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DIAGNOSTIC ROLE OF IMMUNOMARKER EXPRESSIONS IN GALLBLADDER CARCINOMA AND PREMALIGNANT LESIONS

Anshoo Agarwal[1], Rani Bansal[2], Kavita Chauhan[3*], Mamta Gupta[4]

[1,3*]Department of Pathology, Faculty of Medicine, Swami Vivekananda Subharti University, Meerut, India
[2]Department of Pathology, Faculty of Medicine, Government Doon Medical College, Dehradun, India
[4]Department of Pathology, Faculty of Medicine, Northern Border University, Arar; Saudi Arabia

***Corresponding Author:** Kavita Chauhan
*E-mail address – AAKC2024@outlook.com, Phone numbers-00917906510312

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 10% of the patients are found to be eligible for a curative surgical resection.
Material and Methods: All consecutive 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 clinicopathological data of the 100 patients were compiled into a data base and de-identified.
Results: Age distribution of Gall Bladder lesion cases in our study was from 30 years to more than 60 years of age. 46.20% of females in the age group of 45 years to 60 years presented with mass in the gall bladder. Overall, the concordance rates between the immunomarkers was relatively high, ranging from 47% to 88% except between E-cadherin and other immunomarkers. This suggests that the immunomarkers are generally related to each other. The high concordance rate was found between p53 and Ki67, p53 and EGFR, p53 and Cyclin D1, Ki67 and EGFR, Ki67 and Cyclin D1 , EGFR and Cyclin D1., EGFR, HER2/neu and Cyclin D1.
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 in the identification of patients who will benefit tremendously from adjuvant and targeted therapies.

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

Introduction

There are a number of lesions that can affect the gallbladder, ranging from inflammatory to preneoplastic to malignant tumors. Persistent inflammation of the gallbladder is known to cause

preneoplastic abnormalities, which in turn can result in malignant tumors[1] .GBC is linked to an extremely dismal prognosis; therefore, early diagnosis is extremely important to improve the prognosis of patients[2]. A malignant tumor has spread by the time the majority of patients show up, which makes it difficult or impossible to surgically remove the malignant growth completely with surgery[3].

The prevalence of cancer in India has changed due to variables such as abrupt changes in lifestyle and economic conditions[2,4]. The main demographic and epidemiological factors contributing to the change include alcohol use, eating a high-calorie diet, obesity, and inactivity[3].In terms of cancer prevalence rates worldwide, India now ranks third after China and the United States[5]. 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[6-8].

The most prevalent lesions affecting the Gallbladder are Gallbladder Polyps (GBPs), which are benign lesions that begin in the mucosa and have a 0–27% chance of developing into cancer[9]. GBC frequently manifests as polypoidal lesions, which can cause diagnostic uncertainty[10]. GBC is frequently challenging to diagnose early due to its ambiguous symptomatology[7,11]. Cytological investigations like Fine Needle Aspiration Cytology (FNAC) were used in addition to ultrasonography (USG) as the study's main diagnostic tool and were regarded as one of the primary tools available for early detection enhancing overall survival (OS) of patients [12]. The prognosis for GBC depends on the size and histologic type of the tumor. The most important prognostic indicators are histopathological variables like involvement of lymph nodes and local and distant metastases [10-]. GBC commonly presents as a mass or localized wall thickening with induration. Quite frequently, an obstruction of the neck and cystic duct associated with an hourglass malformation of gallbladder may be seen [9,13]. GBC can be classified cytopathological into many other subtypes, such as biliary type adenocarcinoma, papillary adenocarcinoma, intestinal type, mucinous adenocarcinoma, and poorly cohesive carcinoma etc [9,15].75% types of Gallbladder Carcinoma's are biliary type adenocarcinoma[10.16]. Additional histological variants that are frequently seen are papillary adenocarcinoma and mucinous adenocarcinoma; all other subtypes are uncommon [9]. Several staging systems have been described for gallbladder cancer, including the Pathologic Staging (pTNM) of the American Joint Committee on Cancer for GBC [17- 19.] .

Although there has been much progress in understanding the pathogenesis of GBC, molecular concepts are still not fully understood [20-23]. The pathophysiology of GBC has been linked to several mutations that include both passenger and driving variants[24].These hotspot driver mutations are therefore necessary for the clinical use of targeted treatments[25] .There is still much to learn about the precise genetic abnormalities that contribute to the growth of gallbladder cancer[26-27]. Major oncogenes associated with the etiopathogenesis are KRAS, EGFR, HER2/neu, and tumor suppressor genes (TP53, P16, Fragile histidine triad, retinoblastoma) [28,29] .

A key component in the diagnosis and prognosis of GBC is the use of immunomarkers, which are substances made of proteins or components of proteins formed by cancer cells that are readily detectable in the serum, blood, body fluid, or urine of a patient but not in healthy people[24,30] . Clinical trials, novel medicines, and adjuvant oncology treatment methods all rely heavily on immunomarkers, which can additionally provide a prognosis estimate[24,26]. Widespread screening can employ immunomarkers to find asymptomatic patients with cancer in its early stages[13,29].

Many molecular factors linked to GBC pathogenesis have been postulated as prospective immunomarkers that may be helpful in the categorisation of GBC patients in addition to the widely recognized clinical prognostic immunomarkers[30]. Consequently, a poorer prognosis has been associated with overexpression of EGFR, mutations in KRAS, overexpression of p53, and amplification of HER2/neu, among other possible molecular immunomarkers[31]. Immunohistochemical analysis has been used to assess a number of possible immunomarkers in formalin-fixed paraffin-embedded (FFPE) cancer biopsy sections[32]. The 5-year survival rate for individuals with metastases to distant organs is reported to be 2%, indicating a very bad prognosis[33]. Patients whose gallbladder cancer has invaded beyond the muscle layer have a worse prognosis, especially if the cancer is at stage IIIB or higher[34]. In this context, it is generally accepted that

residual disease is a poor prognostic indicator for incidental GBC[35]. Numerous immunomarkers that have been found and suggested to be novel possibilities with prognostic significance have not been used commonly in clinical practice[49].

There are currently no particular specific or reliable immunomarkers available for gallbladder cancer early detection[34] .It has been proposed that combining the use of immunomarker studies and radiographic investigations may help in the early detection of this cancer [33,34].

In response to the increasing prevalence of GBC in north India and a scarcity of publications on relevant immunomarkers study in GBC, the current study was undertaken. The results of this research may form a basis for educating surgeons about improved therapeutic options available due to GBC early identification and also as an instructional guide for pathologists to further improve GBC histopathological diagnosis which may help minimize mortality from GBC late detection.

Aims & Objectives

- To correlate 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 with clinicopathological findings.
- To evaluate diagnostic usefulness of these panel of tumor (immuno) markers expression in neoplastic and non-neoplastic gallbladder lesions.

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 .All cholecystectomy specimens received in the Department of Pathology, Subharti Medical College and associated Chattrapati Shivaji Subharti Hospital (CSSH), Meerut were included in this study. The clinicopathological data of the 100 patients were put together 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

Majority of patients with neoplastic gallbladder lesions belonged to age group 51 to 60 years (40%) followed by 61 to 70 years (26%). 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%) as compared to non-neoplastic Gallbladder lesions (50%) but the difference was not statistically significant. 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.

Site of lesion in 19(38.00%) cases was found to be fundus, in 11(22.00%) cases was found to be body and in 2(4.00%) cases, site of lesion was found to be gallbladder neck. Gross findings in 28(56.00%) of GBC cases was found to be diffuse infiltrative typ of lesione, 13(26.00%) cases had exophytic proliferative type of lesion and 9(18.00%) cases had ulcero-proliferative type of lesion. Histological grade found in 8 (16.00%) cases of GBC was G3 (poorly differentiated}, 40 (80%)

cases was in G2 (Moderately differentiated) and 2(4.00%) cases was of G1 (Well differentiated) adenocarcinoma. Pathologic staging (pTNM) in 34 (68.00%) cases was pT1 (tumor invading lamina propria or muscular layer), 12(24.00%) cases was pT2 (tumor invading perimuscular connective tissue but no extension beyond serosa or into liver) and 2(4.00%) cases was pT3 (tumor perforates serosa, liver, adjacent organ or structures) and 2 (4.00%) cases were categorized in pTis (i,e carcinoma in situ cases).

Regional lymph nodes (pN) involvement in 49 (98.00%) cases was pN0 (lymphnode were not found , so cound not be assessed), and in 1(2.00%) cases it was categorized as pN1 (metastasis to one to three regional lymph node was there)

Statistical significant association was seen between age and EGFR expression. Patients of age group >40 years had significantly higher positive expression of immunomarkers: EGFR (42.35%), as compared to <40 years (0 %). Patients of age group < or = 40 years had significantly higher positive expression of immunomarkers: E-cadherin (53.35%), as compared to >40 (23.53 %). Statistical significant association was seen between Gallbladder wall thickness and all immunomarkers.. Patients with Gallbladder wall thickness >3 mm had significantly higher positive expression of immunomarkers: p53 (59.57%), Ki67 (61.70%), EGFR (65.96%), Cyclin D1 (36.17%) and Her2/Neu (44.68%) and E-Cadherin expression was (6.38%).

The clinico-pathologic analysis revealed a substantial association between gallstones and the expression of various immunomarkers in gallbladder lesions. GallBladder lesions associated with presence of stones demonstrated significantly higher positive expressions of P53 (43.75%), Ki67 (41.67%), EGFR (45.83%), Cyclin D1 (31.25%), and Her2/Neu (33.33%) compared to lesions where no stone were present. Analysis of association of hyperplastic, metaplastic and dysplastic lesions are shown in figure 1& 2 and association of neoplastic gallbladder lesions with immunomarkers details is shown in Table 1.

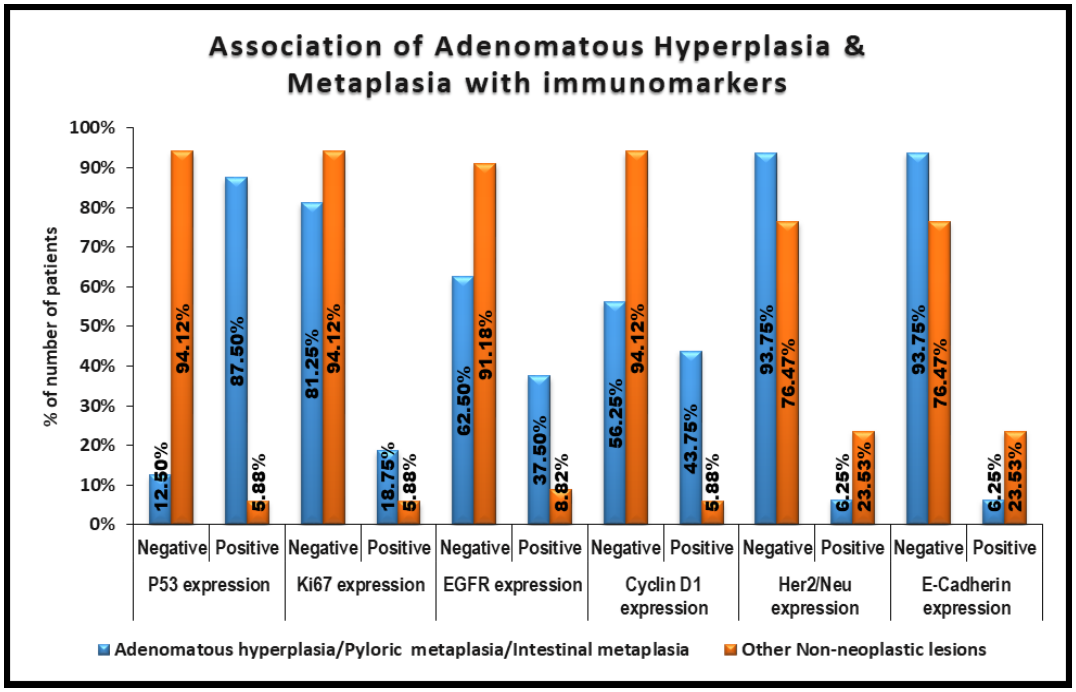

Figure 1:Immunomarker expression in gallbladder lesions with Adenomatous hyperplasia & Metaplasia (Pyloric & Intestinal)

The assessment of p53 expression in Hyperplasia (Adenomatous) and Intestinal and pyloric metaplasia revealed a statistically significant association ($p<0.0001$). Notably, 87.50% of these lesions showed positive p53 expression. Ki67 expression showed a non-significant association ($p=0.311$), with 18.75% of cases showing positive expression.

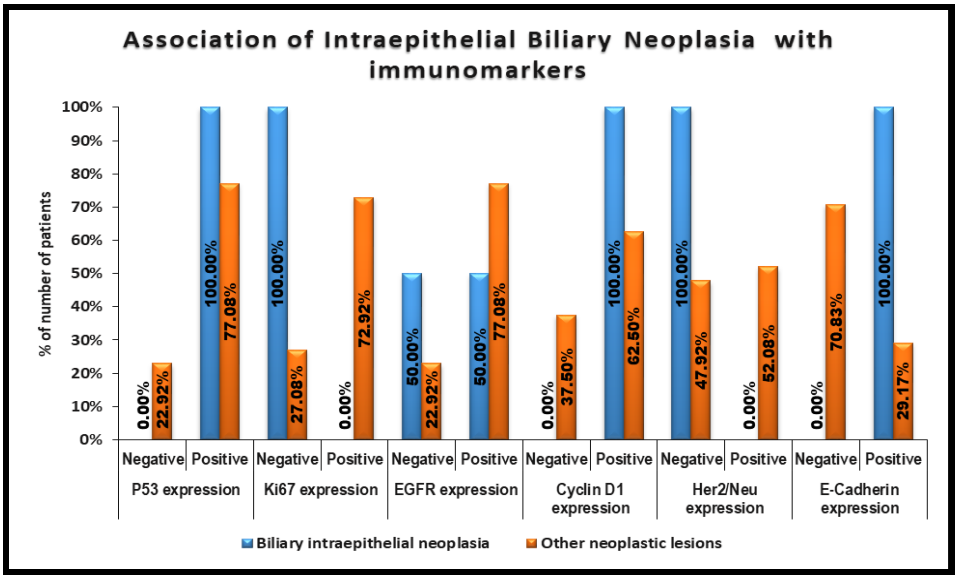

Figure 2: Association of Biliary Intraepithelial Neoplasia with immunomarkers.

Biliary intraepithelial neoplasia (BilIN) cases had positive expression of p53,Cyclin D1, E-Cadherin and negative expression of Ki67, HER2/neu. No significant association was seen in Biliary intraepithelial neoplasia with all immunomarkers. (p>0.05)

Table 1: Analysis of association of neoplastic Gallbladder lesions with immunomarkers

Immunohistochemical expression of immunomarkers in Neoplastic and Non-neoplastic Gallbladder lesions

Gallbladder lesions	p53 expression n (%)			Ki67 expression n(%)			EGFR expression n(%)			Cyclin D1 expression n(%)			HER2/neu expression n(%)			E-Cadherin expression n(%)		
	Negative	Positive	P value	Negative	Positive	P value	Negative	Positive	P value	Negative	Positive	P value	Negative	Positive	P value	Negative	Positive	P value
Adenocarcinoma - Biliary type	5 (29.41)	12 (70.59)	0.364[†]	10 (58.82)	7 (41.18)	0.001[†]	6 (35.29)	11 (64.71)	0.18[†]	8 (47.06)	9 (52.94)	0.242[†]	15 (88.24)	2 (11.76)	0.0002[*]	8 (47.06)	9 (52.94)	0.023[*]
Adenocarcinoma - Intestinal type	1 (100)	0 (0)	0.22[*]	1 (100)	0 (0)	0.3[*]	1 (100)	0 (0)	0.24[*]	1 (100)	0 (0)	0.36[*]	1 (100)	0 (0)	1[*]	0 (0)	1 (100)	0.32[*]
Mucinous adenocarcinoma	1 (20)	4 (80)	1[*]	0 (0)	5 (100)	0.305[*]	2 (40)	3 (60)	0.582[*]	0 (0)	5 (100)	0.145[*]	2 (40)	3 (60)	1[*]	5 (100)	0 (0)	0.163[*]
Adenocarcinoma-NOS (Not otherwise specified)	4 (23.53)	13 (76.47)	1[*]	2 (11.76)	15 (88.24)	0.056[*]	2 (11.76)	15 (88.24)	0.181[*]	7 (41.18)	10 (58.82)	0.584[*]	5 (29.41)	12 (70.59)	0.037[†]	13 (76.47)	4 (23.53)	0.524[*]
Poorly cohesive carcinoma	0 (0)	8 (100)	0.174[*]	0 (0)	8 (100)	0.086[*]	0 (0)	8 (100)	0.173[*]	2 (25)	6 (75)	0.694[*]	0 (0)	8 (100)	0.004[*]	8 (100)	0 (0)	0.045[*]
Biliary intraepithelial neoplasia	0 (0)	2 (100)	1[*]	2 (100)	0 (0)	0.086[*]	1 (50)	1 (50)	0.426[*]	0 (0)	2 (100)	0.53[*]	2 (100)	0 (0)	0.49[*]	0 (0)	2 (100)	0.098[*]
Chronic cholecystitis	7 (100)	0 (0)	0.081[*]	6 (85.71)	1 (14.29)	0.546[*]	6 (85.71)	1 (14.29)	1[*]	7 (100)	0 (0)	0.325[*]	5 (71.43)	2 (28.57)	0.595[*]	3 (42.86)	4 (57.14)	0.21[*]
Acute on chronic cholecystitis (Empyema)	9 (90)	1 (10)	0.138[*]	10 (100)	0 (0)	0.569[*]	9 (90)	1 (10)	0.665[*]	9 (90)	1 (10)	0.665[*]	9 (90)	1 (10)	0.665[*]	6 (60)	4 (40)	0.717[*]
Chronic cholecystitis with cholesterolosis	7 (87.50)	1 (12.50)	0.409[*]	7 (87.50)	1 (12.50)	1[*]	7 (87.50)	1 (12.50)	1[*]	7 (87.50)	1 (12.50)	1[*]	6 (75)	2 (25)	0.623[*]	2 (25)	6 (75)	0.013[*]
Hypereosinophilic cholecystitis	3 (100)	0 (0)	0.542[*]	3 (100)	0 (0)	1[*]	3 (100)	0 (0)	1[*]	3 (100)	0 (0)	1[*]	1 (33.33)	2 (66.67)	0.08[*]	2 (66.67)	1 (33.33)	1[*]
Xanthogranulomatous cholecystitis	3 (100)	0 (0)	0.542[*]	3 (100)	0 (0)	1[*]	3 (100)	0 (0)	1[*]	3 (100)	0 (0)	1[*]	3 (100)	0 (0)	1[*]	3 (100)	0 (0)	0.542[*]
Hyalinising cholecystitis (Porcelain gallbladder)	3 (100)	0 (0)	0.542[*]	3 (100)	0 (0)	1[*]	3 (100)	0 (0)	1[*]	3 (100)	0 (0)	1[*]	2 (66.67)	1 (33.33)	0.456[*]	2 (66.67)	1 (33.33)	1[*]
Adenomatous hyperplasia	2 (20)	8 (80)	0.0007[*]	8 (80)	2 (20)	0.258[*]	5 (50)	5 (50)	0.01[*]	8 (80)	2 (20)	1[*]	9 (90)	1 (10)	0.665[*]	9 (90)	1 (10)	0.134[*]
Pyloric metaplasia	0 (0)	4 (100)	0.008[*]	3 (75)	1 (25)	0.353[*]	3 (75)	1 (25)	0.56[*]	1 (25)	3 (75)	0.016[*]	4 (100)	0 (0)	1[*]	4 (100)	0 (0)	0.285[*]
Intestinal metaplasia	0 (0)	2 (100)	0.098[*]	2 (100)	0 (0)	1[*]	2 (100)	0 (0)	1[*]	0 (0)	2 (100)	0.029[*]	2 (100)	0 (0)	1[*]	2 (100)	0 (0)	0.542[*]

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

Biliary type of adenocarcinoma: Ki67 expression showed a significant association, with 41.18% positive expression in the biliary type of adenocarcinoma with p value=0.001. There was no statistically significant difference found in p53, Ki67, EGFR, and Cyclin D1 expressions with p values being > 0.05.

Adenocarcinoma-NOS (Not otherwise specified): HER2/neu expression showed a significant association, with 70.59% positive expression in the adenocarcinoma NOS (Papillary type of adenocarcinoma) with p value=0.037. There was no statistically significant difference found in p53, Ki67, EGFR, and Cyclin D1 & E-Cadherin expressions with p values being > 0.05.

Mucinous adenocarcinoma :There was no statistically significant difference found in p53, Ki67, EGFR, and Cyclin D1, HER2/neu & E-Cadherin expressions with p values > 0.05.

Poorly cohesive carcinoma : Her 2/Neu expression had significantly higher positive expression (100%, p=0.004).E-Cadherin expression showed 100% negative (loss of expression) expression with p value =0.043. There was no statistically significant difference found in p53, Ki67, EGFR, and Cyclin D1 expressions with p value being> 0.05.

Intestinal type of adenocarcinoma : There was no statistically significant difference found in p53, Ki67, EGFR, and Cyclin D1, HER2/neu & E-Cadherin expressions with p values > 0.05.
In terms of overall diagnostic accuracy, EGFR immunomarker was the best with a value of 80%, emphasizing its potential as a reliable immunomarker for neoplastic Gallbladder lesions. These insights may be useful for decision-making regarding the selection and utilization of immunomarkers for diagnostic purposes in the context of Gallbladder Cancer cases .
Overall, the concordance rates between the immunomarkers was relatively high, ranging from 47% to 88% except between E-cadherin and other immunomarkers. This suggests that the immunomarkers are generally related to each other except E-cadherin. The high concordance rate was found between p53 and Ki67, p53 and EGFR, p53 and Cyclin D1, Ki67 and EGFR, Ki67 and Cyclin D1 , EGFR and Cyclin D1. The relatively low concordance rate was observed between E-cadherin and p53, Ki67, EGFR, HER2/neu and Cyclin D1.

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.
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 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, 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.]

In our study the concordance rates between the immunomarkers was relatively high, ranging from 47% to 88%. This suggests that the immunomarkers are generally related to each other. The high concordance rate was found between p53 and Ki67, p53 and EGFR, p53 and Cyclin D1, Ki67 and

EGFR, Ki67 and Cyclin D1, EGFR and Cyclin D1. The relatively low concordance rate was observed between E-cadherin and p53, Ki67, EGFR, HER- 2/ neu and Cyclin D1 .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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