Asian Journal of Current Research in Clinical Cancer
ISSN: 3062-4444
2023, Volume 3, Issue 1, Page No: 34-45

Available online at: www.galaxypub.co/page/journals

Galaxy Publication

A Broad Perspective on the Role of Immune-Markers in Gallbladder Lesions: Their Clinico-Diagnostic and Prognostic Significance

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Received: 11 September 2023; **Revised:** 27 November 2023; **Accepted:** 06 December 2023

ABSTRACT

Benign gallbladder disorders are frequently observed and generally resolve without long-term effects. However, some of these conditions are associated with an increased risk of gallbladder cancer, while others can lead to malignancy. This review aims to inform surgeons of the potential improvements in treatment strategies enabled by earlier recognition of GBC and to assist pathologists in refining histopathological assessments to reduce fatalities associated with delayed diagnoses. The immune markers identified so far are among the most widely explored in gallbladder cancer-related diseases. Diagnosis often occurs at advanced stages when standard treatments fail to deliver results. This limited response in late-stage gallbladder cancer highlights the need for improved prognostic and therapeutic strategies. Although progress has been made, there is still no prognostic marker with full specificity and sensitivity for gallbladder cancer. Various molecular markers have been investigated for this role, with p53 and HER2 showing significant potential. Alterations in these immune markers contribute to gallbladder carcinogenesis. Further comparative studies on their expression in normal, precancerous, and cancerous tissues are essential. Accurate prognostic markers may support tailored treatments and help reduce gallbladder cancer mortality.

Keywords: Prognostic significance, Gallbladder, Immune markers, Gallbladder cancer, Clinico-diagnostic significance

How to Cite This Article: Agarwal A, AlRawaili AM, AlZalbani MK, AlAnazi GK, AlAnazi SK, AlEnezi SAD. A Broad Perspective on the Role of Immune-Markers in Gallbladder Lesions: Their Clinico-Diagnostic and Prognostic Significance. Asian J Curr Res Clin Cancer. 2023;3(1):34-45. https://doi.org/10.51847/NRbZvHtdPn

Introduction

Gallbladder cancer (GBC) stands as the most frequently diagnosed malignant tumor within the biliary system globally, exhibiting substantial variation in prevalence across different regions [1–4]. While its overall incidence remains low, GBC is recognized as the most lethal form of biliary tract malignancy due to its rapid progression and short median survival following diagnosis. The poor outlook for affected individuals is primarily attributed to the aggressive tumor biology and the absence of effective screening tools capable of early detection. A vast majority of patients present with advanced disease, rendering only a small fraction, around 10%, eligible for surgical resection. The intricate anatomy of the hepato-biliary-portal system contributes significantly to surgical risks and unfavorable postoperative outcomes. In addition, manipulation of the tumor during surgery often leads to dissemination and increases recurrence risk [5].

The development of GBC is influenced by multiple contributing factors, including being female, advancing age, obesity, hereditary predisposition, and gallbladder-related abnormalities. Notably, larger gallstones, especially those exceeding 3 cm, are correlated with a heightened risk of malignancy [5]. GBC progression spans approximately 5 to 15 years and typically follows a sequence starting with metaplasia, progressing to dysplasia, carcinoma in situ, and eventually invasive cancer. The fundus is the most frequent site of origin, followed by the

body and neck of the gallbladder. A unique anatomical characteristic of the gallbladder—absence of a distinct submucosal layer—facilitates rapid local invasion of the tumor. Two primary mechanisms proposed for GBC development include the dysplasia-carcinoma pathway arising from metaplastic epithelium and the adenoma-carcinoma progression model. Epigenetic alterations have also been implicated in GBC, particularly DNA methylation of tumor suppressor genes like p16, APC, MGMT, hMLH1, RARbeta2, and p73 [6].

In many instances, dysplasia within the gallbladder epithelium precedes the onset of invasive carcinoma. Conditions such as gallstones and chronic cholecystitis, including recurrent acute inflammation, are often present alongside GBC. Xantho-granulomatous cholecystitis, characterized by marked fibrosis and a granulomatous texture, can mimic GBC during both preoperative evaluation and surgery. Genetic disruptions are fundamental in epithelial malignancies and commonly involve oncogene activation and tumor suppressor gene silencing. E-cadherin (CDH1), encoded on chromosome 16q22.1, is critical for maintaining calcium-dependent adhesion, epithelial structure, and polarity. Its expression varies markedly across normal, inflamed, and cancerous gallbladder tissues. Dysfunction of p53 contributes to genomic instability and enhanced mutation accumulation, laying the groundwork for malignancy [6].

Advanced-stage GBC frequently exhibits decreased E-cadherin expression, which is associated with reduced programmed cell death (apoptosis) [7]. Among emerging immune markers of prognostic relevance in GBC, the neutrophil–lymphocyte ratio and carcinoembryonic antigen (CEA) have shown potential as independent indicators of poorer survival outcomes [8].

Understanding the natural trajectory of gallbladder conditions may support better allocation of medical resources towards the early management of high-risk patients. In regions with elevated GBC prevalence, prophylactic cholecystectomy in individuals harboring asymptomatic gallstones could play a vital role in secondary prevention. Interestingly, the increased utilization of laparoscopic cholecystectomy for gallstone disease may inadvertently contribute to the rising incidence of GBC detection [9].

Given the aggressive nature and high burden of GBC, there is a pressing need for identifying robust molecular immune markers that can enhance early diagnosis and prognosis [6]. Despite considerable progress, outcomes for patients with GBC remain unsatisfactory [10]. This review aims to inform surgeons of potential advances in therapeutic strategies enabled by earlier recognition of GBC and to assist pathologists in refining histopathological evaluations to reduce fatalities linked to delayed diagnoses.

Results and Discussion

Review of literature

While the overall cancer prevalence remains comparatively lower in developing nations than in Western regions, recent years have witnessed a noticeable shift. This transformation has largely been driven by rapid lifestyle modifications and evolving socioeconomic conditions. Contributing elements such as alcohol intake, sedentary behavior, dietary habits rich in calories, and increasing obesity rates are recognized as significant demographic and epidemiological influencers behind this trend.

In terms of cancer distribution across genders, population-based data indicate the top malignancies in men include those of the lung, oral cavity, stomach, colorectal region, and pharynx (excluding the nasopharynx). Among women, the predominant cancers are breast, cervix uteri, colorectal, ovary, and oral cavity. Notably, gallbladder cancer ranks prominently in several national cancer registries among female patients.

The observed rise in cancer incidence may also be partially attributed to improved diagnostic capabilities, expanded screening programs, and enhanced accessibility to medical services, all of which contribute to greater detection and documentation of disease burden.

Structural anatomy, morphology, and function of the gallbladder

The surge in laparoscopic cholecystectomy procedures as a treatment for gallstone-related conditions has paralleled the rising incidence of gallbladder cancer [9]. The gallbladder itself is a pear-shaped, expandable sac with delicate walls, located within the cystic fossa on the visceral surface of the liver, adjacent to the quadrate lobe. It holds roughly 50 ml of bile. The organ is partially enveloped by the liver's peritoneum on its lower surface, while its upper surface is in direct contact with hepatic tissue due to the absence of intervening peritoneum. On occasion, the gallbladder may exhibit complete peritoneal coverage and be suspended by a mesentery, a variant which can predispose it to torsion.

Physiologically, the gallbladder plays a pivotal role in bile storage and its concentration, achieved through the active reabsorption of electrolytes such as sodium chloride and bicarbonate, along with water. It also secretes mucous, contributing to the bile's viscosity.

From a histological standpoint, smooth muscle fibers are prominent in both the gallbladder and the sphincter of Oddi, unlike the remainder of the biliary tract, which contains minimal musculature. The epithelial lining of the gallbladder and biliary pathways consists of columnar cells, with numerous mucus-secreting goblet cells present in the gallbladder.

The primary vascular supply arises from the cystic artery, complemented by several smaller branches stemming from the right hepatic artery, particularly from the gallbladder bed. Surgical interventions involving this region demand a clear understanding of anatomical landmarks such as Calot's triangle, the epiploic foramen, and the hepatoduodenal ligament. Notably, the cystic artery may have variable origins, including the left hepatic artery or the gastroduodenal artery, which is a critical consideration during hepatobiliary surgeries.

Lymphatic drainage from the gallbladder is directed toward hilar lymph nodes within the porta hepatis, including the cystic lymph node of Lund found within Calot's triangle. Drainage of the common bile duct involves the superior pancreaticoduodenal and retroduodenal nodes, with all these eventually emptying into the celiac lymphatic chain.

Embryogenesis of the gallbladder can give rise to diverse anatomical anomalies, such as an elongated cystic duct that merges with additional hepatic ducts, an absent or shortened cystic duct, or a cystic duct draining into the left side of the common hepatic duct. Other congenital variants include the presence of a common hepatic duct below the duodenum, gallbladder agenesis, bilobed gallbladders, and multiple gallbladders.

Non-neoplastic and neoplastic gallbladder lesions

Among the most frequently encountered biliary conditions are gallbladder polyps (GBPs), which are benign mucosal outgrowths. Though typically non-malignant, the transformation potential into carcinoma has been reported within a wide range, from 0% to 27%. The clinical importance of GBPs lies in their resemblance to polypoidal gallbladder cancer, often resulting in diagnostic dilemmas. These lesions predominantly comprise lipid-based substances such as triglycerides and cholesterol esters deposited within the lamina propria.

In pediatric populations, GBPs can be categorized as primary, such as adenomas or hyperplasia, or secondary, associated with syndromes like Petz-Jegher syndrome, leukodystrophy, or pancreato-biliary malunion. Broadly, benign gallbladder polyps are distinguished into neoplastic (true polyps) and non-neoplastic types, as summarized 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])

True polyps of neoplastic origin primarily consist of adenomas and mesenchymal tumors. These lesions are considered pre-malignant and evolve from dysplastic and flattened epithelial tissue. In contrast, pseudopolyps—or non-neoplastic polyps—comprise cholesterol accumulations and hyperplastic growths arising from inflammatory, granulomatous, heterotopic, or ectopic origins. Among the various types, cholesterol polyps are the most frequently diagnosed and are typically benign with no malignant potential. Most of these polypoid structures remain clinically silent and are commonly detected incidentally during abdominal ultrasonography.

Gallbladder cancer (GBC) has a well-established correlation with cholelithiasis. The increased incidence of GBC in females may be associated with the hormonal influences of estrogen and progesterone, while males are known to have greater susceptibility to cholesterol-induced cytolytic changes. Another rare condition, xanthogranulomatous cholecystitis (XGC), presents as a severe inflammatory disease of the gallbladder, sometimes mimicking or coexisting with malignancy due to its invasive characteristics.

Although GBC was first documented nearly 200 years ago, its early identification and effective treatment continue to present challenges. A retrospective analysis [10] highlighted the diagnostic difficulties associated with this malignancy due to its non-specific presentation. Common initial signs included abdominal mass and pain. Diagnostic confirmation primarily relied on ultrasonography (USG), often followed by fine-needle aspiration cytology (FNAC). Improving outcomes depends largely on early suspicion and proactive public health education to raise awareness.

In the United States, the overall five-year survival rate for gallbladder cancer stands at 18%. When diagnosed at stage I, survival can reach 60%. However, this statistic only applies to the minority of patients whose cancer is detected early while still localized within the gallbladder. Once the disease spreads to regional lymph nodes, survival drops to 25%, and for those with distant metastases, the five-year survival falls below 2%, indicating a starkly unfavorable prognosis [7-9].

Gallbladder cancer arises from a complex interplay of factors, with no single causative agent identified. The development of GBC involves multiple molecular and genetic mechanisms. Two predominant models explain its evolution based on histological and molecular studies [5]:

- Dysplasia-carcinoma sequence: This model suggests that cancer arises from metaplastic epithelial changes progressing through stages of dysplasia to carcinoma in situ (CIS), supported by a variety of molecular and genetic findings. It has been observed that the transition from dysplasia to invasive carcinoma often spans a duration of 15 to 19 years.
- Adenoma-carcinoma sequence: Unlike the previous model, this pathway is thought to play a limited role in GBC pathogenesis, as adenomatous precursors are detected in less than 3% of early-stage carcinomas. Consequently, it has received less focus in recent investigations.

While substantial evidence supports these theories, pinpointing which specific factors ultimately lead to cancer development remains challenging. Clinical observations and research have identified several contributing risk factors for gallbladder cancer [5], which include the following:

Demographic factors

Advanced age, female sex, overweight or obesity, and geographical factors such as populations in South America, India, Pakistan, Japan, and Korea. Ethnic groups at higher risk include Caucasians, Southwestern Native Americans, Mexicans, and Americans. There is also a hereditary component.

Gallbladder-related conditions

Disorders such as porcelain gallbladder, cholelithiasis (gallstones), gallbladder polyps, congenital biliary cysts, and defects in pancreaticobiliary maljunction.

Exposure factors

Environmental and lifestyle exposures like heavy metals, certain medications (e.g., methyldopa, oral contraceptives, isoniazid, estrogen), and smoking.

Infectious agents

Infections caused by *Salmonella* and *Helicobacter* species.

The relative risk associated with these various factors is summarized in **Table 2**.

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])

Studies have highlighted the role of gallstone presence, size, and quantity as critical indicators for patients with gallstone disease requiring surgical intervention. In cases of gallbladder cancer (GBCA), females are 8.48 times more likely than males to develop gallstones. Statistical analysis using the chi-square test revealed a significant link between gender and the occurrence of cholelithiasis. Most patients presented with multiple gallstones, and the analysis showed that most of these stones had a diameter of approximately 3 cm, whether singular or multiple [5, 9-11].

There is an overlap in risk factors for both gallstone disease and gallbladder cancer, including factors such as female gender, fertility, age, and obesity. Long-standing gallstone disease, in particular, is a significant contributor to the development of GBC [5, 10].

The prognosis for gallbladder carcinoma largely depends on the tumor's extent and histological type. The most important prognostic indicators are the degree of tumor invasion and whether there is regional or distant spread [12]. GBC may present as either a bulkier mass, localized wall thickening with associated induration, or as a polypoid growth. Additionally, an hourglass-shaped deformity with obstruction at the cystic duct is commonly seen. Gallbladder cancers are classified cytopathologically into the following types [5]:

- Papillary adenocarcinoma
- Mucinous adenocarcinoma
- Signet ring cell carcinoma
- Adenosquamous carcinoma
- Squamous cell carcinoma
- Neuroendocrine carcinoma
- Undifferentiated carcinoma

Adenocarcinomas account for about 98% of gallbladder cancers, with the majority being moderately to poorly differentiated. Histological variants, including papillary, squamous, mucinous, and adenosquamous types, are relatively common, while other forms are rare. Some tumors may exhibit multiple histological types. Invasive papillary carcinoma is associated with a less aggressive clinical course compared to the more common adenocarcinoma, mucinous, and adenosquamous types. Small cell gallbladder carcinoma has an extremely poor prognosis, with 5- and 10-year survival rates of 8% and 0%, respectively [12].

Several staging systems are used to assess gallbladder cancer, including Nevin's staging **(Table 3)**, the Japanese Biliary Surgical Society staging **(Table 4)**, and the TNM system by the American Joint Committee on Cancer **(Table 5)** [13-15].

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

Stage	Definition
I	The cancer is confined to the mucosal layer.
II	The tumor extends into both the mucosal and muscular layers.
III	The cancer affects the mucosa, muscular layer, and subserosal layer.
IV	The tumor invades all three layers of the gallbladder wall and may affect a cystic lymph node.
V	The cancer spreads to the liver bed or metastasizes to distant sites.

(Reference: [14])

The staging system developed by the Japanese Biliary Surgical Society is presented in **Table 4** [15, 16]

Table 4. Japanese biliary surgical society staging method

Stage	I	II	III	IV
Capsular invasion	No capsular invasion (S 0)	Suspected capsular invasion (S 1)	Visible capsular invasion (S 2)	Direct invasion of surrounding organs (S 3)
Liver invasion	No hepatic invasion (Hinf 0)	Suspected hepatic invasion (Hinf 1)	Noticeable hepatic invasion around the gallbladder (Hinf 2)	Extensive hepatic invasion (Hinf 3)
Bile duct invasion	No extrahepatic bile duct involvement (Binf 0)	Suspected bile duct involvement (Binf 1)	Significant bile duct involvement (Binf 2)	Extensive bile duct involvement (Binf 3)
Lymph node metastasis	No lymph node metastasis (N 0)	Metastasis in lymph nodes near the extrahepatic bile duct (N 1)	Metastasis in hepatoduodenal nodes (N 2) or adjacent areas (N 3)	Distant lymph node metastasis (N 4)
Liver metastasis	No liver metastasis (H 0)	No liver metastasis (H 0)	No liver metastasis (H 0)	One liver lobe metastasis (H 1) or small metastases in both lobes (H 2) or multiple liver metastases (H 3)
Peritoneal dissemination	No peritoneal spread (P 0)	No peritoneal spread (P 0)	No peritoneal spread (P 0)	Peritoneal dissemination near the tumor (P 1) or distant peritoneal spread (P 2 or 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 tumor (T)

T1 refers to tumors that invade the lamina propria, while Tis indicates carcinoma in situ (a). T2 denotes tumors that extend into the perimuscular connective tissue and muscular layer (b). T3 describes tumors that perforate the gallbladder wall and may spread to adjacent structures such as the liver or other organs like the stomach, duodenum, pancreas, or extrahepatic bile ducts. T4 involves tumors that invade major structures like the porta hepatis or hepatic artery and multiple extrahepatic organs.

Regional lymph nodes (N)

N0 signifies the absence of lymph node metastasis. N1 shows metastasis to nodes around the cystic duct, common bile duct, hepatic artery, and/or portal vein. N2 involves metastasis to lymph nodes along the periaortic, pericaval, superior mesenteric artery, and/or celiac artery. N3 refers to further spread to distant nodes.

Distant metastasis (M)

M0 indicates no distant metastasis, while M1 suggests the presence of distant metastasis.

Diagnosis of gallbladder cancer

Clinical symptoms

The symptoms of gallbladder cancer (GBC) are often vague and resemble those of biliary colic or chronic cholecystitis. Common signs include discomfort in the upper abdomen or right upper quadrant, jaundice, nausea, vomiting, weight loss, and lack of appetite. GBC can be suspected before surgery based on these symptoms or may be incidentally discovered during a cholecystectomy for a benign condition.

Imaging techniques for diagnosis

Ultrasound

Ultrasound (USG) is typically the first diagnostic tool for gallbladder issues. Its sensitivity and specificity for advanced GBC are approximately 85%. However, it may struggle to detect tumors in the early stages, particularly if they are accompanied by cholelithiasis. High-resolution contrast-enhanced ultrasonography can detect 70-90% of polypoidal growths. GBC on ultrasound may appear as a mass invading the gallbladder, intraluminal growth, or thickening of the gallbladder wall with asymmetry. Besides diagnosing, ultrasound aids in staging the disease. Endoscopic ultrasonography (EUS) is considered the gold standard for staging, offering precise imaging for fine needle aspiration (FNA) biopsies. Recent advancements include contrast-enhanced harmonic EUS (CEH-EUS).

CT scan

CT scans are vital for diagnosing and staging GBC and assessing liver invasion, lymphadenopathy, and metastasis to distant organs, particularly those around the porta hepatis. Multidetector row CT (MDCT) is valuable for distinguishing between malignant and benign gallbladder thickening. GBC may appear as a polypoid growth, diffuse or localized gallbladder thickening, or as a mass replacing the gallbladder.

Endoscopic retrograde cholangiopancreatography (ERCP)

ERCP is not very effective for diagnosing GBC. While it is useful for identifying filling defects, it cannot detect polypoidal growths.

MRI, MRA, and MRCP

MRI combined with MRA (magnetic resonance angiography) and MRCP (magnetic resonance cholangiopancreatography) is excellent for detecting vascular, biliary tract, liver, and lymph node involvement. The addition of diffusion-weighted imaging (DWI) increases sensitivity and helps differentiate malignant gallbladder conditions from benign ones.

FDG-PET scan

Fluorodeoxyglucose-PET (FDG-PET) scan combines both metabolic and anatomical imaging of gallbladder lesions. It can be helpful both preoperatively to assess the possibility of surgery and postoperatively to stage the disease.

Percutaneous methods

Fine needle aspiration (FNA) through percutaneous transhepatic approaches, such as ultrasound-guided or CT-guided biopsies, offers accurate diagnoses of gallbladder polyps. While these procedures are time-consuming, invasive, and not always well-tolerated by patients, their diagnostic accuracy is 80-90%.

Cytopathological and histopathological diagnosis

Cytopathological testing is essential for the classification of gallbladder cancers, which may include various forms such as papillary adenocarcinoma, mucinous adenocarcinoma, signet ring cell carcinoma, adenosquamous carcinoma, squamous cell carcinoma, neuroendocrine tumors, small cell carcinoma, and undifferentiated carcinoma. Gallbladder cancer (GBC) may be diagnosed either as a preoperatively suspected malignancy, detected incidentally during cholecystectomy for benign conditions, or unexpectedly during post-surgical cytopathological review. A large portion of GBC diagnoses, more than two-thirds, occurs during or after surgery. It is crucial to note that the advanced stage of disease in symptomatic patients has remained unchanged over the past 85 years. Surgical excision of the gallbladder tumor is currently the only curative treatment for GBC [5]. Opinions remain divided regarding the best surgical approach for GBC, which ranges from standard cholecystectomy to more radical procedures such as extensive hepatic resection and pancreaticoduodenectomy. In a comparative study by Fong *et al*. [19], patients who underwent aggressive surgery for large tumors with significant liver invasion demonstrated long-term survival. Even those who had received non-curative surgical interventions in the past had a possibility of long-term survival.

The only curative treatment for GBC is the complete removal of the gallbladder. However, this is often not possible due to late-stage diagnosis. Treatment options include “radical cholecystectomy,” initially introduced by Glenn and Hays in 1954, as well as “extended radical cholecystectomy.” Due to the gallbladder’s anatomical location near important structures like the porta hepatis, and its tendency to spread to the liver and lymphatic system, total resection can be difficult. Depending on the stage of the cancer, surgical treatments can range from a basic cholecystectomy to a more extensive procedure that includes partial hepatectomy and lymph node removal. Chemotherapy is employed in various circumstances for both curative and palliative treatment of gallbladder cancer, including:

- As an adjuvant therapy, either alone or combined with radiation, following surgical resection.
- For patients with locally advanced, non-resectable cancers, either as a standalone treatment or with radiation.
- For patients with metastatic disease in the advanced stage.

The rate of incidental gallbladder cancer (iGBC) following laparoscopic cholecystectomy has been found to range from 0.7% to 2.1%. Despite the possibility of curing iGBC, the prognosis is poor if biliary spillage occurs during the surgery. This high occurrence highlights the challenges in detecting iGBC preoperatively. Indicators like irregular thickening of the gallbladder wall, inability to visualize the gallbladder, large polyps, and lymphadenopathy can help surgeons suspect iGBC.

While there has been significant progress in understanding the molecular mechanisms behind gallbladder cancer, the genetic aspects remain incompletely understood. Several mutations, including both passenger and driver mutations, are involved in GBC development, and targeted therapies are dependent on these mutations [20]. However, the exact genetic alterations responsible for the disease are not fully clarified. Genetic changes in GBC may include oncogene activation, tumor suppressor gene suppression, microsatellite instability, and methylation of gene promoter regions [5]. Key genes implicated in the development of GBC include oncogenes, tumor suppressor genes, adhesion molecules, mucins, and genes involved in angiogenesis and apoptosis regulation. Studies have shown that methylation of tumor suppressor genes increases as the disease progresses from chronic cholecystitis to metaplasia [21]. The role of microsatellite instability (MSI) in GBC remains poorly defined and is an area of ongoing investigation [22-25].

Biomarkers, which are molecules produced by cancer cells, can be detected in the blood or urine of cancer patients but are absent in healthy individuals. These biomarkers are important for the diagnosis and prognosis of GBC [26-28]. They also play a critical role in clinical trials, novel drug development, and adjuvant therapy approaches. Biomarkers hold potential for use in screening programs to detect asymptomatic individuals at an early stage of cancer. The current model of cancer metastasis involving the tumor microenvironment (TME) suggests that the TME supports tumor growth by providing survival signals and pro-angiogenic factors. Biomarkers mediate the

interactions between cancer cells and their microenvironment, leading to advancements in early diagnosis and metastasis prevention [29, 30].

Gallbladder cancer progresses over around 15 years, starting with metaplasia or mild hyperplasia, followed by dysplasia or intraepithelial neoplasia, carcinoma in situ, and finally invasive malignancy [31-34]. GBC has a high mortality rate due to its tendency to present at advanced stages, often linked to undetected genetic changes over extended periods [34-36]. Identifying prognostic biomarkers and potential diagnostic indicators may help identify patients who could benefit from additional treatment options. While several tumor markers are overexpressed in GBC and have been associated with poor survival outcomes, few studies have explored the clinicopathological relationships [37-41]. For example, studies by Tan *et al*. [41] and Doval *et al*. [42] found no statistical correlation between the expression of Her2/neu, p53, Ki-67, and cyclin D1 and factors like tumor stage, grade, lymph node involvement, or distant metastases in GBC. However, tumor markers remain important for early diagnosis, targeted therapy development, and treatment planning for GBC [41, 42].

In a study on tumor markers in gallbladder cancer, researchers observed that serum levels of CA 19-9 increased as the cancer progressed, whereas AFP and CEA did not exhibit similar trends. CA 19-9 was found to be the most sensitive marker for GBC, with a sensitivity of 100%, compared to 50% for AFP and 72.22% for CEA. Another study found the sensitivity and specificity of CA 19-9 to be 0.66 and 0.90, respectively, while CEA had values of 0.75 and 0.71 [31, 43-49].

Biomarkers in gallbladder carcinoma are presented in **Table 6**.

Table 6. Biomarkers in gallbladder 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

Benign gallbladder diseases are relatively common and are typically resolved without long-term complications. However, certain benign conditions are prone to evolving into cancer, while others may present symptoms that resemble malignant diseases. The biomarkers that have been studied extensively about gallbladder disease offer significant insight. Investigating their expression in benign gallbladder conditions is crucial for understanding their role in the onset of gallbladder cancer (GBC). Studies indicate that mutations in the genes responsible for these biomarkers, along with alterations in their expression patterns, are central to the initiation, progression, and development of GBC. These markers also hold promise in predicting the disease's prognosis and aiding in the differential diagnosis, especially when there is a notable discrepancy in expression between benign and malignant growths. Moreover, these biomarkers could serve a broader purpose in forecasting the malignant potential of benign inflammatory conditions, thus facilitating early diagnosis and improved patient outcomes.

GBC is often diagnosed at advanced stages, where traditional treatments fail, leading to higher mortality. The lack of response in advanced GBC cases highlights the need for the development of new prognostic and therapeutic strategies. Emerging prognostic biomarkers could provide an essential breakthrough by helping identify patients most likely to benefit from targeted therapies and adjuvant treatment options.

Despite extensive research, a definitive prognostic marker with 100% sensitivity and specificity for GBC has yet to be discovered. Numerous molecular markers have been evaluated for their potential as prognostic indicators in GBC, with p53 and HER2 being particularly well-studied and promising. However, while these markers show potential for prognostic use in GBC, the available data are still insufficient for their reliable clinical application, especially in distinguishing GBC from other gastrointestinal cancers and benign conditions that share similar clinical features.

The deregulation and accumulation of the molecular markers discussed above play a significant role in gallbladder carcinogenesis. Future research should focus on conducting comprehensive studies to examine the concentration of these markers in normal, precancerous, and cancerous tissues, using standardized assays to generate clinically relevant results. Additionally, exploring factors such as geographical differences, genetic predisposition, gender, and the co-expression of oncogenes in multivariable analyses should be prioritized. Identifying highly specific prognostic markers could greatly enhance individualized treatment approaches and reduce GBC-related mortality.

Acknowledgments: We extend our sincere gratitude to Prof. Anshoo Agarwal, Professor of Pathology at the Faculty of Medicine, Northern Border University, Saudi Arabia, for his unwavering support, guidance, and encouragement throughout the completion of this work.

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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