Original Article

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Pathology Section

p53 Expression in Gallbladder Lesions: A Cross-sectional Study from a Tertiary Care Centre in Northern India

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ABSTRACT

Introduction: Gallbladder lesions comprise inflammatory, benign, pre-malignant, and malignant lesions. The progression of benign lesions into malignant ones involves a complex process. The p53 gene is commonly disrupted in carcinogenesis. Malignant lesions may exhibit p53 overexpression compared to benign and inflammatory lesions.

Aim: To investigate the distribution of gallbladder lesions and the expression of the p53 nuclear protein in these lesions.

Materials and Methods: A cross-sectional study was conducted at the Department of Pathology, Sher-i-Kashmir Institute of Medical Sciences, Srinagar, Jammu and Kashmir, from June 2020 to December 2022. A total of 249 specimens of gallbladder lesions, including congenital, inflammatory, benign, pre-malignant, and malignant lesions, were included. All samples underwent Haematoxylin and Eosin (H&E) staining and Immunohistochemistry (IHC) with p53 antibody using the peroxidase-antiperoxidase method. The association of p53 expression with histopathological diagnosis was analysed using Fisher's Exact test. The final analysis was performed using Statistical Package for Social Sciences (SPSS) software, version 25.0. A p-value <0.05 was considered statistically significant.

Results: Out of 249 specimens, there were 217 (87.14%) inflammatory lesions, 9 (3.6%) benign lesions, 2 (0.8%) pre-malignant lesions, and 21 (9.26%) malignant lesions. The main inflammatory lesions were Chronic Cholecystitis (CC) with 132 cases (60.83%) and CC with cholesterosis with 36 cases (16.59%). The most common pre-malignant lesion was choledochal cyst with 3 cases (3.33%). Benign tumours (leiomyoma) were present in 2 (0.8%) patients. Among the 21 (8.46%) malignant tumours, 4 (19.05%) were moderately differentiated adenocarcinoma, 13 (61.9%) were poorly differentiated adenocarcinoma, and 4 (19.05%) were well-differentiated adenocarcinoma. p53 overexpression was significantly higher in patients with malignant tumours (9 cases, 42.86%) compared to inflammatory lesions (26 cases, 11.98%), benign lesions (0 cases, 0%), and pre-malignant lesions (0 cases, 0%) (p=0.003).

Conclusion: Gallbladder lesions exhibit a wide range of histopathological presentations. Inflammatory lesions are the most common, followed by pre-malignant and malignant lesions. p53 can serve as a novel marker for differentiating inflammatory lesions from malignant lesions in the gallbladder.

Keywords: Carcinoma, Immunohistochemistry, Novel marker, p53

INTRODUCTION

Gallbladder diseases present with a diverse clinical and histopathological spectrum, including congenital, inflammatory, benign, pre-malignant, and malignant conditions [1]. The most common pathology occurring in the gallbladder is cholelithiasis, followed by cholecystitis [2]. Very few studies have been conducted on the North Indian population, and the frequency of gallbladder lesions, especially in the Jammu and Kashmir, is still unclear. There is a significant difference in gallbladder diseases between North India and South India [3].

The diagnosis of gallbladder lesions through radiological methods is not always accurate. The lesions may not be identified during pre-operative imaging. Gallbladder cancer is suspected preoperatively in about 20%-30% of all patients, while the remaining 70%-80% of cases are diagnosed intraoperatively or incidentally by the pathologist.

Simple cholecystectomy is the surgical treatment of choice for inflammatory and benign conditions. In the case of cancer, a simple cholecystectomy or an extended cholecystectomy with liver wedge resection may be curative. Adjuvant treatments, such as radiation, chemotherapy, or a combination, are necessary in advanced stages [4].

The histopathological examination of the specimen enables the correct diagnosis of both pre-malignant and malignant lesions. Therefore, it is crucial to evaluate histopathological changes to determine the occurrence and distribution of the lesions [5]. Gallbladder cancer is suspected preoperatively in about 20%-30% of all patients, with the remaining 70%-80% of cases being diagnosed intraoperatively or incidentally by the pathologist. Simple cholecystectomy is the preferred surgical treatment for inflammatory and benign conditions. In cases of cancer, a simple cholecystectomy or an extended cholecystectomy with liver wedge resection may be curative. Adjuvant treatment such as radiation, chemotherapy, or a combination is necessary in advanced stages. Overall, the prognosis remains poor.

However, even histopathologically, there may be a diagnostic dilemma in differentiating certain pre-malignant lesions from malignant lesions [6]. The evaluation of the association between chronic inflammatory conditions and carcinogenesis has shown that the disruption of the p53 gene is the most common mechanism, leading to the terminology of "Guardian of the Genome" [7]. p53, a tumour suppressor gene, is present on the short arm of chromosome 17 and helps prevent the build-up of potentially destructive mutations in Deoxyribonucleic Acid (DNA). p53 plays a significant role in the initiation and progression of cancer [8]. Moreover, the expression of p53 is significant in carcinogenesis in chronic inflammatory lesions like Chronic Cholecystitis (CC) [6,8]. Since it can be difficult to identify malignant transformation in inflammatory and pre-malignant lesions, recognition of p53 mutation through Immunohistochemistry (IHC) can help with diagnosis [9]. Additionally, IHC analysis of p53 mutations in gallbladder lesions could be beneficial for screening, early detection of recurrence in the remaining biliary system, liver metastasis, and follow-up of high-risk patients [10,11]. Therefore, this study was undertaken to identify

the distribution of the spectrum of gallbladder lesions (congenital, inflammatory, benign, pre-malignant, and malignant lesions) in the Jammu and Kashmir region of North India. The objectives of the study were to determine the association of p53 expression with the spectrum of gallbladder lesions.

MATERIALS AND METHODS

A cross-sectional study was conducted in the Department of Pathology at Sher-i-Kashmir Institute of Medical Sciences, Srinagar, Jammu and Kashmir, India, from June 2020 to December 2022. A total of 249 specimens of gallbladder lesions were included, comprising congenital, inflammatory, benign, pre-malignant, and malignant lesions. Since it was a cross-sectional study involving pathological specimens, ethical clearance was not required or waived. Furthermore, no patient data were disclosed.

Inclusion criteria: All cholecystectomy specimens obtained in the histopathological department of the hospital were included in the present study during the study period.

Exclusion criteria: Those patients with any other major co-morbid illness or those for whom a specific diagnosis regarding inflammatory, pre-malignant, benign or malignant conditions could not be made and patients who refused to participate in the study were excluded from the study.

Sample size: The study by Pavani M et al., observed that p53 expression was present in 16.67% of patients [9]. Taking this value as a reference, the minimum required sample size, with a 5% margin of error and a 5% level of significance, is 214 patients. To reduce the margin of error, a total sample size of 249 was chosen.

Procedure

Age, gender, and presenting complaints of each patient were noted. Histopathological diagnoses were retrieved from the records. The histopathological specimens were fixed in 10% formalin and embedded in wax. Slides were prepared and stained with haematoxylin and eosin. The slides were independently examined by a histopathologist under light microscopy [12]. The grading of cell differentiation for malignant types was performed according to World Health Organisation (WHO) criteria, classifying them as well-differentiated, moderately differentiated, or poorly differentiated [11]. Blocks and sections were retrieved from the collection for performing p53 IHC to assess nuclear positivity.

p53 immunostaining was conducted using the biotin-streptavidin-alkaline phosphatase method. Two sections, each 2-4 µm thick, were prepared, with one serving as a negative control and the other used for immunostaining. p53 positive control cell slides from CELL MARQUE were used alongside each slide during the p53 expression assessment. The sections were de-paraffinised with xylene, rehydrated with graded alcohol, and washed with deionised water. Citrate buffer pretreatment was performed for antigen retrieval. Primary anti-p53 antibodies (Dako, Clone DO-7) were applied at a dilution of 1:50 and incubated for two hours at room temperature. Subsequently, incubation was carried out for 20 minutes with biotinylated anti-mouse/anti-rabbit secondary antibodies, followed by incubation with alkaline phosphatase-conjugated streptavidin (Alkaline Phosphatase Kit, Biogenex) for 20 minutes. Phosphate buffer saline was used for the negative controls. The sections were counterstained with Harris haematoxylin, covered with coverslips, and examined under the microscope for p53 expression, which was scored using a semi-quantitative method based on the incidence of positively stained cells [11].

Scoring was performed based on intensity, with values of 0, 1, 2, and 3 representing absent, mild, moderate, and intense staining, respectively. The percentage positivity was graded as absent, Grade-1, Grade-2, and Grade-3 for 0% cells, <10% cells, 10-50% cells, and >50% positive cells. Therefore, the total score ranged from 0 to 6, and a score of ≥3 was considered as positive overexpression of p53 [11]. The final histopathological diagnosis and the association of p53 expression in inflammatory, benign, pre-malignant, and malignant lesions were determined statistically.

STATISTICAL ANALYSIS

The categorical variables were presented as "number and percentage (%)". Meanwhile, the quantitative data were presented as "means±SD" and as the "median with 25th and 75th percentiles" (interquartile range). The association between p53 expression and histopathological diagnosis was analysed using Fisher's Exact test. Data entry was performed in a Microsoft Excel spreadsheet, and the final analysis was conducted using SPSS software, version 21.0, manufactured by IBM in Chicago, USA. A p-value of less than 0.05 was considered statistically significant for determining statistical significance.

RESULTS

The mean age of the patients was 45.22±14.4 years. In the present study, 165 (66.27%) of the patients were females, and 84 (33.73%) were males. The symptoms reported included palpable mass in 210 cases (84.34%), jaundice in 206 cases (82.73%), and right upper quadrant pain in 200 cases (80.32%) [Table/Fig-1].

Demographic and clinical characteristics	Frequency	Percentage
Gender		
Female	165	66.27%
Male	84	33.73%
Symptoms		
Right upper quadrant pain	200	80.32%
Jaundice	206	82.73%
Palpable mass	210	84.34%
Age (years)		
Mean±SD		
Females	45.22±14.4	
Males	50.44±10.2	

[Table/Fig-1]: Distribution of demographic and clinical characteristics of study subjects.

The main inflammatory lesions (n=217, 87.14%) consisted of 132 cases (60.83%) of Chronic Cholecystitis (CC), 36 cases (16.59%) of CC with cholesterosis, and 27 cases (12.44%) of CC with cholelithiasis. Among the pre-malignant lesions (n=9, 3.6%), three cases (33.33%) were choledochal cysts, and two cases (22.22%) each were CC with antral metaplasia, CC with focal adenomyomatosis, CC with intestinal metaplasia, CC with pyloric metaplasia, and CC with tubular metaplasia. Two cases (0.8%) of benign tumours (leiomyoma) were observed. Out of 21 cases (8.46%) of malignant tumours, four cases (19.05%) were moderately differentiated adenocarcinoma, 13 cases (61.9%) were poorly differentiated adenocarcinoma, and four cases (19.05%) were well-differentiated adenocarcinoma [Table/Fig-2].

Histopathological diagnosis	Frequency (Total N=249)	Percentage (100%)
Inflammatory	217	87.14
Acute on Chronic Cholecystitis (CC)	3	1.38
Acute on CC with abscess formation	1	0.46
Acute on CC with gangrenous changes	1	0.46
Acute suppurative cholecystitis	1	0.46
Adenomatous hyperplasia	2	0.92
Cholesterosis	1	0.46
Chronic atrophic cholecystitis	2	0.92
CC	132	60.83
CC with cholelithiasis	27	12.44
CC with cholesterosis	36	16.59

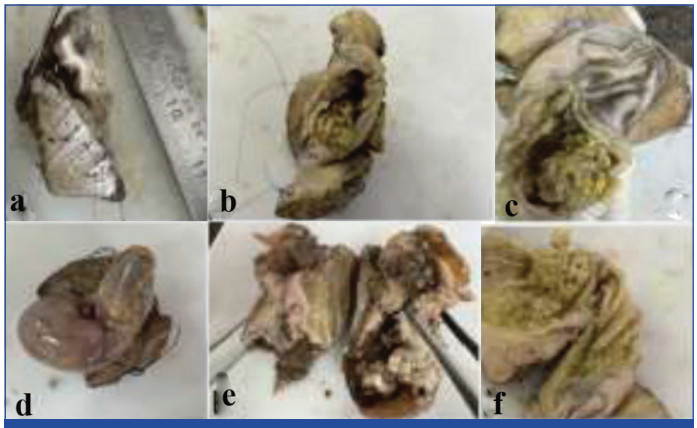
CC with cyst wall showing foci of haemorrhage and inflammation	1	0.46
CC with cystic duct showing hypertrophied muscle layer	1	0.46
CC with reactive lymphadenitis	1	0.46
Empyema	1	0.46
Necrotic GB with thickened muscularis	1	0.46
Xanthogranulomatous cholecystitis	6	2.76
Benign tumour	2	0.8
Leiomyoma	2	100
Pre malignant	9	3.6
Choledochal cyst	3	33.33
CC with antral metaplasia	2	22.225
CC with focal adenomyomatosis	1	11.11
CC with intestinal metaplasia	1	11.11
CC with pyloric metaplasia	1	11.11
CC with tubular metaplasia	1	11.11
Malignant tumour	21	8.46
Moderately differentiated adenocarcinoma	4	19.05
Poorly differentiated adenocarcinoma	13	61.9
Well differentiated adenocarcinoma	4	19.05

[Table/Fig-2]: Distribution of histopathological diagnosis of study subjects.

There was a significant overexpression of p53 in malignant gallbladder lesions (p=0.003). p53 overexpression was observed in 26 cases (11.98%) of inflammatory lesions and nine cases (42.86%) of malignant tumours. There was no p53 overexpression in benign tumours and pre-malignant lesions. Among the malignant tumours, p53 overexpression was present in eight cases of poorly differentiated carcinoma and one case of moderately differentiated carcinoma [Table/Fig-3]. Representative case images are shown in [Table/Fig-4-7].

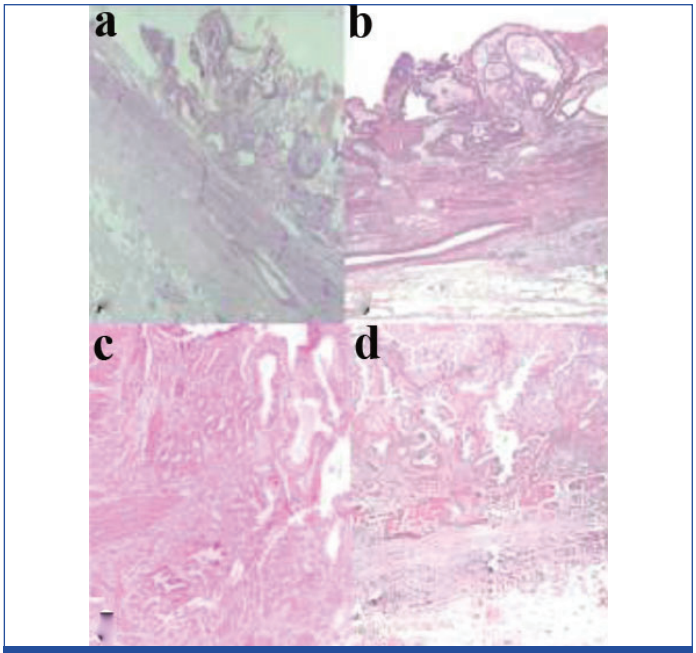
p53 overexpression	Inflammatory (n=217)	Pre-malignant (n=9)	Benign tumour (n=2)	Malignant tumour (n=21)	p-value
Absent	191 (88.02%)	9 (100%)	2 (100%)	12 (57.14%)	0.003*
Present	26 (11.98%)	0 (0%)	0 (0%)	9 (42.86%)	

[Table/Fig-3]: Association of p53 overexpression with histopathological diagnosis.
*Fisher's-exact test

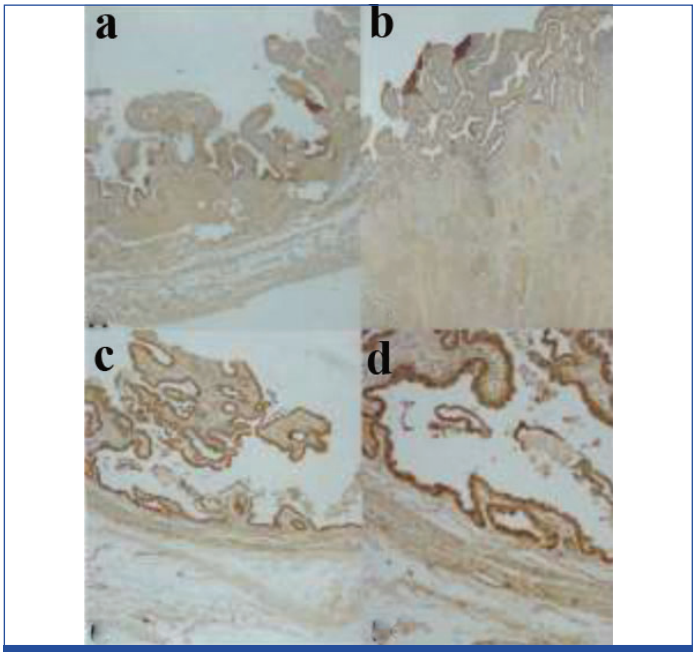


[Table/Fig-4]: Gross photographs of radical cholecystectomy specimens of Carcinoma gallbladder; (a) showing markedly thickened gall bladder wall with grey white area; (b,c) showing cut open gall bladder with a papillary growth; (d,e) showing external surface and cut section of a friable intraluminal growth invading the hepatic bed; (f) Cut open gallbladder with multifocal papillary proliferation in the fundus and body of the gallbladder.

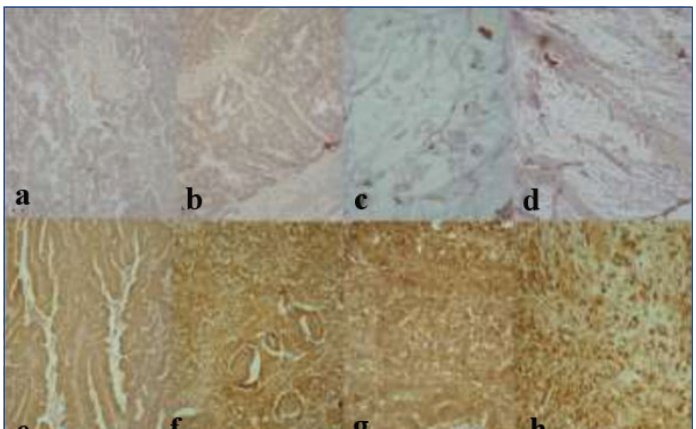
Gross photographs of radical cholecystectomy specimens of carcinoma gallbladder mostly showed a markedly thickened gallbladder wall with grey-white areas and friable intraluminal growth [Table/Fig-4]. Microscopically, carcinoma of the gallbladder exhibited wall infiltration with malignant glands [Table/Fig-5]. Photomicrographs showed p53 nuclear staining in benign [Table/Fig-6] and malignant lesions [Table/Fig-7].



[Table/Fig-5]: Photomicrograph of carcinoma gallbladder showing full thickness gallbladder wall with malignant glands infiltrating (a) Lamina propria (H&E, 10X); (b-d) muscle layer (H&E,10X).



[Table/Fig-6]: Photomicrograph showing p53 staining. (a) nuclear p53 negativity in chronic cholecystitis (p53,4X); (b) nuclear p53 negativity in chronic cholecystitis with adenomyomatosis (p53, 4X); (c) nuclear p53 positivity in a papillary adenocarcinoma (p53,4X); (d) nuclear p53 positivity in a case of chronic cholecystitis (p53,4X).



[Table/Fig-7]: Photomicrograph showing negative p53 staining in well differentiated; (a,b) (p53; 20x); Poorly differentiated Signet ring cell carcinoma; (c,d) (p53; 20x); strong nuclear p53 positivity in moderately differentiated; (e,f) (p53; 40x); poorly differentiated adenocarcinoma gallbladder; (g,h) (p53; 40x).

DISCUSSION

The spectrum of gallbladder lesions exhibited a wide variation from non-malignant to malignant conditions. Overall, the mean age of all the patients was 45.22±14.4 years. This is in line with a study conducted in South India by Pavani M et al., where the age of patients ranged from 18 to 72 years, with a mean age of 46.3 years at the time of presentation [9]. In patients with malignant lesions, the mean age was 51.5 years. This finding is consistent with the mean age of 45.77±14.65 years at presentation reported in studies by Mushtaq M et al., and Vahini G and Premalatha P. [1,13]. Gallbladder diseases are more commonly observed in women, and middle-aged individuals are most frequently affected.

Females (165, 66.27%) outnumbered males (84, 33.73%) in the present study, as the incidence of gallbladder lesions was higher in females compared to males. This finding is consistent with the study conducted by Pavani M et al., where females were found to be more affected than males, with a male-to-female ratio of 1:1.2 [9]. Similar findings were reported by Patel AM et al., (M:F=1:1.25), Jha RK and Yadav DP (M:F=1:4), and Selvi RT et al., (M:F=1:1.6) [14-16]. Factors that may contribute to the formation of gallstones in females include female sex hormones, particularly estrogen, and a sedentary lifestyle [17]. Estrogen has been shown to increase the secretion of biliary cholesterol in the liver, leading to increased bile cholesterol saturation and the formation of cholesterol gallstones [18].

The majority of cases in the present study were inflammatory lesions (217, 87.14%). The main inflammatory lesions observed were Chronic Cholecystitis (CC) in 132 cases (60.83%), CC with cholesterosis in 36 cases (16.59%), and CC with cholelithiasis in 27 cases (12.44%). These findings are similar to those reported by Pavani M et al., who found that the predominant lesions were inflammatory lesions (84.70%), precursor lesions (10.70%), and malignant lesions (4.58%) [9]. CC is the most common gallbladder disease and the primary indication for cholecystectomy. In their study, they found that patients presented with acute cholecystitis as well as acute-on-chronic cholecystitis. Similar findings were also reported by Kumari NS et al., where the most commonly observed gallbladder lesions were chronic calculous cholecystitis (405, 73.64%) [5]. Other lesions included chronic cholecystitis (85, 15.45%), acute-on-chronic cholecystitis with stones (18 cases, 3.27%), and without stones (3, 0.55%) [Table/Fig-8] [1,2,5,9,13-16,19-22].

Studies	Year	Place of study	N	Mean age	Males N(%)	SpectrumI, B, PM, M	p53 expression in I, B, PM, M
Patel AM et al., [14].	2022	Wardha, Maharashtra	72	47.11 years	32 (44.44%)	-	-
Pavani M et al., [9]	2020	Hyderabad, Telangana	262	51.5 years	Male: Female 1:1.2	I: 84.7%, PM: 10.68%, M: 4.58%	I: 11.8%, PM: 0%, M: 50%
Shukla SK et al., [19]	2019	Uttarakhand	25	25-80 years	-	All malignant	44%
Mushtaq M et al., [1]	2017	Islamabad, Pakistan	360	Majority cases (38.33%) in 5th decade	93(25.83%)	Non-neoplastic lesions {354 (98.33%)}, neoplastic lesion {6 (1.66%)}.	-
Jha RK and Yadav DP [15]	2017	Nepal	200	22-81 years	Male: Female 1:4	Acute cholecystitis {3 (1.5%)}, Chronic Cholecystitis (CC) {179 (89.5%)}, polyps {9 (4.5%}, gall bladder carcinoma in {5 (2.5%)}	-
Kumari NS et al., [5]	2016	Hyderabad	170	Majority {64 (37.6%)} 41-60 years	1:1	CC {79 (46.47%)}, CC with cholelithiasis {59 (34.7%)}, gangrene {13 (7.67%)}, gangrene with perforation (3), empyema (2), xanthogranulomatous cholecystitis {3 (1.8%)}, adenomyosis {4 (2.35%)}, and follicular cholecystitis, cholesterolosis, eosinophilic cholecystitis, porcelain gall bladder, mucocele, and carcinoma in 1 case each	-
Singh A et al., [30]	2016	-	60	>55 years {33 (68.75%)}	15 (25%)	Malignant: 100%	22 (36.67%)
Vahini G et al., [13]	2015	Mumbai, India	110	45.2 years	77 (70%)	CC {80 (72.7%)}, acute cholecystitis 20 (18.3%), cholesterolosis 2 (1.8%), adenomatous hyperplasia 2 (1.8%), tubular adenomatous polyp 1 (0.9%), carcinoma 5 (4.5%)	
Tereda T [2]	2013	-	540	64.75±14.43 years	213 (39.44%)	Acute cholecystitis {8 (1.5%)}, CC {508 (94.1%)}, adenocarcinomas {12 (2.2%)}, cystadenocarcinoma {1 (0.2%)}	-
Ghosh M et al., [28]	2013	New Delhi, India	80	-	-	GBC (n=80), CC (n=60), gall bladder controls (n=10)	GBC: 45 (56.25%), cholecystitis (0%), gall bladder controls (0%)
Selvi RT et al., [16]	2011	Tamil Nadu	78	45.90 years	28 (35.89%)	Acute cholecystitis {2 (2.5%}, CC {67 (87%)}, polyp {2 (2.5%)}, granulomatous cholecystitis {1 (1.2%)}, empyema {1 (1.2%)}, eosinophilic cholecystitis {4 (3.8%)}, carcinoma {1 (1.2%)}	-
Chaube A et al., [27]	2006	Varanasi, India	78	53.62±12.46 years	3:1	Gallbladder cancer 40 (51.28%),	Gallbladder cancer: p53 over expression 8 (20%), benign: 0%
Our study	2023	Jammu and Kashmir region of North India		45.22±14.4 years	84 (33.73%)	I: 87.14%, B: 0.8% PM: 3.6%, M: 8.46%	11.98% , 0%, 0%, 42.86%

[Table/Fig-8]: Review of literature [1,2,5,9,13-16,19,27,28,30].
I: Inflammatory, B: Benign, PM: Pre-malignant, M: Malignant
The data is represented in N (%) or mean±SD

The majority of cholecystitis cases are reported to be associated with cholelithiasis, which is a common disorder affecting 10% to 20% of adults [13,15]. Recurrent inflammation leads to fibrosis and thickening of the gallbladder wall. CC and other conditions such as adenomyosis, gallstones, and xanthogranulomatous reactions have been reported to be associated with metaplasia [9,13,15,16,19]. Several risk factors related to cholecystitis include advanced age, female gender, obesity, ethnicity, pregnancy, use of oral contraceptives, and diabetes mellitus [15,16]. Recurrent episodes of acute cholecystitis can lead to the development of CC, with the majority of cases being associated with gallstones [23].

In the present study, a total of nine cases (3.6%) of pre-malignant lesions were identified. Among these, three cases (33.33%) were choledochal cysts, two cases (22.22%) were CC with antral metaplasia, and CC with focal adenomyomatosis, CC with intestinal metaplasia, CC with pyloric metaplasia, and CC with tubular metaplasia were each present in one case (11.11%). Similar findings were reported by Pavani M et al., who found cases of pre-malignant lesions such as choledochal cysts (n=8, 3.05%), cystadenomas (n=3, 11.4%), and polyps (n=2, 0.76%) [9]. Consistent results were also reported in the study by Shukla SK et al., [Table/Fig-8] [19].

Gallbladder polyps larger than 1.5 cm are associated with a 50% risk of malignancy [24]. Choledochal cysts are also linked to a greater risk of progressing to malignancy, even in the absence of inflammatory risk factors such as gallstones. Patients with these lesions may develop Gallbladder Cancer (GBC) at an earlier age [18]. GBC is a rare but the most common malignancy of the biliary tract, accounting for 80% to 95% of all biliary tract carcinomas and ranking as the fifth most common gastrointestinal malignancy [25]. In the present study, the authors focused on the incidence of gallbladder carcinoma in the Jammu and Kashmir region of North India. Among the 21 (8.46%) malignant tumours identified, 4 (19.05%) were poorly differentiated adenocarcinoma, 13 (61.90%) were moderately differentiated adenocarcinoma, and 4 (19.05%) were well-differentiated adenocarcinoma. In a study by Pavani M et al., 4.6% (n=12) of the cases were malignant, including well-differentiated adenocarcinoma, moderately differentiated adenocarcinoma, and poorly differentiated carcinomas [9]. Additionally, two malignant cases were incidentally discovered during cholecystectomy procedures performed for cholecystitis. The overall mean survival was six months, with a 5-year survival rate of 5%. These findings are consistent with the results reported by Terada T and Sharma A et al., [2,25]. Early diagnosis is crucial for appropriate management and a better prognosis. GBC progresses silently, and with delayed diagnosis, it is often fatal.

Surgery is the only curative option for gallbladder cancer. Early detection is extremely rare and typically occurs incidentally. Late presentation is associated with advanced staging, nodal involvement, and a higher risk of recurrence after resection [25]. There is a close association between chronic inflammation and neoplasia, as repeated or persistent inflammation can induce, promote, or increase susceptibility to carcinogenesis. Chronic inflammation damages DNA, stimulates tissue reparative proliferation, and creates a stromal environment enriched with cytokines and growth factors [26].

The p53 gene, encoded on chromosome 17p, plays a key role in regulating cell proliferation, and mutational inactivation of p53 promotes carcinogenesis and the progression of malignancy [9]. While the role of the p53 tumour suppressor gene has been studied in various cancers, its specific role in the pathogenesis of gallbladder cancer is still unknown [27]. Abnormalities in p53 are commonly observed in gallbladder cancer pathogenesis, starting from chronic cholecystitis. Inflammation may contribute to early alterations in p53, possibly through increased cell turnover and oxidative stress, although the exact mechanism is not yet fully understood. The most common genetic alteration in gallbladder cancer is inactivation of the TP53 gene, which can occur through mutation or deletion. Alterations in TP53 disrupt the architecture of the epithelium, leading to the progression of metaplasia to invasive carcinoma [27].

The authors found that p53 expression was significantly associated with the histopathological diagnosis. p53 expression was more prevalent in patients with malignant lesions compared to those with inflammatory, benign, and pre-malignant lesions (42.86% vs. 11.98% vs. 0% vs. 0%, p=0.003). Furthermore, p53 expression was higher in poorly differentiated and moderately differentiated adenocarcinomas compared to well-differentiated adenocarcinoma. These findings are consistent with some previous studies. Pavani M et al., found p53 expression present in 50% of malignant lesions [9]. No p53 expression was found in early or well-differentiated adenocarcinomas, but strong p53 expression was observed in poorly differentiated and moderately differentiated carcinomas. This is similar to the observations of Roa I et al., who also reported p53 expression in nearly 50% of advanced cancers compared to early carcinomas [28]. In the study by Oohashi Y et al., it was mentioned that p53 protein overexpression is an early event in carcinogenesis and this alteration is maintained during its progression [29]. Similar findings were reported by Kaur D et al., who found that overexpression of p53 is associated with an increased grade of gallbladder cancer, suggesting a role for p53 in tumour progression rather than initiation [30]. Wang HH et al., also reported a role of aberrant p53 expression in the occurrence of gallbladder cancer [18]. Ghosh M et al., found that there was an increase in p53 expression with increasing tumour grade [21]. p53 overexpression was present in 56.25% of gallbladder cancer cases, while no overexpression was observed in cases of chronic cholecystitis or control gallbladders. Kaur D et al., reported an inverse relationship between p53 overexpression and tumour grade [30]. The histological grade of p53-positive adenocarcinomas was significantly different from p53-negative adenocarcinomas, with a higher proportion of poorly differentiated adenocarcinomas in patients with p53 overexpression. Singh A et al., reported that among 60 cases of gallbladder carcinoma, 22 (36.67%) cases demonstrated strong HER-2 overexpression, while 20 (33.3%) cases showed positivity for p53 in tumour cases, which was associated with a poor outcome [22].

The authors found that p53 expression was positive in 26 (11.98%) out of 217 inflammatory lesions. Among other studies, Pavani M et al., reported that 11.8% of the cases had p53 expression in chronic cholecystitis cases with a thickened and inflamed gallbladder wall [9]. Similar findings were observed in the study by Kanoh K et al., where p53 expression was present in 14.3% of the inflammatory conditions [31]. It was suggested that chronic cholecystitis with a thick and sclerotic wall, resulting from repeated inflammation, may be an early change leading to carcinogenesis. Yanagisawa N et al., found the presence of sporadic p53 transition mutations in non-neoplastic lesions such as severe cholecystitis, suggesting the significance of the sequence from chronic cholecystitis to gallbladder cancer carcinogenesis [8]. In a study evaluating the effect of chronic inflammation and gallstones on gallbladder carcinogenesis, it was proposed that chronic inflammation of the gallbladder at the molecular level may result in p53 heterozygosity loss and excessive p53 protein expression [32]. Kanoh K et al., also reported that chronic contracted cholecystitis with a thickened and sclerotic wall was a risk factor for carcinogenesis [31]. Among the pre-malignant lesions, p53 expression was not positive in any case in the present study. Similarly, Chaube A et al., found that p53 overexpression was not observed in any pre-malignant lesions [20]. It was suggested that the role of p53 is limited in determining the initiation of gallbladder carcinogenesis.

Limitation(s)

Firstly, the authors did not assess the expression of any other markers. Additionally, the authors did not conduct a follow-up of the patients to determine the outcomes and their association with p53 expression.

CONCLUSION(S)

In conclusion, p53 can be considered as a novel marker for differentiation between benign and malignant lesions of the gallbladder, as its expression is significantly increased in malignant lesions. It may also serve as a supportive marker for distinguishing between different grades of differentiation. This has important implications in clinical practice, where the frequent use of p53 as a diagnostic tool can help prevent the misdiagnosis of malignant gallbladder lesions and ultimately improve patient outcomes.

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