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RESEARCH ARTICLE

Clinicopathologic Characteristics and Survival Outcomes of Gallbladder and Biliary Tract Cancers: A 21-Year Single-Center Retrospective Cohort in Northern Iran

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Abstract

Introduction: Gallbladder cancer (GBC) and biliary tract cancers (BTC) are uncommon but aggressive malignancies often diagnosed at advanced stages and associated with poor survival. Their epidemiological patterns vary geographically, and long-term survival data from referral centers in Iran remain limited. This study aimed to evaluate clinicopathologic characteristics, survival outcomes, and identify independent prognostic factors in patients with GBC and BTC over a 21-year period in northern Iran. **Materials and Methods:** This retrospective cohort included all patients diagnosed with primary GBC or BTC and treated at the Shahid Rajaee Radiotherapy Center, Babolsar, between 2004 and 2024. Demographic, clinical, and Pathologic variables were retrieved from medical records. Survival status was confirmed through telephone follow-up and civil registry verification. Kaplan–Meier analysis and log-rank tests were used to compare survival, while Cox proportional hazards regression identified predictors of mortality. **Results:** A total of 63 patients met the eligibility criteria. The mean age was 58.16 ± 11.86 years, with nearly equal sex distribution. GBC accounted for 55.6% of cases. The overall survival and 1-, 2-, and 5-year survival rates were 38.1%, 81.0%, 61.9%, and 46.0%, respectively. The median overall survival was 20 months for GBC and 54 months for BTC (p=0.508). Higher tumor grades (Grade 3 and Grade 4) were associated with significantly increased hazard of death (all p≤0.002), whereas age, sex, histological subtype, and metastatic status did not independently predict mortality in multivariable analysis. **Conclusion:** Tumor grade emerged as the strongest prognostic factor in GBC and BTC. multi-center studies with detailed staging and treatment data are needed to refine prognostic models.

Keywords: Gallbladder Cancer- Biliary Tract Cancer- Clinicopathologic Characteristics- Survival Analysis- Tumor Grade

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Introduction

Biliary tract cancers (BTCs) represent some of the rarest and most aggressive malignancies of the gastrointestinal system, typically diagnosed at advanced stages and associated with a poor prognosis [1, 2]. Among them, gallbladder cancer (GBC) is the most frequent neoplasm of the biliary tract, accounting for approximately 80–95% of all biliary tract malignancies [3]. According to the GLOBOCAN 2022 report, an estimated 122,462 new cases of GBC were recorded worldwide, ranking 22nd in incidence and 20th in mortality among all cancers [4]. The highest incidence rates have been reported in Chile, China, India, Japan, and South Korea [5], whereas the disease remains relatively uncommon in Western Europe and North America [6].

In Iran, BTCs constitute around 0.5% of all newly diagnosed cancers as of 2020. Trend analyses indicate

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a decreasing incidence between 2000 and 2016, accompanied by an increasing mortality rate from 1990 to 2015 [7, 8]. Given this discrepancy, identifying epidemiological patterns of incidence, mortality, and survival among high-risk populations is crucial for developing effective prevention strategies and guiding national cancer control programs [9].

BTCs encompass a heterogeneous group of malignancies, including intrahepatic cholangiocarcinoma (iCCA), extrahepatic cholangiocarcinoma (eCCA), gallbladder cancer (GBC), and ampulla of Vater carcinoma (AVC) [10, 11]. Cholangiocarcinomas are adenocarcinomas arising from the epithelial lining of intrahepatic and extrahepatic bile ducts. Approximately two-thirds of cases are extrahepatic, occurring either at the hepatic hilum (Klatskin tumors) or more distally along the biliary tree [12, 13].

Most GBCs are adenocarcinomas originating from the gallbladder mucosa, progressing through a metaplasia–dysplasia–carcinoma sequence [14]. Due to the gallbladder's anatomical location and the absence of specific symptoms in early stages, diagnosis is often delayed [15]. By the time of detection, the disease typically invades the liver or regional lymph nodes of the hepatic portal region [1]. Consequently, the prognosis remains poor, with a median overall survival of about six months following diagnosis [3, 14].

Several established risk factors contribute to BTC development, including gallstones, congenital biliary cysts, and chronic Helicobacter pylori infection. Gallstones are the predominant risk factor, observed in approximately 80% of GBC cases. Moreover, chronic liver conditions such as cirrhosis and viral hepatitis further increase the susceptibility to these malignancies [16-18].

Despite the clinical significance of BTCs, long-term epidemiological data on incidence, mortality, and survival trends remain limited in Iran, particularly within oncology and radiotherapy referral centers. Although international cohorts have consistently identified tumor grade and stage as major prognostic determinants, outcome data specific to Iranian patient populations especially those treated in tertiary radiotherapy centers remain scarce. This gap limits understanding of how regional epidemiology, patterns of referral, and treatment availability may shape prognostic profiles in this setting.

Therefore, this study aimed to characterize the 21-year clinicopathologic patterns and survival outcomes of patients with biliary tract cancers treated at the Shahid Rajaee Radiotherapy Center in Babolsar, northern Iran. We hypothesized that clinicopathologic patterns and key prognostic factors in this cohort would reflect both the unique regional epidemiology of northern Iran and the treatment context of a tertiary radiotherapy referral center.

Materials and Methods

Study Design and Setting

This retrospective cohort study included all patients treated at the Shahid Rajaee Radiotherapy Center in Babolsar, Mazandaran Province, northern Iran, between March 2004 and December 2024, encompassing a 21-year period. This center is one of the major oncology and radiotherapy referral hospitals in northern Iran, serving not only local patients from Mazandaran Province but also referred cases from neighboring provinces.

All eligible patients diagnosed with primary gallbladder or biliary tract cancers during the study period were identified through a census sampling approach. Medical records with incomplete information, Pathologic findings unrelated to gallbladder or biliary tract malignancies, or cases of secondary (metastatic) cancers were excluded. The primary outcome was overall survival (OS) and mortality, assessed at 1-, 2-, and 5-year intervals. Associations between patients' demographic, Pathologic, and clinical characteristics and survival outcomes were also evaluated.

The study protocol was approved by the Ethics Committee of Babol University of Medical Sciences (Approval Code: IR.MUBABOL.HRI.REC.1403.303). All procedures complied with ethical research standards, and patients' data were anonymized and analyzed with strict confidentiality.

Data Collection

Data were retrospectively extracted from the electronic medical records of patients diagnosed with primary gallbladder and biliary tract cancers at the Shahid Rajaee Center. The following variables were collected:

• Demographic variables: age, sex (male vs. female), marital status (single vs. married), and place of residence (urban vs. rural);

• Pathologic and clinical characteristics: histopathologic type (adenocarcinoma for gallbladder; adenocarcinoma, cholangiocarcinoma, or neuroendocrine carcinoma for biliary tract), tumor grade, and presence of distant metastasis;

• Survival and mortality information: vital status and date of death (if applicable).

Because the exact timing of metastasis (at diagnosis, during treatment, or after treatment) was not consistently documented in medical records, it was recorded as a binary variable (present/absent).

Overall survival (OS) was defined as the time interval from the initial Pathologic diagnosis to death from any cause or the last known follow-up. OS rates were calculated at 1-, 2-, and 5-year intervals. To obtain complete follow-up data on survival status and cause of death, structured telephone interviews were conducted with patients' families. When further confirmation of death date was required, official records were retrieved from the Mazandaran Civil Registration Organization under authorized access.

Statistical Analysis

Descriptive statistics were used to summarize study data. Continuous variables were presented as mean ± standard deviation (SD) or median (interquartile range, IQR), while categorical variables were reported as frequencies and percentages. The Kolmogorov–Smirnov test was applied to assess the normality of data distribution.

Associations between categorical variables were analyzed using the Chi-square test or Fisher's exact test (when expected cell counts were<5). For ordinal variables, the Cochran–Armitage trend test was employed. Survival analysis was performed using the Kaplan–Meier method, and group differences were compared using the log-rank test. Cox proportional hazards regression was applied to examine the effects of prognostic variables on survival, and the proportional hazards assumption was evaluated to confirm the appropriateness of the model.

All data management was performed in Microsoft Excel 2019, and statistical analyses were conducted using SPSS software, version 27.0 (IBM Corp., Armonk, NY, USA). A two-tailed p-value<0.05 was considered statistically significant.

Results

A total of 105 patient records were initially identified in the hospital archives. After applying the inclusion and exclusion criteria, 63 cases were eligible for analysis, while 42 were excluded. These 105 records represent the subset of retrievable cases from the hospital's electronic registry, from which only patients with accurately documented and pathology-confirmed GBC or BTC were included based on predefined eligibility criteria. Also, Secondary malignancies were ascertained through integrated review of pathology reports, clinical assessment, and documentation provided by the radiation oncology specialist (Figure 1).

During the study period (2004–2024), the highest annual number of gallbladder and biliary tract cancer (GBC and BTC) cases was observed in 2022, with seven cases recorded, whereas in some years only a single case was reported. Overall, males accounted for a slightly higher proportion of cases compared to females (Figure 2).

Among the 63 included patients, 32 (50.8%) were male and 31 (49.2%) were female (male:female ratio ≈ 1:1). The mean age of the patients was 58.16 ± 11.86 years (range: 33–82). Figure 3 illustrates the age distribution. The mean age was 58.78 ± 12.50 years among males and 57.52 ± 11.33 years among females (Table 1).

Regarding marital status, 5 patients (7.9%) were single and 58 (92.1%) were married. Additionally, 42 patients

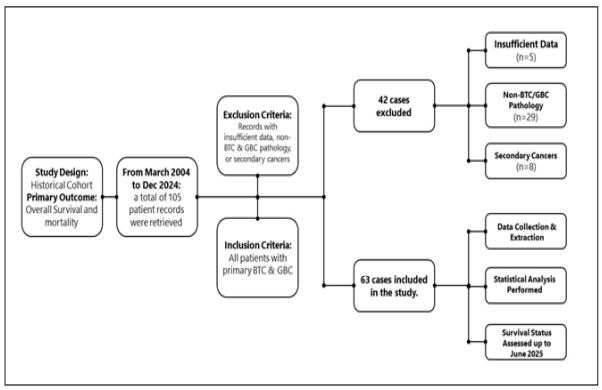

Figure 1. Flowchart Describing the Patient Selection Process, Including Inclusion and Exclusion Criteria and the Final Number of Analyzed Cases

(66.7%) resided in urban regions and 21 (33.3%) lived in rural areas. Among the pathology-confirmed cases, 35 (55.6%) were diagnosed with GBC (predominantly adenocarcinoma), while 28 (44.4%) had BTC, including adenocarcinoma (n=22), cholangiocarcinoma (n=5), and neuroendocrine carcinoma (n=1), arising from both intrahepatic and extrahepatic bile ducts (with 22 cases originating from extrahepatic ducts). Among BTC adenocarcinomas, 17 cases were extrahepatic, while 5 demonstrated both intrahepatic and extrahepatic involvement. Also, pathologic diagnosis was based solely on morphology and routine pathology reporting; many earlier cases lacked immunohistochemical (IHC) profiling due to limited availability of diagnostic resources at the time. Older pathology reports contained less detail, and several variables were recorded in a standardized manner only in more recent years.

As shown in Table 1, no significant differences were observed between males and females regarding mean age, marital status, residence type, histological subtype, tumor grade, metastatic status, or overall survival (p>0.05).

Tumor grading was extracted from pathology reports across a 21-year period, during which multiple diagnostic centers were involved; therefore, uniform grading standards may not have been consistently applied. Because the study was retrospective, it is also unclear whether assessments were performed by the same or by different pathologists.

Among the 63 patients, 29 cases (46.0%) were classified as Grade 1, 20 cases (31.7%) as Grade 2, 10 cases (15.9%) as Grade 3, and 4 cases (6.3%) as Grade 4, with Grade 1 being the most frequent. Overall, 12 patients (19.0%) had metastatic disease, while 51 patients (81.0%) had no evidence of metastasis. The most common metastatic sites were the liver (n=8), followed by bone (n=3) and lung (n=1).

As of June 2025, patient survival was assessed through telephone follow-up and verification with the Civil Registration Office of Mazandaran Province. Older reports lacked complete documentation of key variables such as TNM stage, lymph-node involvement, and treatment details; due to their limited numbers, these cases did not meet the threshold for inclusion in the survival analysis.

A total of 39 patients (61.9%) had died, while 24 (38.1%) were alive at the time of follow-up (Table 2). The 1-year, 2-year, and 5-year mortality rates were 19.0%, 38.1%, and 54.0%, respectively. The mean age did not significantly differ between deceased patients and survivors (58.90 ± 11.59 vs. 56.96 ± 12.45 years; p=0.533).

No significant associations were observed between sex, marital status, residence type, or histological subtype and mortality across any of the evaluated time intervals (p>0.05). Although mortality was slightly higher in BTC compared to GBC (64.3% vs. 60.0%), this difference was not statistically significant (p=0.728).

Tumor grade demonstrated a significant association with overall mortality (p=0.045), as well as with 1-year (p<0.001) and 2-year mortality (p=0.005). However, the association with 5-year mortality was not statistically significant (p=0.103). Metastatic status did not show a

Table 1. Demographic and Clinical Characteristics of Patients with Gallbladder and Biliary Tract Cancers, Overall and Stratified by Sex

			Sex		
Variables		Total	Male (n=32, 50.8%)	Female (n=31, 49.2%)	p-value
Age (year) (mean±SD)		58.16±11.86	58.78±12.50	57.52±11.33	0.676
Marital Status N (%)	Single	5 (7.9)	3 (9.4)	2 (6.5)	1
	Married	58 (92.1)	29 (90.6)	29 (93.5)	
Type of Residence N (%)	Urban	42 (66.7)	21 (65.6)	21 (67.7)	1
	Rural	21 (33.3)	11 (34.4)	10 (32.3)	
Tumor Side N (%)	GB	35 (55.6)	17 (53.1)	18 (58.1)	0.693
	BT	28 (44.4)	15 (46.9)	13 (41.9)	
Grade N (%)	Grade 1	29 (46.0)	15 (46.9)	14 (45.2)	0.873
	Grade 2	20 (31.7)	9 (28.1)	11 (35.5)	
	Grade 3	10 (15.9)	6 (18.8)	4 (12.9)	
	Grade 4	4 (6.3)	2 (6.3)	2 (6.5)	
Metastasis N (%)	No	51 (81.0)	25 (78.1)	26 (83.9)	0.561
	Yes	12 (19.0)	7 (21.9)	5 (16.1)	
Overall Mortality N (%)	Alive	24 (38.1)	11 (45.8)	13 (54.2)	0.537
	Death	39 (61.9)	21 (53.8)	18 (46.2)	

significant relationship with mortality at any follow-up interval (p>0.05).

The overall survival rate was 38.1%, while the 1-year, 2-year, and 5-year survival rates were 81.0%, 61.9%, and 46.0%, respectively. The median overall survival was 20 months for GBC and 54 months for BTC, with no statistically significant difference (Log-Rank p=0.508). Survival differed significantly by tumor grade, with median survival of 60 months (Grade 1), 30 months (Grade 2), 18 months (Grade 3), and 1 month (Grade 4) (Log-Rank p<0.001). Median overall survival was 26 months in males and 40 months in females (Log-Rank p=0.318).

Similar patterns were observed in 1-year, 2-year, and 5-year survival analyses: tumor grade remained significantly associated with survival, while histological category and sex did not (all Log-Rank p>0.05). (Figure 4)

In the Cox model (Table 3), age and sex were not significant predictors of mortality (p>0.05). Tumor grade, modeled with Grade 1 as the reference, showed a stepwise increase in mortality risk. Grade 2 was associated with increased overall mortality (HR=2.366; 95% CI: 1.039–5.384; p=0.040), while Grade 3 and Grade 4 showed markedly elevated mortality risks across all survival endpoints (all p<0.05). Histological subtype was associated with 2-year mortality (HR=2.834; 95% CI: 1.134–7.082; p=0.026), but not with overall, 1-year, or 5-year mortality. Metastatic status was not significantly associated with mortality at any interval (p>0.05).

Discussion

Principal findings

In this single-center retrospective cohort of 63 patients with gallbladder and biliary tract cancers (GBC and BTC), tumor grade emerged as the strongest and most consistent predictor of mortality across multiple time horizons. Higher grades were associated with markedly worse survival (Grade 2 → increased overall mortality; Grade 3 and 4 → large and statistically significant hazard increases across overall, 1-, 2- and 5-year endpoints). By contrast, histologic category (GBC vs. BTC) had a limited effect significant only for 2-year mortality and neither metastatic status nor patient sex or age were independently associated with mortality in multivariable Cox models. Overall survival in our cohort was poor (overall survival rate 38.1%; median OS 20 months for GBC vs 54 months for BTC), and 5-year mortality exceeded 50% in this sample.

Because this study was conducted in a tertiary cancer referral center, where most patients are referred from neighboring northern provinces, the findings may not be fully generalizable to the broader Iranian population. Additional studies from other healthcare settings and diverse geographic regions of Iran are needed to validate

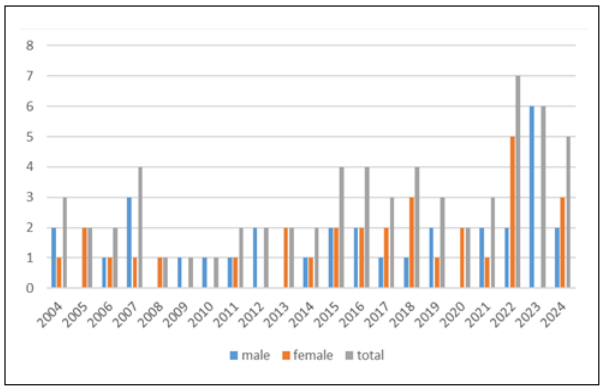

Figure 2. Annual distribution of GBC and BTC cases by sex at Shahid Rajai Radiotherapy Center, Babolsar (2004–2024).

Table 2. Association between Demographic and Clinical Variables and Overall, 1-year, 2-year, and 5-year Mortality among Patients with GBC and BTC.

Variables	Type	Total	Overall Mortality N (%)		1-Year Mortality N (%)		2-Year Mortality N (%)		5-Year Mortality N (%)	
			Alive (n=24)	Death (n=39)	Alive (n=51)	Death (n=12)	Alive (n=39)	Death (n=24)	Alive (n=29)	Death (n=34)
Sex	Male	n=32	11 (45.8)	21 (53.8)	24 (47.1)	8 (66.7)	18 (46.2)	14 (58.3)	14 (48.3)	18 (52.9)
	Female	n=31	13 (54.2)	18 (46.2)	27 (52.9)	4 (33.3)	21 (53.8)	10 (41.7)	15 (51.7)	16 (47.1)
	P-Value		0.537		0.222		0.348		0.712	
Marital Status	Single	n=5	1 (4.2)	4 (10.3)	5 (9.8)	0 (0.0)	1 (2.6)	4 (16.7)	1 (3.4)	4 (11.8)
	Married	n=58	23 (95.8)	35 (89.7)	46 (90.2)	12 (100)	38 (97.4)	20 (83.3)	28 (96.6)	30 (88.2)
	P-Value		0.641		0.573		0.065		0.363	
Type of Residence	Urban	n=42	17 (70.8)	25 (64.1)	35 (68.6)	7 (58.3)	29 (74.4)	13 (54.2)	21 (72.4)	21 (61.8)
	Rural	n=21	7 (29.2)	14 (35.9)	16 (31.4)	5 (41.7)	10 (25.6)	11 (45.8)	8 (27.6)	13 (38.2)
	P-Value		0.582		0.513		0.099		0.371	
Histology Category	GBC	n=35	14 (58.3)	21 (53.8)	28 (54.9)	7 (58.3)	19 (48.7)	16 (66.7)	16 (55.2)	19 (55.9)
	BTC	n=28	10 (41.7)	18 (46.2)	23 (45.1)	5 (41.7)	20 (51.3)	8 (33.3)	13 (44.8)	15 (44.1)
	P-Value		0.728		0.83		0.164		0.955	
Grade	Grade 1	n=29	15 (62.5)	14 (35.9)	27 (52.9)	2 (16.7)	23 (59.0)	6 (25.0)	17 (58.6)	12 (35.3)
	Grade 2	n=20	8 (33.3)	12 (30.8)	18 (35.3)	2 (16.7)	12 (30.8)	8 (33.3)	9 (31.0)	11 (32.4)
	Grade 3	n=10	1 (4.2)	9 (23.1)	6 (11.8)	4 (33.3)	4 (10.3)	6 (25.0)	3 (10.3)	7 (20.6)
	Grade 4	n=4	0 (0.0)	4 (10.3)	0 (0.0)	4 (33.3)	0 (0.0)	4 (16.7)	0 (0.0)	4 (11.8)
	P-Value		0.045		<0.001		0.005		0.103	
Overall Metastases	No	n=51	20 (83.3)	31 (79.5)	41 (80.4)	10 (83.3)	30 (76.9)	21 (87.5)	23 (79.3)	28 (82.4)
	Yes	n=12	4 (16.7)	8 (20.5)	10 (19.6)	2 (16.7)	9 (23.1)	3 (12.5)	6 (20.7)	6 (17.6)
	P-Value		1		1		0.345		1	

and extend these observations.

Our epidemiologic observations (rare tumor type, geographic variation, modest male predominance) align with global surveillance reports and regional registries. GLOBOCAN 2022 places gallbladder cancer among relatively uncommon cancers globally (≈122,462 new cases in 2022), with important geographic heterogeneity (highest incidence reported in parts of Asia and South America) [4, 19]. Regional Iranian registry analyses similarly report BTCs as uncommon (≈0.5% of cancers in Iran) and demonstrate variable temporal trends by province [9, 20].

The prognostic power of tumor grade observed here is consistent with multiple prior studies showing tumor differentiation/grade as an adverse prognostic factor in GBC and BTC. Several prognostic models and nomograms include histologic grade among key predictors of survival, and higher grade has repeatedly associated with shorter median OS [21-23]. Our graded survival estimates (Grade 1→medians long; Grade 4→ extremely poor) mirror published stage/grade-specific survival patterns in tertiary datasets [23, 24].

That histologic category (GBC vs BTC) was not a consistent predictor across all endpoints in our sample is not unexpected in smaller, heterogeneous cohorts. Larger population-based studies show survival differences by anatomic site and stage (e.g., extrahepatic BTC vs intrahepatic BTC vs GBC), but effect sizes vary by stage mix and treatment patterns. Some modern series report better mid-term survival for extrahepatic BTC compared with gallbladder primaries when adjusted for stage and resectability [25, 26]. Our finding of a 2-year effect but not a persistent 5-year difference may reflect small numbers, stage distribution, or differential early-mortality patterns.

The lack of an independent association between metastatic status and mortality in our adjusted models may appear surprising given the well-established adverse impact of distant metastases on prognosis. However, this can be explained by multiple factors: (1) limited sample size and low numbers of metastatic events (n=12) reduce statistical power; (2) potential misclassification or incomplete timing data for metastasis in retrospective records can attenuate associations; and (3) heterogeneity in metastatic sites (liver, bone, lung) which carry differing

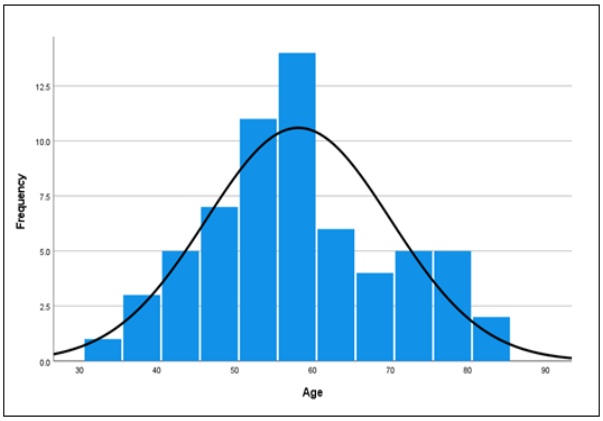

Figure 3. Age Distribution among Patients Diagnosed with GBC and BTC (n=63).

Table 3. Cox Proportional Hazards Analysis for Predictors of Overall and Time-specific Mortality in Patients with GBC and BTC.

Variables	Overall Mortality		1-Year Mortality		2-Year Mortality		5-Year Mortality	
	HR (95.0%CI for HR)	P-Value	HR (95.0%CI for HR)	P-Value	HR (95.0%CI for HR)	P-Value	HR (95.0%CI for HR)	P-Value
Age	1.014 (0.987-1.043)	0.31	0.964 (0.908-1.023)	0.228	1.007 (0.968-1.047)	0.744	1.01 (0.980-1.041)	0.509
Sex	1.691 (0.851-3.362)	0.134	2.828 (0.697-11.475)	0.146	1.966 (0.824-4.693)	0.128	1.635 (0.793-3.371)	0.183
Grade (2/1)	2.366 (1.039-5.384)	0.04	1.263 (0.171-9.340)	0.819	2.635 (0.882-7.872)	0.083	2.297 (0.967-5.460)	0.06
Grade (3/1)	4.126 (1.684-10.113)	0.002	7.301 (1.304-40.873)	0.024	4.592 (1.449-14.545)	0.01	3.458 (1.302-9.184)	0.013
Grade (4/1)	30.984 (7.165-133.981)	<.001	108.236 (9.558-1225.664)	<.001	51.4 (9.869-267.707)	<.001	32.724 (7.392-144.870)	<.001
Histology Category	1.739 (0.872-3.469)	0.116	2.609 (0.621-10.965)	0.19	2.834 (1.134-7.082)	0.026	1.856 (0.899-3.831)	0.094
Metastases	1.438 (0.616-3.353)	0.401	3.77 (0.482-29.478)	0.206	2.46 (0.629-9.621)	0.196	1.715 (0.664-4.432)	0.266

prognoses and treatment options. Larger cohorts and pooled analyses typically demonstrate a strongly adverse effect of metastasis on OS in GBC/BTC [22, 27].

Although distant metastasis is widely recognized as a strong adverse prognostic indicator in biliary malignancies, its lack of independent significance in our Cox models is plausibly attributable to several study-specific factors rather than to a true absence of effect. First, grade and metastatic status are biologically and clinically intertwined: poorly differentiated tumors are more likely to disseminate, so in a small cohort a strong grade effect can mask the incremental contribution of metastasis through confounding. Second, our metastatic subgroup was small (n=12), yielding limited statistical power and wide, unstable hazard estimates; such underpowered comparisons commonly fail to detect established associations even when clinically meaningful differences exist. Third, retrospective and heterogeneous documentation over a 21-year span introduces misclassification (incomplete staging, lack of synchronous vs. metachronous timing), which attenuates observed associations [28-30].

These data limitations likely also explain the absence of age or sex effects in our models: population-based series have variably reported age and sex associations with GBC/BTC outcomes, but such effects are often modest and become non-significant in small, referral-based samples where referral patterns, stage mix, and treatment availability differ from population registries [9, 31].

Our observed overall and stage-specific survival estimates are broadly comparable to earlier institutional series from Iran and international reports, though direct comparison is limited by differences in case mix and completeness of staging and treatment data. Iranian tertiary-center reports and regional series have documented short median survivals for advanced BTC/GBC and emphasize late presentation and limited access to curative surgery as major drivers of poor outcomes [32].

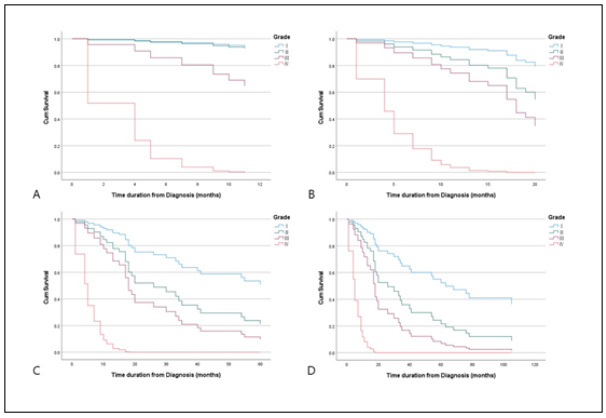

Figure 4. Kaplan–Meier Survival Curves Stratified by Tumor Grade (I–IV). Higher tumor grade was consistently associated with poorer survival across all follow-up periods.

Biological and clinical interpretation

Pathologic grade reflects tumor differentiation, proliferative activity and underlying biology (mutational burden, stromal reaction, tumor budding (clusters of detached tumor cells at the invasive front)) that drive aggressiveness and treatment resistance. The stepwise hazard increase across grades in our data supports the biological plausibility that poorly-differentiated tumors (high grade) behave more aggressively, produce earlier dissemination, and respond less favorably to standard therapies. Recent molecular characterizations of BTC/GBC identify genomic subsets (e.g., TP53 alterations, ERBB2 amplifications, FGFR2 fusions in cholangiocarcinoma) that correlate imperfectly with histologic features but further explain heterogeneity in outcomes and may guide targeted therapies [33, 34].

The absence of an independent age or sex effect in our Cox models likely reflects limited sample size and possible confounding by stage and grade. Population studies sometimes report female predominance in GBC

incidence (often related to gallstone prevalence), but sex differences in survival are inconsistent after adjustment [35, 36].

Strengths and limitations

Strengths of this study include a consecutive, hospital-based cohort with pathology-verified diagnoses, systematic survival ascertainment through civil registration linkage, and the use of multivariable Cox regression across multiple time horizons (overall, 1-, 2-, and 5-year endpoints). The setting within a radiotherapy referral center also offers clinically meaningful insights into real-world oncology patients, particularly those who ultimately undergo oncologic evaluation or treatment.

However, several important limitations must be acknowledged:

• Sample size and statistical power: With only 63 analyzable cases and very small numbers in key subgroups (e.g., 12 metastatic cases and 4 Grade 4 tumors), the study was underpowered to detect modest associations and yielded wide confidence intervals for several hazard ratios. The lack of statistical significance for clinically important predictors such as metastatic status likely reflects low event counts rather than a true absence of effect. This pattern is commonly observed in single-center studies of rare biliary tract tumors [23, 24].

• Retrospective design and data completeness: As with most retrospective cohorts, the dataset was limited by missing or inconsistently documented clinical variables, including the timing of metastasis, performance status, and systemic therapy details. Because the timing of metastatic spread was incompletely reported, metastasis had to be coded as a binary variable, which reduced temporal granularity and increased the potential for misclassification and residual confounding.

• Heterogeneity in tumor sites and treatment: BTC encompasses a biologically diverse group of malignancies (intrahepatic and extrahepatic cholangiocarcinoma, ampullary cancers, and gallbladder adenocarcinoma) with distinct natural histories and therapeutic pathways. Although we adjusted for histological category, residual heterogeneity in stage, resectability, and treatment modality (surgery, chemotherapy, radiotherapy, and targeted therapies) likely influenced survival outcomes. Contemporary multi-institutional registries typically stratify cohorts by resectability and treatment receipt to address this source of variability [25, 28, 37].

• Single-center referral bias: As a tertiary radiotherapy center, the institution may overrepresent patients with more advanced, recurrent, or treatment-refractory disease, limiting the generalizability of findings to population-based cohorts or to earlier-stage presentations.

Future research should prioritize larger, multi-center cohorts to increase statistical power, enable robust subgroup analyses, and improve external validity. Incorporating granular clinical variables including TNM stage, resection status, systemic treatment regimens, and performance status will enhance prognostic modeling. Additionally, integrating molecular and genomic profiling may help identify biologically meaningful pathways and support the development of personalized therapeutic strategies.

In conclusion, in this 21-year single-center cohort of patients with gallbladder and biliary tract cancers, tumor grade was the dominant determinant of survival, with higher grades conferring substantially higher hazards of death. Histologic site and metastatic presence showed inconsistent associations in adjusted analyses likely reflecting limited sample size and data granularity. These findings underscore the importance of accurate Pathologic grading, and collaborative multi-institutional studies in Iran and globally to refine prognostication and improve outcomes for these rare but lethal cancers.

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Clinical trial registration

Not applicable.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest related to this study.

Data Availability Statement

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

This study did not use any custom code. All analyses were performed using SPSS version 27 and Microsoft Excel.

Author Contributions

Dr. Danial Fazelat-Panah contributed to the conceptualization, funding acquisition, investigation, methodology development, project administration, supervision, drafting of the original manuscript, and critical revision and editing of the final version.

Mr. Seyed Reza Najafi, as the corresponding author, was responsible for data curation, investigation, drafting of the original manuscript, and critical review and editing of the paper.

Dr. Hoda Shirafkan contributed to the study methodology and performed the statistical analyses.

Nima Noori, Fahime Khoshparast, and Dr. Alireza Taji contributed to data collection and curation.

Ethics approval statement

This study was approved by the Ethics Committee of Babol University of Medical Sciences (Approval Code: IR.MUBABOL.HRI.REC.1403.303). All patient data were obtained in accordance with institutional guidelines and remained confidential throughout the study.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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Declaration on generative AI and AI-assisted technologies in the writing process

The authors declare that no generative artificial intelligence (AI) or AI-assisted technologies were used in the writing, editing, data analysis, or preparation of this manuscript. All components of the work including conceptualization, methodology, data extraction, statistical analysis, interpretation of findings, and manuscript drafting were performed solely by the authors.

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