

Molecular Profiling of EGFR and KRAS Alterations in carcinoma Gallbladder across disease stages : Clinicopathological and Therapeutic Implications

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Abstract

Background

Gallbladder carcinoma (GBC) is the most frequent biliary malignancy in northern India, often presenting late with limited therapeutic options. Molecular alterations such as EGFR overexpression and KRAS mutations have emerged as important oncogenic drivers in several solid tumors. This study aimed to evaluate the frequency and clinicopathological relevance of these alterations in Indian patients with GBC.

Methods:

This prospective observational study included 85 patients with histologically confirmed gallbladder carcinoma (GBC) treated between 2018 and 2021 at a tertiary center in northern India. Clinical, radiological, and pathological data were systematically recorded. Disease extent was categorized as resectable, unresectable, or metastatic based on imaging and intraoperative findings. EGFR expression was assessed by immunohistochemistry, while KRAS mutations were analyzed using real-time polymerase chain reaction. Associations between molecular alterations and clinicopathological variables were evaluated.

Results:

Among 85 patients, 66 (77.6%) were female and 19 (22.4%) male, with a mean age of 53.1 ± 11.8 years. At presentation, 49 (57.6%) patients had resectable disease, while 36 (42.4%) were unresectable/metastatic. EGFR overexpression was observed in 28 (32.9%) patients and KRAS mutations in 17 (20%). EGFR positivity was associated with LVI and higher tumor grade ($p = 0.03$). KRAS mutations were significantly associated with advanced disease, with higher prevalence in unresectable/metastatic cases compared to resectable tumors ($p < 0.001$), and were also correlated with poor differentiation ($p = 0.01$).

Conclusion:

EGFR and KRAS alterations are frequent in Indian patients with GBC and correlate with aggressive clinicopathological features. Incorporating molecular testing may improve prognostication and support precision-targeted therapy development.

Keywords: Gallbladder carcinoma, EGFR, KRAS, molecular profiling, prognosis, targeted therapy

Introduction

Gallbladder carcinoma (GBC) remains one of the most lethal malignancies of the biliary tract, with pronounced geographical variation in incidence^{1,2}.

Northern India is recognized as a global hotspot, with high disease burden attributed to genetic, environmental, and dietary factors³. Late diagnosis,

nonspecific symptoms, and limited effective therapies contribute to dismal survival outcomes⁴. Radical cholecystectomy is the only potentially curative option, yet most patients present at unresectable stages⁵.

Advances in molecular oncology have identified several signalling pathways that drive GBC progression. Among these, the **epidermal growth factor receptor (EGFR)** and **KRAS** genes play key roles in tumor cell proliferation and invasion^{6,7}. EGFR overexpression and KRAS activating mutations have been associated with aggressive behaviour and therapeutic resistance in colorectal, pancreatic, and biliary cancers^{8,9}. However, the prevalence and prognostic relevance of these alterations in Indian GBC cohorts remain underexplored^{10,11}. This study evaluates the frequency and clinicopathological associations of EGFR and KRAS alterations in gallbladder carcinoma and its relevance across disease stage, aiming to provide region-specific molecular insight relevant to Indian patients.

Materials and Methods

This prospective observational study was conducted at a tertiary hepatobiliary center in northern India from 2018 to 2021, in collaboration with the Departments of Pathology and Radiology, after obtaining approval from the Institutional Ethics Committee. A total of 85 consecutive patients aged 18–70 years with histologically confirmed gallbladder adenocarcinoma were included after obtaining written informed consent. Patients with non-adenocarcinoma histology or those unwilling to participate were excluded.

Baseline demographic, clinical, and biochemical parameters were recorded for all patients. Radiological evaluation was performed using contrast-enhanced computed tomography to assess local tumor extent, lymph node involvement, and distant metastasis. Based on clinical and imaging findings, patients were categorized as resectable, unresectable, or metastatic. Patients with resectable disease underwent radical cholecystectomy with standard lymphadenectomy, while selected cases required bile duct excision or limited hepatic resection depending on tumor extent to achieve R0 resection. Patients with unresectable or metastatic disease underwent biopsy or appropriate palliative interventions. Histopathological evaluation included tumor differentiation, lymphovascular invasion,

perineural invasion, nodal status, and resection margins. Tumors were staged according to the American Joint Committee on Cancer (AJCC) 8th edition TNM classification. For molecular analysis, EGFR expression was assessed on paraffin-embedded tissue sections (3–5 µm) using the Ventana Benchmark XT system with OptiView DAB detection. Membranous staining in ≥30% of tumor cells with moderate to strong intensity (2+/3+) was considered positive. KRAS mutation analysis was performed on DNA extracted from tumor tissue using real-time polymerase chain reaction targeting hotspot mutations in codons 12 and 13 (including G12D, G12V, and G13D).¹² Statistical analysis was performed using IBM SPSS Statistics, Version 25.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation, while categorical variables were presented as frequencies and percentages. Associations between variables were analyzed using the chi-square test or Fisher's exact test, as appropriate. A p-value of <0.05 was considered statistically significant.

Results

A total of 85 patients were included in the study, of which 19 (22.4%) males and 66 (77.6%) female patients. Their mean age was 53.1±11.82 years, range 24 – 81 years (Graph 1 and 2). Patients were symptomatic for a mean duration of 2.3±1.9 months (range 0.4 – 16 months) prior to presentation. The commonest presenting symptom was abdominal pain, followed by anorexia, jaundice and Gastric outlet obstruction (Table 1,2). Cholelithiasis was present in 44 (51.8%) patients. Among the patients presenting with pain, only 17.6% patient had previous episode of biliary colic. At presentation, 49 patients (57.6%) were categorized as resectable, 14 (16.5%) as unresectable, and 22 (25.9%) as metastatic based on imaging (Table 3, Graph 3). On imaging in the Resectable group, polypoidal growth and irregular thickening were the most common finding. Most common location of tumour-fundus and body. Duodenum and colon are the most common organ involved apart from liver bed (14.5%). In Unresectable group, most tumour present as mass replacing the gall bladder (92.2%) with vascular involvement in 21.4% and other organ involvement in 35.6% patients and in metastatic group, Liver

metastasis was picked up on Contrast Enhanced computed imaging (CECT) in 27.3% and ascites in 13.6% patients. Vascular involvement in 13.3%. All the patients had positive peri choledochal nodes and 50% had positive inter-aortocaval nodes.(Table 4) Median CA 19-9 levels at presentation in potentially resectable group were 190.5 U/mL while the mean value of CA 19.9 in unresectable and metastatic group were 577.20 U/ml and 29200 U/ml (Table 5, Graph 4). Among the resectable group, 40 (47.1%) patients underwent radical cholecystectomy only and 6 patients (7.1%) required extra-hepatic bile duct excision, 1 with positive cystic duct margin, 4 due to large nodes causing Common Bile Duct(CBD) compression with jaundice, 1 due to tumour embolus in CBD. 3.5% (n=3) underwent additional organ resection for negative margins(Table 6). Sixteen patients were taken up for staging as on pre-operative imaging appearing resectable. On staging laparoscopy , 13 patient was metastatic so only laparoscopic biopsy was done. 3 patient had positive inter-aortocaval nodes positive on frozen section and the procedure was abandoned. Two patient underwent palliative cholecystectomy in view of empyema gall bladder and one underwent loop gastrojejunostomy for symptomatic Gastric outlet obstruction. Major morbidity rate for operable patients (Clavien Dindo Grade >III) was 1.2% (n=1) and mortality rate 3.5 % (n = 3) with a median hospital stay of 6 days (range 4-34 days). Seventeen patient had minor complication including Superficial Surgical Site Infection (SSSI) in 11 patients , Minor Bile leak from liver bed in 5 patients which was managed conservatively. One patient with colonic resection due to involvement developed mild leak on Post Operative day (POD) 4 and was managed conservatively(Table 7). One patient with Roux en Y Hepaticojejunostomy(HJ) had bile in POD 2 which was followed by severe haemorrhage on POD7 and needed emergency re exploration. Bleeding was from Right hepatic artery which was controlled and patient survived . Out of 3 patients with Child-Turcotte-Pugh (CTP) A , one decompensated in post-operative period and died on POD 12.

TNM staging of the resected specimen as shown in Table -8, with majority of resectable tumours were T1b,T2 and T3 disease. Stage II disease was the most common (30.6%), followed by Stage I (26.5%) and Stage IIIB (18.4%).

Histopathological examination revealed that 42.4% of tumors were well differentiated, 32.9% moderately differentiated, and 24.7% poorly differentiated(Table 9). Lymphovascular invasion and perineural invasion were present in 57.6% and 34.1% of cases, respectively(Table 10,Graph 5), while lymph node metastases were identified in 30.6%.

Molecular analysis demonstrated EGFR overexpression in 28 (32.9%) patients and KRAS mutations in 17 (20%). EGFR positivity was observed across disease stages, including 30.6% of resectable, 35.7% of unresectable, and 36.4% of metastatic cases(Table 11). In contrast, KRAS mutations were more frequently associated with advanced disease, being present in only 4% of resectable cases compared to 42.8% of unresectable and 40.9% of metastatic cases. The most common KRAS variants were G12D and G12V, while co-expression of EGFR and KRAS alterations was uncommon (<5%) (Table 12,13). On correlation analysis, EGFR overexpression showed a significant association with lymphovascular invasion ($p = 0.03$) and higher tumor grade, but not with other baseline parameters. KRAS mutations demonstrated a strong association with unresectability and advanced disease stage ($p < 0.001$), as well as with poor tumor differentiation ($p = 0.01$). Co-expression of EGFR and KRAS alterations did not show statistical significance ($p = 0.21$) (Table 14,15).

Discussion

GBC remains a highly aggressive malignancy with a marked geographical predilection for northern India⁽⁶⁾. The demographic profile in our cohort demonstrated a strong female predominance and a mean age in the fifth decade, consistent with established epidemiological trends in high-incidence regions of India^{1,3}. The mean age of presentation of GBC in India is younger than their counterparts in the USA and western European countries. The average age at diagnosis in India was 51 ± 11 years in contrast to 71.2 ± 12.5 years in the West⁽³⁾. The average age of presentation 53.5 years (range 24-81) in our study. Hence, a high index of suspicion is required even in younger patients in high incidence areas. There is an increasing trend in GBC incident rates in Northern Indian in both genders⁽⁶⁾.

The high prevalence of gallstones (52%) further supports their well-recognized role as a major etiological factor in gallbladder carcinogenesis. Most

patients presented with advanced disease, with nearly 42% being unresectable or metastatic at diagnosis, underscoring the aggressive nature of this malignancy and the challenges associated with early detection⁽⁷⁾. Jaundice was present in 4.1% in resectable patients whereas in locally advanced unresectable and metastatic group, jaundice was present in 85.7% and 59.1%. respectively(**p=0.001**). The presence of jaundice suggests malignant invasion of the biliary tree and majority will have disseminated disease. These patients usually require resection of common bile duct and are associated with positive margins in almost 40% cases⁽⁸⁾. However, in a study by Nundy *et al.*⁽⁹⁾, curative resection with negative margins was possible in 50% cases of gallbladder cancer patients presenting with jaundice. In view of these contrasting results, gallbladder cancer patients presenting with jaundice should not be denied a curative resection, rather a meticulous evaluation for resectability should be considered.

Abdominal lump was present in 28.2% although it was more in unresectable group (**p=0.002**). Median CA 19-9 levels at presentation in potentially resectable group were 190.5 U/mL while the mean value of CA 19.9 in unresectable and metastatic group were 577.20 U/ml and 29200 U/ml(**p<0.001**). High resolution cross-sectional imaging is essential for adequate staging. A good imaging would avoid unnecessary laparotomies in patients with advanced disease⁽¹⁰⁾. Both CECT and MRI with MRCP are helpful in identifying metastatic disease, as well as defining the extent of primary lesion. In our study, most common finding on CECT in resectable group was polypoidal mass(42.9%) or irregular thickening (40.8%) whereas in unresectable group mass replacing the Gall bladder fossa with other organ involvement was the most common findings(92.9%) (**p <0.001**)

FDG-PET scanning with CT (PET/CT) combines metabolic and anatomical localizations of suspicious lesions. It has been shown to detect 95.9% of primary gallbladder cancers, 85.7% of lymph node involvement, and 95.9% of metastatic disease⁽¹¹⁾. is combination of diagnostic imaging can be used (a) preoperatively to define the possibility of curative surgery and (b) in patients postoperatively for restaging. Positron emission tomography (PET)-CT scan may help detecting metastatic disease which is occult on CT scan. However, PET scan has significant false positive results and therefore histological

confirmation of metastases should always be considered before denying a patient curative surgery.

Surgery

Complete surgical tumour resection is the only curative treatment for GBC. A complete resection is challenging as the gallbladder has anatomically neighbouring vital structures such as the porta hepatica, and this malignancy has a propensity for hepatic invasion with early lymphatic metastases. The role of Staging laparoscopy cannot be stressed more as even in the era of advanced imaging especially in cases of locally advanced resectable patients on imaging due to aggressive tumour biology. It has high accuracy for detecting peritoneal and liver surface metastases. In a study⁽¹⁰⁾ staging laparoscopy obviated a non-therapeutic laparotomy in 23% of gallbladder cancer patients. We had 12.3% inoperability rate on staging laparoscopy in which peritoneal/liver metastasis were present. Our low rate as compared to other studies can be explained on increased use of PET- CT scan and most patient had positive Interoartocaval nodes which were detected after open sampling.

The depth of tumour invasion, lymph node metastasis, histologic type of cancer cells, and TNM stages are the prognostic factors affecting clinical prognosis of patients with gallbladder carcinoma. Although most traditional gastrointestinal adenocarcinomas are classified as either differentiated or undifferentiated, biliary tract cancer is predominately a well-differentiated adenocarcinoma with a minor component of poor differentiation, thus allowing venous, lymphatic, and perineural invasion in “advanced disease” that is not as readily apparent in “early” biliary cancers. Intramural invasion can be classified as either in infiltrative growth-type or destructive growth-type, the latter conferring a worse overall prognosis. The gross finding, in addition to TNM classification, is known as an important prognostic factor. If the gross finding of gallbladder carcinoma is infiltrative after curative resection, it can be expected that tissue differentiation is bad, and the carcinoma is likely to be at an advanced stage⁽⁵⁾. In our study, resectable tumours were polypoidal or nodular on gross appearance while the unresectable were diffusely infiltrating or scirrhous. More evidence of both LVI and PNI was present in patients with poorly differentiated tumour. LVI (34.7% vs 95.5% **p<0.001**) in resectable as compared to metastatic group. Similarly PNI (20.4% vs 59.1% ,**p 0.005**) in resectable

as compared to metastatic group. EGFR overexpression was identified in approximately one-third of patients in our study, which is comparable to previously reported rates ranging from 25% to 60% in biliary tract cancers⁽¹⁵⁾. EGFR plays a central role in tumor proliferation, angiogenesis, and invasion through activation of downstream signaling pathways such as RAS-RAF-MEK-ERK and PI3K-AKT^{6,7}. In our cohort, EGFR positivity showed a significant association with lymphovascular invasion and higher tumor grade, suggesting its role in promoting aggressive tumor biology. However, its lack of strong association with stage may indicate that EGFR-driven pathways contribute more to tumor invasiveness than to metastatic spread alone. Mutation of K-ras was present in 20% cases in our study. Most common were Mutations in the second nucleotide of exon 2, 4 single nucleotide variant (SNP). In our study, the frequency of KRAS mutation in BTCs is similar to the reports of previous studies. As most of previous studies, we failed to observe a correlation between KRAS mutations with clinicopathological factors in patients with GBC.⁽¹⁹⁾

KRAS mutations were observed in 20% of cases and demonstrated a strong association with advanced disease, unresectability, and poor differentiation. These findings are consistent with prior studies indicating that KRAS mutations are linked to tumor progression and adverse prognosis in gastrointestinal malignancies^(12,14). Notably, KRAS-mutant tumors were predominantly seen in unresectable and metastatic groups, reinforcing their role as a marker of aggressive disease. The predominance of codon 12 mutations (G12D and G12V) aligns with molecular patterns described in other gastrointestinal cancers. We examined the relation between gene mutation status and various clinicopathological features, including age at diagnosis, degree of differentiation, stage of tumour and gender. There was no significant association of any of the studied mutations with clinicopathological features except that KRAS mutations were found to be statistically significantly associated with advanced stage GBC patient. ($p=0.007$). An important observation in our study was the low frequency of co-expression of EGFR and KRAS alterations, suggesting that these represent distinct molecular subsets of GBC. This molecular heterogeneity has significant therapeutic implications. EGFR overexpression may identify a subgroup of

patients who could potentially benefit from EGFR-targeted therapies, whereas the presence of KRAS mutations is known to confer resistance to such treatments by activating downstream signaling independent of receptor inhibition⁽¹¹⁾. From a therapeutic standpoint, EGFR-targeted agents such as erlotinib, gefitinib, cetuximab, and panitumumab have shown variable efficacy in biliary tract cancers and warrant further evaluation in selected GBC patients⁽¹⁹⁻²¹⁾. Conversely, KRAS-mutant tumors may benefit from alternative strategies targeting downstream pathways, including MEK inhibitors such as trametinib or multikinase inhibitors like sorafenib and regorafenib⁽²²⁻²⁴⁾. Although these approaches are not yet standard of care in GBC, the growing understanding of tumor genomics provides a strong rationale for incorporating molecular profiling into routine clinical practice and for designing biomarker-driven clinical trials.

The findings of this study have important clinical implications, particularly in high-incidence regions such as northern India. Routine assessment of EGFR and KRAS status could help stratify patients based on tumor biology, guide therapeutic decision-making, and identify candidates for targeted therapy. This is especially relevant given the limited effectiveness of conventional chemotherapy in advanced GBC. However, this study has certain limitations. It is a single-center study with a relatively modest sample size, which may limit generalizability. Additionally, long-term survival outcomes and response to targeted therapies were not assessed. Future multicentric studies with larger cohorts and incorporation of advanced genomic techniques such as next-generation sequencing are needed to validate these findings and further elucidate the molecular landscape of GBC.

Conclusion

EGFR and KRAS alterations are frequent in gallbladder carcinoma and correlate with tumor aggressiveness.

Their identification not only refines prognostication but also provides a framework for exploring **targeted agents** such as **EGFR inhibitors (erlotinib, gefitinib, cetuximab)** in EGFR-positive cases and **MEK-pathway inhibitors (trametinib)** in KRAS-driven tumors. Integration of molecular testing into the routine diagnostic workflow can help personalize therapy and

improve outcomes in high-incidence regions like northern India.

ABBREVIATIONS

GBC- Gallbladder carcinoma

EGFR- Epidermal growth factor receptor

AJCC- American Joint Committee on Cancer

CECT- Contrast Enhanced Computed Imaging

CBD- Common Bile Duct

SSSI- Superficial Surgical Site Infection

POD- Post Operative day

HJ- Hepaticojejunostomy

CTP- Child-Turcotte-Pugh

BTC- Biliary Tract cancer

PET- Positron emission tomography

SNP- single nucleotide variant

Additional Note on Study Period

The study was conducted between 2018 and 2021. Data analysis and manuscript preparation were completed subsequently following extended molecular validation and COVID-19-related delays.

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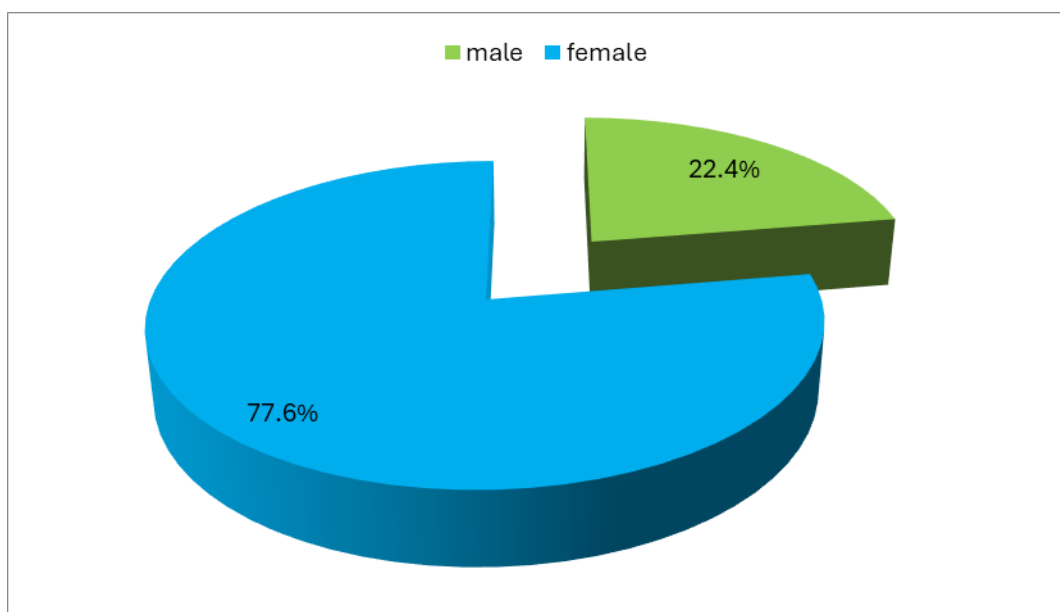
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GRAPH 1- Sex distribution of overall patient population



GRAPH 2- Age-wise distribution of study population

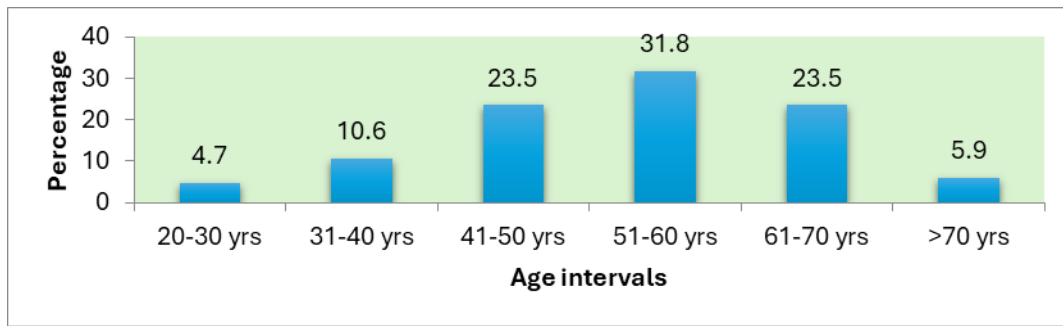


TABLE 1- Symptoms and Presentations of Study Population

Symptoms	Resectable	Unresectable	Metastatic
Abdominal Pain	85.70%	57.10%	72.70%
Palpable Lump	14.3%	57.1%	40.9%
Juandice	4.1%	85.7%	59.1%
Gastric outlet obstruction	2.0%	21.4%	4.5%
Loss of weight/Loss of appetite	26.5%	92.9%	68.2%

TABLE- 2 Univariate Analysis: Preoperative Factors

PARAMETER		Group								p-value
		resectable		unresectable		metastatic		Total		
		N	%	N	%	N	%	N	%	
Lump	absent	42	85.7%	6	42.9%	13	59.1%	61	71.8%	0.002
	present	7	14.3%	8	57.1%	9	40.9%	24	28.2%	
Jaundice	absent	47	95.9%	2	14.3%	9	40.9%	58	68.2%	<0.001
	present	2	4.1%	12	85.7%	13	59.1%	27	31.8%	
GOO	absent	48	98.0%	11	78.6%	21	95.5%	80	94.1%	0.024
	present	1	2.0%	3	21.4%	1	4.5%	5	5.9%	
LOA/LOW	absent	36	73.5%	1	7.1%	7	31.8%	44	51.8%	<0.001
	present	13	26.5%	13	92.9%	15	68.2%	41	48.2%	
H/o GSD	absent	38	77.6%	13	92.9%	19	86.4%	70	82.4%	0.353
	present	11	22.4%	1	7.1%	3	13.6%	15	17.6%	

TABLE 3- Distribution of patients into groups

Groups	Frequency	Percentage
Resectable	49	57.6
Unresectable	14	16.5
Metastatic	22	25.9

GRAPH 3- Subdivision of groups

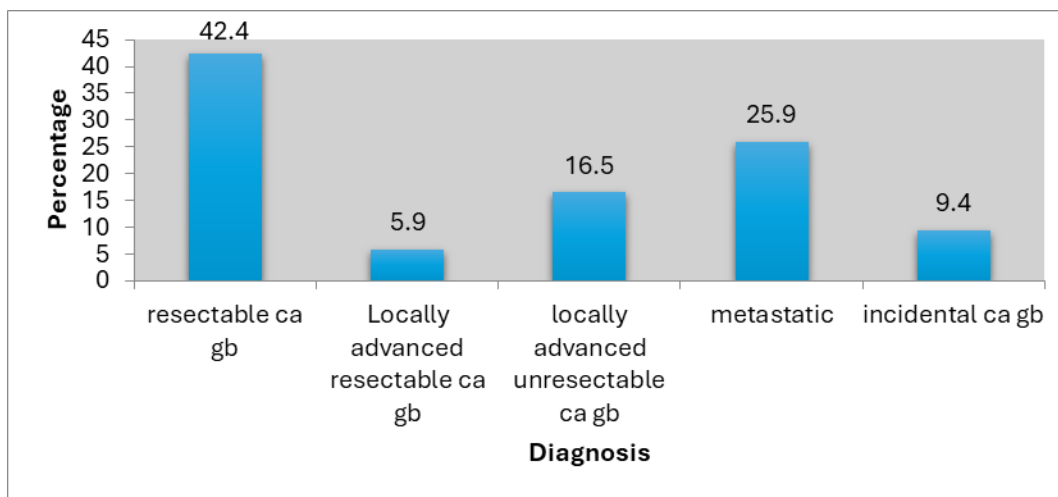


TABLE 4- Univariate Analysis : Preoperative Imaging

		Group								p-value
		resectable		unresectable		metastatic		Total		
		N	%	N	%	N	%	N	%	
CECT growth	polypoidal	21	42.9%	1	7.1%	2	9.1%	24	28.2%	<0.001
	Irregular thickening/ Mass replacing GB	20	40.8%	13	92.9%	20	90.9%	53	62.4%	
	no residual mass	8	16.3%	0	0%	0	.0%	8	9.4%	
location on CT	fundus	17	34.7%	0	0%	5	22.7%	22	25.9%	0.001
	body	8	16.3%	1	7.1%	3	13.6%	12	14.1%	
	neck	2	4.1%	4	28.6%	3	13.6%	9	10.6%	
	fundus body	13	26.5%	3	21.4%	4	18.2%	20	23.5%	
	neck body	2	4.1%	6	42.9%	7	31.8%	15	17.6%	
	No residual mass	7	14.3%	0	0%	0	.0%	7	8.2%	
LN status	absent	28	57.1%	0	.0%	0	.0%	28	32.9%	<0.001
	present	21	42.9%	14	100.0%	22	100.0%	57	67.1%	
Vascular	absent	49	100.0%	11	78.6%	19	86.4%	79	92.9%	0.008
	present	0	.0%	3	21.4%	3	13.6%	6	7.1%	
other organ	No organ	42	85.7%	9	64.3%	9	40.9%	60	70.6%	<0.001
	duo	5	10.2%	2	14.3%	4	18.2%	11	12.9%	
	colon	0	.0%	1	7.1%	3	13.6%	4	4.7%	
	Duodenum & colon	2	4.1%	2	14.3%	0	.0%	4	4.7%	
	liver metastasis	0	.0%	0	.0%	6	27.3%	6	7.1%	
IAS	No	40	81.6%	12	85.7%	6	27.3%	58	68.2%	<0.001
	positive	0	.0%	0	.0%	11	50.0%	11	12.9%	
	Subcentric LN	9	18.4%	2	14.3%	5	22.7%	16	18.8%	
Ascitis	absent	49	100.0%	14	100.0%	19	86.4%	82	96.5%	0.012

	present	0	.0%	0	.0%	3	13.6%	3	3.5%
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TABLE- 5 Univariate Analysis: Preoperative Factors- Tumour markers

	Group						p-value
	resectable		unresectable		metastatic		
	Mean	SD	Mean	SD	Mean	SD	
CA 19.9	190.50	828.67	577.20	1461.23	29200	121884.47	<0.001
CEA	2.00	3.05	9.83	9.60	112.51	292.51	<0.001

GRAPH 4- Tumour markers- CA 19-9 levels in different groups

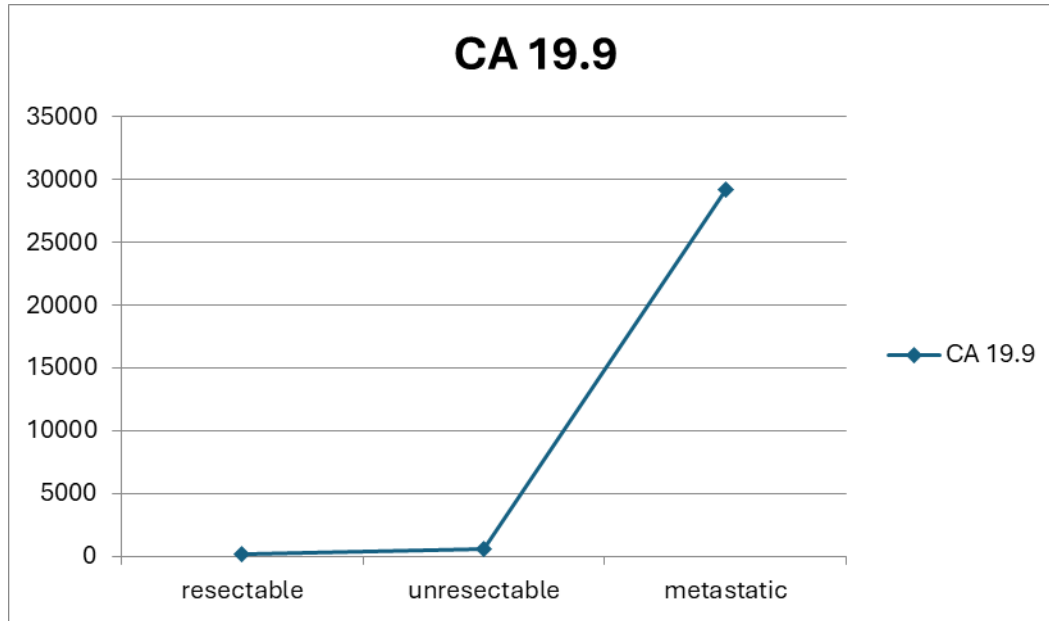


TABLE 6- Interventions in study population

Radical cholecystectomy	40	47.1%
Radical cholecystectomy with CBD excision	6	7.1%
Radical cholecystectomy with other organ excision	3	3.5%
Only Biopsy	16	18.8%
PTBD with biopsy	16	18.8%
Palliative resection	3	3.5%
Conservative	1	1.2%

TABLE 7- Complications in Surgery

No complication	92.90%
Expired	3.50%
Bile leak	1.20%
Colonic leak	1.20%
Post-operative bleed (hepatic artery)	1.20%

TABLE 8- TNM Staging in resectable group

T	T1a	3	6.1%
	T1b	9	18.4%
	T2	20	40.8%
	T3	14	28.6%
	T4	2	4.1%
	Tis	1	2.0%
N	N0	34	69.4%
	N1	10	20.4%
	N2	5	10.2%
M	M0	49	100.0%
	M1	0	.0%

TABLE 9- Tumour differentiation among different groups

		Group								p-value
		resectable		unresectable		metastatic		Total		
		N	%	N	%	N	%	N	%	
differentiation	WD	30	61.2%	4	28.6%	2	9.1%	36	42.4%	<0.001
	MD	14	28.6%	5	35.7%	9	40.9%	28	32.9%	
	PD	5	10.2%	5	35.7%	11	50.0%	21	24.7%	

GRAPH 5 Tumour Differentiation between different groups

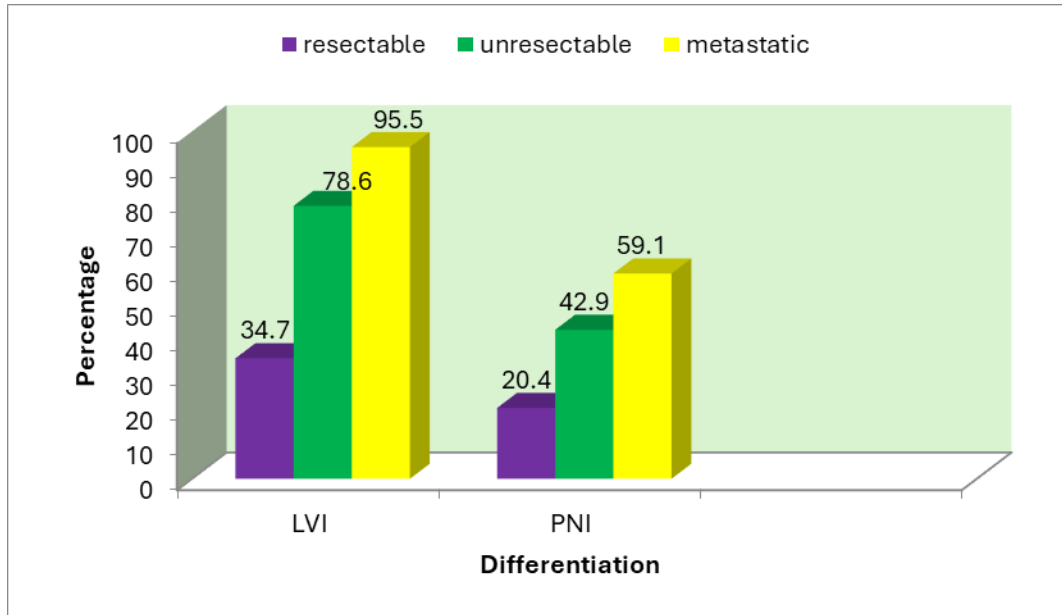


TABLE 10- Difference Of LVI And PNI Among Different Groups

		Group								p-value
		resectable		unresectable		metastatic		Total		
		N	%	N	%	N	%	N	%	
LVI	ABSENT	32	65.3%	3	21.4%	1	4.5%	36	42.4%	<0.001
	PRESENT	17	34.7%	11	78.6%	21	95.5%	49	57.6%	
PNI	ABSENT	39	79.6%	8	57.1%	9	40.9%	56	65.9%	0.005
	PRESENT	10	20.4%	6	42.9%	13	59.1%	29	34.1%	

TABLE 11- EGFR And KRAS Difference Among Different Groups

		resectable		unresectable		metastatic		Total		
		N	%	N	%	N	%	N	%	
EGFR	ABSENT	34	69.4%	9	64.3%	14	63.6%	57	67.1%	0.867
	PRESENT	15	30.6%	5	35.7%	8	36.4%	28	32.9%	
KRAS	ABSENT	47	95.91%	8	57.1%	13	59.1%	68	80.0%	0.007
	PRESENT	2	4.09%	6	42.8%	9	40.9%	17	20.0%	

TABLE 12- LIST OF COMMON MUTATIONS IN EGFR

EGFR Mutation

type	Exon	Genotype	AA change	Locus
SNV	19	c.2252C>T	p.Thr751Ile	chr7:55249063
SNV	19	c.2268C>T	p.Asn756=	chr7:55242479
SNV	19	c.2227G>A	p.Ala743Thr	chr7:55242457
SNV	20	c.2369C>T	p.Thr790Met	chr7:55249071
SNV	19	c.2227G>A	p.Ala743Thr	chr7:55242457
synonymous	20	c.2361G>A	p.Gln787=	chr7:55249063
synonymous	20	c.2361G>A	p.Gln787=	chr7:55249063
missense	18	c.2092G>A	p.Ala698Thr	chr7:55241644
missense	19	c.2236G>A	p.Glu746Lys	chr7:55242462
missense	18	c.2092G>A	p.Ala698Thr	chr7:55241644
missense	18	c.2092G>A	p.Ala698Thr	chr7:55241644

TABLE 13 - LIST OF COMMON MUTATIONS IN KRAS

KRAS Mutation				
type	Exon	Genotype	AA change	Locus
SNV	2	c.40G>A	p.Val14Ile	chr12:25398279
SNV	2	p.Gly12Asp	c.35G>A	chr12:25398280
SNV	2	p.Ala11Val	c.32C>T	chr12:25398287
SNV	4	c.413G>A	p.Gly138Glu	chr12:25378585
SNV	2	c.35G>A	p.Gly12Asp	chr12:25398284
SNV	2	c.40G>A	p.Val14Ile	chr12:25398279

Table 14. Correlation of EGFR Expression with Clinicopathological Parameters

Parameter	EGFR Positive (n = 28)	EGFR Negative (n = 57)	p-value
Age > 50 years	18	30	0.21
Gender (Female)	23	43	0.41
Gallstones present	16	28	0.53
Poor differentiation	12	9	0.03*
Lymphovascular invasion	20	29	0.03*
Stage (III–IV)	17	22	0.06

Table 15. Correlation of KRAS Mutation with Clinicopathological Parameters

Parameter	KRAS Positive (n = 17)	KRAS Negative (n = 68)	p-value
Resectable disease	2	47	< 0.001*
Unresectable/metastatic	15	21	< 0.001*
Poor differentiation	11	10	0.01*
Lymphovascular invasion	13	36	0.04*