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Expressive levels of imuunomarkers and their clinicopathologic significance in the benign and malignant lesions of gallbladder.

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Abstract:

Background:

Worldwide gall bladder cancer (GBC) is known to be the commonest malignant tumour of the biliary tract .It is the most aggressive carcinoma of the biliary tract with short median survival from the time of diagnosis. The aggressive biologic behavior of the carcinoma and non-availability of sensitive screening tests for early detection may be responsible for the poor prognosis associated with GBC. Owing to the delayed diagnosis at an advanced stage, only few of the patients are found to be eligible for a curative surgical resection.

Material and Methods:

All patients diagnosed with neoplastic and non-neoplastic gallbladder lesions in the Department of Pathology, Subharti Medical College were included in the study between the year 2017 -2019. The Hematoxylin and Eosin stained biopsies of 320 patients were assessed and out of them 100 patients were chosen as the sample for the study. The clinicopatholgical data of the 100 patients were compiled into a data base and de-identified.

Results:

In adenocarcinoma - biliary type, there was positive EGFR expression seen in 64.71% of cases compared to negative expression seen in 35.29% cases, the difference was not statistically significant ($p = 0.18$). Adenocarcinoma - papillary ype showed positive expression. Adenocarcinoma –intestinal type showed negative EGFR expression, but again, without statistical significance ($p = 0.24$).

Conclusions:

The minimal response of advanced cases of GBC to traditional treatments calls for new prognostic and treatment perspectives to be identified. Novel prognostic biomarkers could bring about the needed breakthrough in this regard as they will help

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in the identification of patients who will benefit tremendously from adjuvant and targeted therapies.

Key words: Cyclin D1, E-cadherin, EGFR, HER- 2, Ki67, p53 tumor marker ,neoplastic ,non -neoplastic Gall bladder lesions

Introduction

The most prevalent biliary tract cancer with greatest geographic disparity is gallbladder cancer (GBC), which is known to occur worldwide[1]. Gallbladder (GB) is a pear-shaped sac with thin and distensible walls. Superior side of the GB and its surrounding peritoneum is apparent by its absence. Fundus, body, and neck make up gallbladder, which opens into the cystic duct[2]. The fundamental purpose of the GB is to concentrate bile by storing it and by actively reabsorbing sodium chloride, bicarbonate, and water. In certain Indian states, GBC is reportedly the 4th most prevalent cancer, including Uttar Pradesh[1]. It is the most deadly cancer with only a short median survival after diagnosis.

Gallbladder carcinoma, which is epithelial in origin, makes for 98% of all gallbladder malignancies[1]. Adenocarcinomas account for about 90% of cases[2,3]. Microscopic features of adenocarcinomas include glands bordered by columnar or cuboidal cells that may include mucus. These malignant tumors can be classified as well differentiated, moderately differentiated and poorly differentiated carcinoma's. . Common histologic variants of gallbladder cancer that are frequently seen include-biliary-type adenocarcinoma, adenocarcinoma not otherwise specified, (NOS), intestinal-type adenocarcinoma, mucinous adenocarcinoma & poorly cohesive carcinoma; all other subtypes are uncommon. There may be multiple histological variants in tumors[3] .The poor prognosis associated with GBC could be explained by destructive nature of the cancer and the absence of early detection methods. The mortality and morbidity after surgical intervention are further increased by the porto-biliary-hepatic system's anatomical complexity. The possibility of the chance of cancer recurrence adds to the disease burden [2].

Gender being female, elderly age, body mass index, genetic predisposition, and anomalies of the GB which has a complex pathophysiology e.g. cholelithiasis, porcelain gallbladder, cysts, polyps, anomalies of maljunction of pancreatobiliary tract, exposure to heavy metals, bacterial infectivity causing chronic bacterial cholangitis , larger size of gallstones have a much higher risk of getting GBC [2].

The gallbladder is notable for lacking submucosa, and as a result, the cancer is more likely to directly invade the local area. Two possible pathways for the formation of GBC can be determined by morphological, genetic, and molecular evidence: an adenoma-cancer sequence and a dysplasia-cancer sequence resulting from the epithelium that has undergone metaplasia. An evidence that epigenetics has been linked to tumor suppressor genes in GBC is through Kras, and TP53's methylation patterns [3]. In advanced GBC, expression of E-cadherin was often downregulated (61%), and this downregulation was correlated with a lower apoptotic rate [4]. Tumor

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marker expressions have been established to be other predictive indicators for overall poor survival (OS) in GBC [5].

Due to the high prevalence of GBC in northern and central India, it is of the utmost importance to find reliable molecular prognostic indicators that can help with early diagnosis and better prognosis [3]. The effectiveness of various drugs for targeted therapy against proteins including EGFR, HER2, and N-cadherin that have detectable mutations is being studied. Even with these advancements, the GBC patients' survival rate is only barely improving [6-7].

According to several cancer registries in India, among female cancers, GBC has high prevalence [8]. The increased disease burden in recent years may however be justified by advancements in cancer screening, diagnostics, and treatment accessibility.

According to an analysis of the stone sizes leading to the risk of developing GBC, stones larger than 3 cm in size were tfound to be associated with GBC [8]. Regardless of the size and quantity of the stone(s), females in their sixth decade who present with gallstones should be given precedence for surgical treatment, which includes cholecystectomy as they may has a higher risk of developing GBC[2, 8].

According to reports, the incidence of incidental gallbladder cancer (iGBC), where GBC is discovered on postoperative microscopic examination of gallbladder specimen without any pre- or intraoperative symptoms indicative of cancer, is between 0.7% and 2.1% after cholecystectomy [9-10]. Large polyps, lymphadenopathy, non-visualization of the gallbladder, uneven gallbladder wall thickening, and other warning symptoms may serve as a reminder to surgeons about iGBC [12-13]. In addition to age, it is known that the presence of GB wall thickening and an elevated alkaline phosphatase level are useful indicators of GBC's [10, 11].

In response to the increasing prevalence of GBC in north India, the current study was initiated because of scarcity of publications about relevant immunomarkers in GBC. The results of this research are intended to be the basis for educating surgeons about improved therapeutic options due to GBC early identification and as an instructional guide for pathologists to further improve GBC histological diagnosis and minimize mortality from GBC late detection.

In terms of cancer prevalence rates worldwide, India now ranks third after China and the United States[8]. 27 cancer registries in India reported a total of 1.4 million cancer cases for the year 2015, and it has been predicted that this figure will increase to 1.74 million cases for the year 2020 [8]. It is also important to note that there is a minimal incidence of ethnic and cultural differences between Indians and Westerners [6-9]. GBC is frequently challenging to diagnose early due to its ambiguous symptomatology [7].

The prognosis for GBC depends on the histologic type of the tumor. The most important prognostic indicators are histopathological variables like grading, staging, involvement of lymph nodes and local and distant metastases [9]. GBC commonly presents as a mass, polypoidal growth, or localized wall thickening with induration. Histological variants of gallbladder carcinoma that are frequently seen are biliary, adenocarcinoma not otherwise specified, intestinal and mucinous adenocarcinoma; all other subtypes are uncommon[9]. There is still much to learn about the precise genetic abnormalities that contribute to the growth of gallbladder cancer. Major

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oncogenes associated with the etio-pathogenesis are KRAS, EGFR, HER-2/neu and tumor suppressor genes (TP53, & P16)[10-11]. It has been reported that methylation pattern changes throughout the progression from beginning of inflammation in gallbladder till the development of GBC[12]. These hotspot driver mutations are therefore necessary for the clinical use of targeted treatments [13,14].

There aren't many studies on the association between clinicopathology and particular immunomarkers, despite the fact that it is clear that they are frequently expressed in GBC and can be indicators of bad prognosis. According to previous studies, immuno marker expression have been shown to play a beneficial role in early diagnosis, tailored treatments for managing GBC [12]. Importance of immunomarkers in GBC studied by us had been reported previously in few studies [13].

Cyclin D1: It has been shown that cyclin D1 overexpression shortens the G1 phase of the cell cycle, which encourages the malignant transformation of the cell [14].

E-cathedrin: A tumor suppressor gene called E-cadherin (CDH1) controls the adhesion, polarity, and differentiation of epithelial cells. Between healthy, inflamed, and malignant gallbladder tissues, there are claimed to be significant changes in E-cadherin expression[15]. It has been noted that the expression of E-cadherin varies significantly between healthy, inflammatory, and malignant gallbladder tissues; together with the disappearance of adhesion processes, may be accountable for the development of cancer in GBC [16]. It was found that a decrease in E-cadherin expression was linked with cancer progression[17].

EGFR & Her-2/neu: Transmembrane receptor epidermal growth factor receptor (EGFR) plays a role in the prevention of apoptosis and the development of cancer cells , the activation of metastasis, and the stimulation of tumor-induced neovascularization through autophosphorylation [18-22]

p53: Immunohistochemistry can be used to identify these mutations [23]. P53 expression can serve as an indicator of disease progression to incurable invasive disease [24]. In GBC patients with p53 overexpression, the 5-year survival rate was 17.2[25].

Ki-67: In a study it was concluded that expression of Ki-67 had a worse postoperative prognosis[26].

Combining various immune markers together for diagnostic purpose led to the highest levels of sensitivity and specificity. It is studied that the immnomarkers indicators have lower diagnostic precision when used alone, but when combined, they had very high percentage of sensitivity and specificity [27].

Aims & Objectives

- To evaluate and compare the tumor (immuno) marker expressions of Epidermal growth factor receptor (EGFR), Human epidermal growth factor receptor-2 (HER- 2), Kiel 67 (Ki67), E-cadherin, Cyclin D1, and p53 in neoplastic and non-neoplastic gallbladder lesions.
- To correlate these panel of tumor (immuno) markers expression with clinicopathological findings.

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Materials and methods

Our study was done at the Subharti Medical College and the affiliated Chhatrapati Shivaji Hospital in Meerut. A prospective study of three years duration was conducted in the Department of Pathology. Cholecystectomy specimens received during this period in the Department of Pathology were included in study.

In this study, a total of 100 cases were studied to analyze the significance of different tumor (immuno) markers in neoplastic and non-neoplastic lesions of the Gallbladder.

Relevant clinical details of patients undergoing cholecystectomy were recorded from requisition form as well as from patients' records.

Clinicopathological findings of patients were recorded from the medical records and paraffin-embedded blocks of Gallbladder specimen available in the Pathology department of Subharti Medical College were retrieved for immunomarker study. The clinicopathological data of the 100 patients were compiled into a database from the records available in the Department of Pathology, Subharti Medical College and Hospital, Swami Vivekanand Subharti University, Meerut (U.P.).

Results

The present study comprised of total hundred cases of neoplastic and non-neoplastic lesions of the gallbladder as shown in Table 1 and Association of HPE's variable with Neoplastic and Non-neoplastic Gallbladder lesions are shown in Table 2.

Table1. Association of demographic characteristics with Neoplastic and Non-neoplastic Gallbladder lesions.

Demographic characteristics	Neoplastic Gallbladder lesions (n=50) n (%)	Non-neoplastic Gallbladder lesions (n=50) n (%)	Total (n=100) n (%)	P value
Age(years)				
<20	0 (0)	2 (4)	2 (2)	0.495*
20 to 30	2 (4)	4 (8)	6 (6)	0.678*
31 to 40	1 (2)	6 (12)	7 (7)	0.112*
41 to 50	10 (20)	5 (10)	15 (15)	0.161†
51 to 60	20 (40)	11 (22)	31 (31)	0.052†
61 to 70	13 (26)	16 (32)	29 (29)	0.509†
>70	4 (8)	6 (12)	10 (10)	0.741*
Gender				
Female	32 (64)	25 (50)	57 (57)	0.157†
Male	18 (36)	25 (50)	43 (43)	

Fisher's exact test, † Chi square test

Majority of patients with neoplastic gallbladder lesions belonged to age group 51 to 60 years (40%) followed by 61 to 70 years (26%). Very few patients were in the age group of

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<40 years and >70 years. On the other hand, majority of patients with non-neoplastic Gallbladder lesions belonged to age group 61 to 70 years (32%) followed by 51 to 60 years (22%) of age group. Though number of neoplastic gallbladder lesions were found to higher in females (64%) were higher as compared to non-neoplastic Gallbladder lesions (50%) but the difference was not statistically significant. No significant difference was seen in the distribution of age and gender with neoplastic and Non-neoplastic gallbladder lesions. (P value>0.05) .

Table 2. Association of HPE's variable with Neoplastic and Non-neoplastic Gallbladder lesions (n=50 in each group).

HPE variable	Neoplastic Gall bladder lesions(n=50) n (%)	Non-neoplastic Gallbladder lesions(n=50) n(%)	Total (n=100) n (%)	P value
Gallbladder wall thickness				
>3 mm	44 (88)	3 (6)	47 (47)	<.0001*
<3 mm	6 (12)	47 (94)	53 (53)	
Associated stones				
Stones present	29 (58)	19 (38)	48 (48)	0.045†
Stones absent	21 (42)	31 (62)	52 (52)	
Neoplastic Gallbladder lesions				
Histopathology diagnosis	**Neoplastic Gall bladder lesions(n=50) n (%)**	**Non-neoplastic Gallbladder lesions(n=50) n(%)**	**Total n (%)**	**P value**
Adenocarcinoma - Biliary type	17 (34)		17 (17)	
Adenocarcinoma-NOS (Not otherwise specified)	17 (34)		17 (17)	
Poorly cohesive carcinoma	8 (16)		8 (8)	
Mucinous adenocarcinoma	5 (10)		5 (5)	
Adenocarcinoma - Intestinal type	1 (2)		1 (1)	
Biliary intraepithelial neoplasia	2 (4)		2 (2)	
Non-neoplastic Gallbladder lesions				
Chronic cholecystitis		7 (14)	7 (7)	
Acute on chronic cholecystitis(Empyema)		10 (20)	10 (10)	
Chronic cholecystitis with cholesterolosis		8 (16)	8 (8)	
Hypereosinophilic cholecystitis		3 (6)	3 (3)	

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Xanthogranulomatous cholecystitis		3 (6)	3 (3)	
Hyalinising cholecystitis (Porcelain gallbladder)		3 (6)	3 (3)	
Adenomatous hyperplasia		10 (20)	10 (10)	
Pyloric metaplasia		4 (8)	4 (4)	
Intestinal metaplasia		2 (4)	2 (2)	

*** Fisher's exact test, † Chi square test**

Compared to non-neoplastic Gallbladder lesions, in neoplastic Gallbladder lesions the Gallbladder wall thickness was >3 mm in 88% cases (p value<.0001)) and association of Gallbladder lesions with stones were seen in 58% cases (p value=0.009) respectively. Significant association was seen between **Gallbladder wall thickness and presence of stones with** Gallbladder lesions. Among **Neoplastic Gallbladder lesions,** most of the patients (34%) had adenocarcinoma - biliary type and adenocarcinoma-NOS (Not otherwise specified) each followed by poorly cohesive carcinoma (16%), Mucinous adenocarcinoma (10%), Biliary intraepithelial neoplasia (4%). Only 1 case (2%) had adenocarcinoma - Intestinal type.

Among **Non-neoplastic Gallbladder lesions**, 20% of the patients had acute on chronic cholecystitis (Empyema) and 20% of them had adenomatous hyperplasia followed by cases of chronic cholecystitis with cholesterolosis (16%), chronic cholecystitis (14%),pyloric metaplasia (8%). hypereosinophilic cholecystitis, xanthogranulomatous cholecystitis. Hyalinising cholecystitis (Porcelain gallbladder) were observed in 6% patients each and 2 patients had intestinal metaplasia .

Immunohistochemical expression of immunomarkers in Neoplastic and Non-neoplastic gallbladder lesions – 1.p53 expression i.Neoplastic lesions of gallbladder: Predominantly, positive p53 expression was observed in adenocarcinomas - biliary type, mucinous adenocarcinoma, adenocarcinoma-NOS (papillary), poorly cohesive carcinoma, and biliary intraepithelial neoplasia. Adenocarcinoma -intestinal type showed negative p53 expression. While these findings suggest potential roles for p53 in the pathogenesis of these lesions, statistical significance was lacking. (p value>0.05) ii.Non-neoplastic lesions of gall bladder:: Adenomatous hyperplasia showed a significant positive p53 expression (P = 0.0007). Pyloric metaplasia also showed statistical significance positive p53 expression (P = 0.008). Other non-neoplastic gallbladder lesions showed no statistically significant differences in p53 expression and had a predominant negative expression except for Intestinal metaplasia that has 100% positive expression of p53. **2. Ki67 expression** i.Neoplastic lesions of gallbladder: Adenocarcinoma - biliary type showed significant difference in Ki67 expression, with positive expression in 41.18% cases compared to negative expression seen in 58.82% (P = 0.001). Adenocarcinoma - intestinal type showed negative Ki67 expression, although the difference was not statistically significant (P = 0.3). Mucinous adenocarcinoma, adenocarcinoma-NOS (papillary), and poorly cohesive carcinoma exhibited predominantly positive Ki67 expression, albeit without statistical significance (P > 0.05). Biliary intraepithelial neoplasia showed predominantly negative Ki67 expression, again without statistical significance (P > 0.05). ii. Non-neoplastic lesions of gallbladder:- Chronic

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cholecystitis, acute on chronic cholecystitis (empyema), chronic cholecystitis with cholesterolosis, hypereosinophilic cholecystitis, xanthogranulomatous cholecystitis, hyalinising cholecystitis (porcelain gallbladder), predominantly showed negative Ki-67 expression. Adenomatous hyperplasia showed a relatively higher percentage of positive Ki-67 expression compared to other lesions, albeit without statistical significance. Intestinal metaplasia, however, showed negative Ki-67 expression.

3. EGFR expression i.Neoplastic lesion of gall bladder:- In adenocarcinoma - biliary type, there was positive EGFR expression seen in 64.71% of cases compared to negative expression seen in 35.29% cases, the difference was not statistically significant ($p = 0.18$). Adenocarcinoma - papillary ype showed positive expression. Adenocarcinoma –intestinal type showed negative EGFR expression, but again, without statistical significance ($p = 0.24$). ii. Non-neoplastic lesion of gall bladder:- EGFR expression varied, with no statistically significant differences observed ($P > 0.05$) except for adenomatous hyperplasia cases. Notably, chronic cholecystitis, acute on chronic cholecystitis (empyema), chronic cholecystitis with cholesterolosis, hypereosinophilic cholecystitis, xanthogranulomatous cholecystitis, and hyalinising cholecystitis (porcelain gallbladder) predominantly showed negative EGFR expression. Adenomatous hyperplasia had significantly higher positive expression compared to other non-neoplastic lesion ($p=0.01$) and pyloric metaplasia showed higher negative EGFR expression. Similarly, intestinal metaplasia showed just negative EGFR expression. **4. HER2/neu expression** i.Neoplastic lesions of gallbladder: Significant differences in HER2/neu expression were observed in adenocarcinoma - biliary type which showed predominantly negative HER2/neu expression (88.24%).Adenocarcinoma-NOS(papillary type) showed a significant predominance of positive HER2/neu expression (70.59%) compared to negative expression (29.41%) ($p = 0.037$). Furthermore, poorly cohesive carcinoma also exhibited a significant predominance of positive HER2/neu expression (100%) ($p = 0.004$). ii.Non-neoplastic lesions of gallbladder:- Chronic cholecystitis, acute on chronic cholecystitis, chronic cholecystitis with cholesterolosis, xanthogranulomatous cholecystitis, hyalinising cholecystitis , adenomatous hyperplasia, pyloric metaplasia, and intestinal metaplasia predominantly showed negative HER2/neu expression. Hypereosinophilic cholecystitis showed positive HER2/neu expression in 66.67%, though the difference was not statistically significant ($P = 0.08$). 5. **Cyclin D1 expression** i. Neoplastic lesions of gallbladder:- Adenocarcinoma - biliary type , adenocarcinoma-NOS(papillary type) and poorly cohesive carcinoma showed positive Cyclin D1 expression . Mucinous adenocarcinoma demonstrated positive Cyclin D1 expression, yet again without statistical significance. Biliary intraepithelial neoplasia showed positive Cyclin D1 expression, without statistical significance. Adenocarcinoma -intestinal type displayed exclusively negative Cyclin D1 expression, although without statistical significance.ii.Non-neoplastic lesions of gallbladder:- Pyloric metaplasia showed notably higher positive Cyclin D1 expression (75%) compared to negative expression (25%), with a significant difference ($p = 0.016$). Similarly, intestinal metaplasia showed positive Cyclin D1 expression, with a significant difference compared to negative expression ($p = 0.029$).

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Other non-neoplastic lesions, including chronic cholecystitis, acute on chronic cholecystitis , chronic cholecystitis with cholesterolosis, hypereosinophilic cholecystitis, xanthogranulomatous cholecystitis, hyalinising cholecystitis , and adenomatous hyperplasia, did not show statistically significant differences in Cyclin D1 expression.**6. E-cadherin expression** i. Neoplastic lesions of gallbladder:- Statistically significant differences in E-cadherin negative (loss) of expression were observed in adenocarcinoma - biliary type, adenocarcinoma NOS -papillary type and poorly cohesive carcinoma ($P < 0.05$). Poorly cohesive carcinoma showed negative (loss) of E-cadherin expression, with a significant difference compared to positive expression ($p = 0.043$). Other neoplastic lesions of gallbladder, including adenocarcinoma - intestinal type, mucinous adenocarcinoma and biliary intraepithelial neoplasia, did not exhibit statistically significant differences in E-cadherin expression. ii.Non-neoplastic lesions of gallbladder:- Adenomatous hyperplasia and metaplasia showed positive E-cadherin expression though was not statistically significant. Majority of non-neoplastic lesions, including chronic cholecystitis, acute on chronic cholecystitis, hypereosinophilic cholecystitis, xanthogranulomatous cholecystitis, hyalinising cholecystitis did not exhibit statistically significant differences in E-cadherin expression ($P > 0.05$).

Discussions

Overall, gallbladder cancer is the most prevalent bile tract cancer and one of the most aggressive tumors with a dismal prognosis [28.] With age, the incidence rises and at the time of diagnosis, 90% or more of the patients are 50 years or older[3-5]. Similar to earlier studies, majority of patients with neoplastic gallbladder lesions in the present study belonged to age group 51 to 60 years (40%) followed by 61 to 70 years (26%) of age group. Majority of patients in our study with non-neoplastic gallbladder lesions belonged to age group 61 to 70 years (32%).Though number of neoplastic gallbladder lesions were found to higher in females (64%) which is in concordance with previous study [3] as compared to non-neoplastic gallbladder lesions (50%) but the difference was not statistically significant.

Grossly, gallbladder cancer may appear as a polypoidal (30%) or diffusely growing (70%) mass. Usually, it tends to be an invading grey-white mass that may be accompanied by widespread thickening and induration of the gallbladder wall [29-30.] In our study significant association was seen between gallbladder wall thickness and presence of stones with gallbladder lesions.In 60% of cases, gallbladder cancer is found in the fundus, 30% in the gallbladder's body, and 10% in the neck[10.] Our study showed findings similar to earlier studies with gallbladder lesions site distribution with fundus being the most common site in neoplastic gallbladder cancer cases.

According to reports, patients with associated gallstones had a four to seven time's higher risk of developing GBC cancer [29.] In the present study significant association was seen between cases having stones in gallbladder and its association with neoplastic gallbladder lesions which was in concordance with a previous study[6].

The majority of gallbladder disorders are benign, and they can either be symptomatic or asymptomatic. Frequently, they may have gallstones, polyps and cholecystitis [30.]In

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our study, most of the patients (34%) had adenocarcinoma - biliary type and adenocarcinoma-NOS (not otherwise specified) type followed by poorly cohesive carcinoma (16%), mucinous adenocarcinoma (10%), biliary intraepithelial neoplasia (4%). Only 1 case (2%), had adenocarcinoma - intestinal type. The majority of the tumors (80%) had a moderate degree of differentiation, with well-differentiated and poorly-differentiated adenocarcinma accounting for 4% and 16% of cases, respectively. Among non-neoplastic lesions in our study, 20% of the patients had acute on chronic cholecystitis and 20% of them had adenomatous, hyperplasia followed by cases of chronic cholecystitis with cholesterolosis (16%), chronic cholecystitis (14%), pyloric metaplasia (8%). hypereosinophilic cholecystitis, xanthogranulomatous cholecystitis. Hyalinising cholecystitis were observed in 6% patients each and 2 patients had intestinal metaplasia. Sometimes in gallbladder lesions, cellular features almost entirely can get obscured by necrosis and can give misleadingly false-positive findings of GBC. Confusion may result from some pathologic characteristics that are shared by both benign and malignant lesions, such as necrosis or extracellular mucus. An aggressive neoplastic process can be misdiagnosed as acute cholecystitis with parietal necrosis.

With a diverse profile of protein expression in GBC suggests it as a key center for this lethal malignancy[28]. There are still many challenges in making a precise diagnosis of gallbladder cancer, and immunomarkers are being tested for disease-specificity. In the present study P53 expression in Hyperplasia (adenomatous) and Intestinal and pyloric metaplasia revealed a statistically significant association. Notably, 87.50% of these lesions showed positive P53 expression. Cyclin D1 expression also was found to be significantly associated with 43.75% of cases showing positive expression. Biliary intraepithelial neoplasia (BilIN) cases had positive expression of p53, Cyclin D1, E-Cadherin and negative expression of Ki67, Her2/Neu in the present study

Accurate and prompt diagnosis is essential, followed by alternatives for targeted therapy to increase the effectiveness of treatment for GBC. It has become crucial to treat GBC by detecting particular prognostic and diagnostic immunomarkers for tailored therapy. Recent advancements in our understanding of gallbladder carcinogenesis have created opportunities for the creation of effective immunological markers that may be used to track the evolution of disease and provide patients with more individualized and focused care.In this study we have tried to explore the role of some immunomarkers which may be useful in early diagnosis of GBC lesions and detecting these cases at an early stage. Our study results might be able to comprehend the carcinogenesis process better by comparing the differences in their expression levels under normal, benign, and malignant lesions as well as during disease progression via metastasis. This knowledge is crucial for creating individualized treatment plans that may work better. Our study results also presents a comprehensive analysis of expression of p53, Ki67, EGFR, HER-2/neu, Cyclin D1, E-cadherin immunomarkers in context of neoplastic gallbladder lesions.

We can better comprehend their function in GBC if we are aware of how they are expressed in benign gallbladder conditions. According to studies, each of these markers' altered expression and gene alterations has a unique function in the onset,

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development, and progression of GBC [31].They can also be used to predict the course of the disease and assist in differential diagnosis when there is noticeable distinction in the expression levels between benign and malignant lesions. They may play a bigger part in anticipating the potential for malignancy in benign inflammatory diseases, which could lead to early management and improved patient outcomes.They can play a larger role in identifying the potential for malignancy in benign inflammatory disorders, leading to early treatment and better patient outcomes.

Reliable and timely diagnosis is required, followed by alternatives for focused therapy, to increase treatment effectiveness. The identification of specific prognostic indicators and viable candidates for specific therapy has become important in the treatment of GBC. An effective early detection and staging system can be established by understanding the molecular and genetic variables that cause benign inflammatory diseases to turn into carcinomas[32.] .Finding of useful and unique immunomarker panel for GBC [33-34] can transform the diagnostic and therapeutic approach, as how the GBC can be detected and handled in the future. Role of immunomarkers and its association with GBC have undergone substantial research to differentiate GBC from other forms of cancers and benign conditions that may mimic cancer[35]

Conclusions

Gallbladder carcinoma is a fatal cancer with few therapeutic options. Finding GBC-specific prognostic signals might impact the way clinical practice is now carried out, may open avenues for more effective prognostic and patient-centered therapy approaches in situations when traditional therapeutic alternatives may not be possible. It will be possible to develop a better prognostic approach to GBC and improve patient survival times by studying the association of expression of various immuno markers with GBC .

In the present study positive expression of all the immuno markers were found to be more in neoplastic lesions of gallbladder as compared to non-neoplastic lesions of gallbladder for all the immunomarkers. This study reveals that in terms of overall diagnostic accuracy, EGFR scores stood out with a value of 80.00%, emphasizing its potential as a reliable immunomarker for neoplastic gallbladder lesions.

Patients with BiLIN, adenomatous hyperplasia and metaplasia had significantly higher positive p53 expression. These insights are vital for informed decision-making regarding the selection and utilization of immunomarkers for diagnostic purposes in in the precusros lesions of gallbladder for detecting such cases at early stages.

GBC patients typically experience increased mortality rates as a result of delayed diagnosis and ineffectiveness of standard therapies. The limited response of advanced GBC cases to conventional treatments necessitates the identification of new prognostic and therapeutic options. A breakthrough in this area may be possible because to novel prognostic immunomarkers, may allow for an assessment of patients who are going to benefit most from targeted and adjuvant therapy. Immnomarkers that may be specific and sensitive to GBC had not yet been developed, despite the years of study. Our study results may give useful insights about panel of immunomarkers that may have great prognostic and diagnostic usefulness in GBC cases.

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