved in the development of GBC are oncogene activation, tumor suppressor gene suppression, microsatellite instability, and methylation of gene promoter regions [5]. Major genes implicated in the pathogenesis are oncogenes, tumor suppressor genes, adhesion molecules and mucins, angiogenesis, and apoptosis regulator genes. Methylation patterns of the tumor

suppressor genes have been extensively studied and Methylation levels have been shown to build during the transition from chronic cholecystitis to metaplasia [21]. The significance of microsatellite instability (MSI) in gallbladder cancer carcinogenesis is unclear and poorly understood [22-25].

Biomarkers are protein or protein fragment molecules produced by malignant cells that may be easily identified in a patient's blood or urine but not in the blood or urine of a healthy individual and play an important role in the diagnosis and prognosis of GBC [26-28]. Biomarkers are important in clinical trials, novel medicines, and adjuvant therapy modalities in oncology, and they also offer a prognosis estimate: Biomarkers have the potential to be employed in mass screening to detect asymptomatic people in the early stages of cancer. The current tumor microenvironment (TME) model of cancer metastasis is based on this notion. TME exhibits survival signals as well as pro-angiogenic factors, both of which are required for tumor development and metastasis. Biomarkers mediate interactions between tumor cells and their microenvironment, resulting in breakthroughs in the early diagnosis or prevention of metastasis [29, 30].

Gallbladder cancer progresses through three stages: metaplasia of normal epithelium or less hyperplasia, dysplasia or intraepithelial neoplasia, carcinoma in situ, and invasive malignancy in around 15 years [31-34]. GBC patients have a significant death rate because they frequently emerge advanced due to underlying genetic changes that might go undetected for decades [34-36]. Prognostic biomarkers and new potential indicators might aid in identifying individuals who could benefit from additional therapy. Although it has been evident that certain tumor markers are largely overexpressed in gallbladder cancer and are predictors of poor survival, few studies are available on its clinicopathological correlation [37-41]. Studies conducted by researchers Roa, *et. al*., and Doval, *et. al.,* have reported that they did not any statistical correlation between Her2/neu, p53, Ki-67, cyclin D1 positivity, in gallbladder cancer, as well as clinicopathological markers such as tumor stage, grade, lymph node metastasis, or distant metastases. Nevertheless, a positive role of tumor markers **(Table 6)** has been demonstrated in early diagnosis, and development of targeted therapeutics, and adds to the armamentarium of management of GBC [41, 42].

A study was done to evaluate the significance of tumor markers in cancer of the gallbladder and it was discovered that the serum concentration of CA 19-9 rose with the progression of the stage, however, this was not seen A alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA). The study concluded that CA 19-9 was the most sensitive of the three tumor markers (CA 19-9 at 100%, AFP at 50%, and CEA at 72.22%) in the detection of gall bladder cancer. In another study done on tumor markers in GBC, Ca 19-9 sensitivity and specificity were reported to be 0.66 and 0.90, respectively. CEA, on the other hand, had values of 0.75 and 0.71 [31, 43-49].

Table 6. Biomarkers in Gall Bladder Carcinoma [50]

Gallbladder Carcinoma Biomarkers		
1. ABCG2	16. CEA	31. MUC 1
2. Annexin A3	17. COX 2	32. MUC 2
3. ALDH	18. Cyclin D1	33. NANOG OCT-4
4. ALCAM gene	19. E-Cadherin	34. P-53
5. CA19-9	20. EGFR	35. Prosaposin
6. CA15-3	21. Eph B1	36. RAS
7. CA-125	22. Ephrin B	37. RAF
8. CA-242	23. ERBB 3	38. RCAS 1
9. CD -4	24. ERBB 4	39. SOX
10. CD-24	25. GLUT 1	40. SOX-2
11. CD-34	26. GLUT 3	41. SPOCK1
12. CD-44	27. H1F1 Alpha	42. T-Cadherin
13. CD-133	28. HER-2	43. TNF alpha
14. CD-147	29. Ki-67	44. Transgelin
15. CD-166	30. MEKERK ½	45. VEGF

(References: [20])

CONCLUSION

Gallbladder benign diseases are common and usually cured without further consequences. However, certain benign illnesses have a high chance of developing cancer, while others mimic malignant disorders. The biomarkers examined thus far are among the most extensively researched concerning gallbladder disease.

Understanding their expression levels in benign gallbladder illnesses can help us comprehend their involvement in GBC. According to research, mutations in the genes encoding these indicators, as well as their altered expression, play distinct roles in GBC initiation, development, and progression. They can also be utilized to forecast disease prognosis and help in the differential diagnosis when there is a substantial variation in expression levels between benign and malignant tumors. Their responsibilities can be broadened further by forecasting the malignant potential of benign inflammatory disorders, leading to early intervention and a better prognosis for patients.

Gallbladder cancer patients are usually diagnosed in later stages when conventional treatments are ineffective, resulting in higher mortality rates. The lack of responsiveness of advanced instances of GBC to existing therapies necessitates the identification of novel prognostic and therapeutic approaches. Novel prognostic biomarkers may provide a crucial breakthrough in this area by assisting in the selection of patients who will benefit greatly from adjuvant and targeted therapy.

Despite the available data and years of research, a prognostic marker that is 100% specific and sensitive to GBC is not yet available. A diverse number of molecular markers have been studied for their potential to be prognostic markers in GBC. Of these p53 and HER2 have been studied very extensively and have shown promise. Though these can be used as prognostic markers in GBC, the current data available is insufficient for their efficient clinical use to demarcate GBC from other forms of GI cancers and benign conditions that mimic malignancy.

The deregulation and accumulation of the molecular markers we have discussed so far impact the carcinogenesis of the gall bladder significantly. Further analytical studies on the concentration levels of these markers in normal vs precancerous vs cancerous tissues should be carried out with standardized assays to achieve clinically applicable results. Multivariable analysis that includes geographical variations, genetic predisposition, gender, co-expression of oncogenes, etc., also needs to be explored in detail. Highly specific prognostic markers can help individualize treatment options and bring down the mortality rate in GBC.

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