ORIGINAL RESEARCH

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Prognostic Factors in Resected Biliary Tract Cancers and the Impact of Cytokeratin 20 Expression

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ABSTRACT Objective: Surgical resection is the mainstay of treatment in biliary tract cancers. However, the prognostic factors after resection are not very well defined. Cytokeratins are a group of intermediate filaments present in epithelial tissues that play a crucial role in cell differentiation. Cytokeratin 20 (CK20) is frequently expressed in different types of cancers, but its effect on survival varies considerably. The current study aimed at assessing the prognostic factors as well as CK20 expression for survival outcomes, which has not yet been studied in this group of patients. **Material and Methods:** We performed a multicenter, retrospective analysis of patients diagnosed with biliary tract cancer and treated them with upfront surgery. In total, 81 patients from four oncology centers were involved in this study. We aimed to determine the impact of known clinicopathologic factors and CK20 expression on survival rates using Cox regression analysis. **Results:** Median follow-up was 21.6 months. Surgical margin status and lymph node metastases were independent factors for both disease-free (p=0.006 and p<0.001, respectively) and overall survival (OS) (p=0.004 and p<0.001, respectively), while perineural invasion (PNI) significantly influenced disease-free survival (DFS) (p=0.013). Patients with CK 20 expression had better DFS and OS; however, its prognostic effect was not demonstrated in multivariate analysis (p>0.05). **Conclusion:** Our study demonstrated that lymph node metastasis, positive surgical margins, and PNI were found to be potential prognostic factors for survival outcomes, whereas the CK20 expression was not precise enough to be considered as an independent factor affecting the prognosis.

Keywords: Biliary tract cancer; cytokeratin 20; prognosis

Biliary tract cancers (BTC) are a group of malignant tumors originating from the bile duct epithelium and include intrahepatic cholangiocarcinoma (ICC), extrahepatic cholangiocarcinoma (ECC), and gallbladder carcinoma (GBC).[1,2] They constitute 0.7% of all cancers and 3% of all gastrointestinal cancers; approximately 250,000 new patients were diagnosed in 2020.[3-5] Unfortunately,70% of BTCs are unresectable at the time of diagnosis, and their 5-year survival rate ranges from 5% to 15%.[6,7]

Although surgery being the only definitive treatment, R0 resection is possible in only 70% of patients. Moreover, the median overall survival (OS) duration of operated patients is less than 5 years despite the usage of modern surgical methods and adjuvant therapies.[8]

Since cytokeratins (CK) are keratin proteins present in the cytoplasmic skeleton of epithelial tissues, their primary role is to maintain the structural integrity of the epithelium.[9,10] More than 20 subtypes

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of CK have been identified to date, and they can be differentiated from each other by their isoelectric pH and molecular weight.[11] CKs are mainly used as a marker to determine the origin of tumors in clinical practice. CK20 is expressed in various malignant tumors, including the biliary duct, gallbladder, lung, pancreas, urothelium, and lower gastrointestinal tract.[12-16] Although its prognostic effect has been assessed in many previous studies, yet the results vary in different types of cancers.[17-19]

Since both GBCs and cholangiocarcinomas (CCs) share the same embryological origin, all adjuvant treatment trials in the past have always included patients from these two groups, but unfortunately, these adjuvant treatment trials did not show any significant difference in terms of OS.[20-22] Although the prognostic impact of clinicopathologic factors remains conflicting in the literature, a better understanding of various prognostic factors will make it easier to overcome this problem.[23,24] In this study, we aimed to assess the cytokeratin 20 (CK20) expression and other prognostic factors involved in resected BTCs.

MATERIAL AND METHODS

We conducted a multicenter, retrospective, observational study and reviewed the medical records of 81 patients who underwent surgical resection for ICC, ECC, or GBC from four oncology centers (Kartal Dr. Lütfi Kırdar City Hospital, Marmara University Pendik Training and Research Hospital, Ümraniye Training and Research Hospital, Bakırköy Dr. Sadi Konuk Training and Research Hospital). The following clinical information was reviewed from the patient records: age, sex, Eastern Cooperative Oncology Group performance score (ECOG PS), stage at diagnosis, T and N stages according to **TNM staging 8th edition,** surgical margins, CK7 and CK20 expressions, lymphovascular invasion (LVI), perineural invasion (PNI), preoperative carbohydrate antigen (CA) 19-9 values, adjuvant radiotherapy, chemotherapy regimens for adjuvant and metastatic settings and all metastatic sites while involving entire metastatic site count.

STATISTICAL ANALYSIS

All the obtained parameters such as clinicopathological features, disease-free survival (DFS), and OS rates were analyzed. SPSS version 27.0 software was used for all the analyses. Descriptive statistics were applied to organize the clinical data in terms of mean, standard deviation, median with range, frequency, and rate. OS rate was measured from the date of surgery to the date of death from any cause. DFS was measured from the date of surgery to the date of local recurrence, distant metastases, or death from any cause. Categorical variables were compared using the Fisher's exact test. Survival probabilities were predicted by Kaplan-Meier method. Exploratory multivariate analysis for OS and DFS was performed using the Cox proportional hazards model, adjusting for known baseline prognostic factors. Differences were considered to be statistically significant at $p<0.05$.

The study protocol was approved by the Clinical Research Ethics Committee of the University of Health Sciences Umraniye Training and Research Hospital, İstanbul. All the procedures in the report have been in accordance with the ethical principles of the Institutional Research Committee, the 1964 Helsinki declaration, and the subsequent amendments.

RESULTS

Eighty-one patients were included in the analysis. Patient demographics and clinical characteristics are listed in Table 1. The median follow-up after surgery was 21.6 months. The median age of patients was 63 (range: 39-82), out of which 58% of patients were male. The most frequent primary tumor location was the gallbladder (44.4%). Most of the patients had R0 resection (66.7%), while 21% had microscopic and 12.3% had macroscopic residual disease after resection. CK20 staining was positive in 24.7% of all the cases.

In univariate analysis, female gender, higher ECOG PS, later stage at diagnosis, positive surgical margins, higher T and N stages, positive CK20 staining, higher preoperative CA 19-9 levels, LVI, PNI, and adjuvant radiotherapy were correlated with shorter DFS whereas in multivariate analysis, surgical margin [hazard ratio (HR): 1.75, 95% 22 confidence interval (CI): 1.18-2.61, p=0.006)], N stage (HR: 3.03, 95% CI: 1.86-4.91, $p<0.001$) and PNI (HR: 2.39, 95% CI: 1.21-4.72, p=0.013) were found to be independent factors for DFS (Table 2).

TABLE 1: Baseline demographics and clinical characteristics.

		Range	Median	Mean±SD/n-%	
Age		39 -82	63.00	62.0±10.0	
Sex	Male			34	42.0%
	Female			47	58.0%
Follow-up (months)		5.0 -93.2	21.6	24.68±14.05	
ECOG PS	0			28	34.6%
	I			40	49.4%
	II			11	13.6%
	III			2	2.5%
Primary tumor location	Gallbladder			36	44.4%
	ICC			23	28.4%
	ECC			22	27.2%
Stage at diagnosis	I			10	12.3%
	II			25	30.9%
	III			39	48.1%
	IV			7	8.6%
Surgical margin	R0			54	66.7%
	R1			17	21.0%
	R2			10	12.3%
T stage	I			12	14.8%
	II			33	40.7%
	III			32	39.5%
	IV			4	4.9%
N stage	0			43	53.1%
	I			25	30.9%
	II			13	16.0%
Positive CK7 staining				75	92.6%
Positive CK20 staining				20	24.7%
Lymphovascular invasion				39	48.1%
Perineural invasion				42	51.9%
Preoperative CA 19-9 level		1-2,714	54.0	270.1±489.4	
Adjuvant RT	(-)			58	71.6%
	(+)			23	28.4%
Adjuvant CT regimen	Gemcitabine			5	6.2%
	Gemcitabine+capecitabine			12	14.8%
	Capecitabine			33	40.7%
	FUFA			1	1.2%
	CapeOX			4	4.9%
	GemOX			1	1.2%
				n	**%**
Metastatic site					
Liver				34	42.0%
Lung				6	7.4%
Bone				2	2.5%
Brain				0	0.0%
Pleura				2	2.5%
Mediastinal LN				2	2.5%
Intraabdominal LN				35	43.2%
Peritoneum				20	24.7%
Involved metastatic site count	0			28	34.6%
	1			14	17.3%
	2			30	37.0%
	3 or more			9	11.1%
First-line chemotherapy regimen for advanced disease	Cisplatin+gemcitabine			38	71.7%
	Gemcitabine			8	15.1%
	FOLFOX			4	7.5%
	CapeOX			1	1.9%
	Capecitabine			1	1.9%
	Carboplatin+gemcitabine			1	1.9%

SD: Standard deviation; ECOG PS: Eastern Cooperative Oncology Group performance score; ICC: Intrahepatic cholangiocarcinoma; ECC: Extrahepatic cholangiocarcinoma; CK7: Cytokeratin 7; CK20: Cytokeratin 20; CA 19-9: Carbohydrate antigen 19-9; RT: Radiotherapy; CT: Chemotherapy; FUFA: 5-fluorouracil+folinic acid; CapeOX: Capecitabine+oxaliplatin; GemOX: Gemcitabine+oxaliplatin; LN: Lymph nodes; FOLFOX: 5-fluorouracil+folinic acid, oxaliplatin.

TABLE 2: Univariate and multivariate analyses of disease-free survival.

	Univariate analysis					Multivariate analysis				
	HR	95% CI			p value	HR	95% CI			p value
Age	1.01	0.98	-	1.04	0.573					
Sex	0.55	0.32	-	0.95	***0.033***					
ECOG PS	1.52	1.05	-	2.20	***0.028***					
Primary tumor location	1.23	0.89	-	1.69	0.211					
Stage at diagnosis	3.46	2.17	-	5.50	***0.000***					
Surgical Margin	2.95	2.04	-	4.26	***0.000***	1.75	1.18	-	2.61	***0.006***
T Stage	2.28	1.62	-	3.22	***0.000***					
N stage	4.29	2.82	-	6.53	***0.000***	3.03	1.86	-	4.91	***0.000***
Positive CK7 staining	5.80	0.80	-	42.10	0.082					
Positive CK20 staining	2.73	1.47	-	5.04	***0.001***					
Preoperative CA 19-9 level	1.00	1.00	-	1.00	***0.000***					
Lymphovascular invasion	2.04	1.15	-	3.59	***0.014***					
Perineural invasion	3.57	1.96	-	6.48	***0.000***	2.39	1.21	-	4.72	***0.013***
Adjuvant radiotherapy	3.68	1.99	-	6.83	***0.000***					
Adjuvant chemotherapy regimen	0.86	0.68	-	1.08	0.195					

Cox Regression (Forward LR); HR: Hazard ratio; CI: Confidence interval; ECOG PS: Eastern Cooperative Oncology Group performance score; Cytokeratin 7; CK20: Cytokeratin 20; CA 19-9: Carbohydrate antigen 19-9.

Predicted DFS for R2 and R1 resected groups were significantly lower than R0 resected group (8.6, 11.2, 33.8 months, respectively, p<0.05). DFS between R1 and R2 resected patients was not statistically significant (Figure 1). Patients with node N2 and N1 disease had lower DFS as compared to node-negative patients (median DFS: 9.3, 11.2, 40.1 months, respectively, p<0.05) (Figure 2). Multivariate analysis showed no prognostic impact of CK20 staining on DFS either (p>0.05).

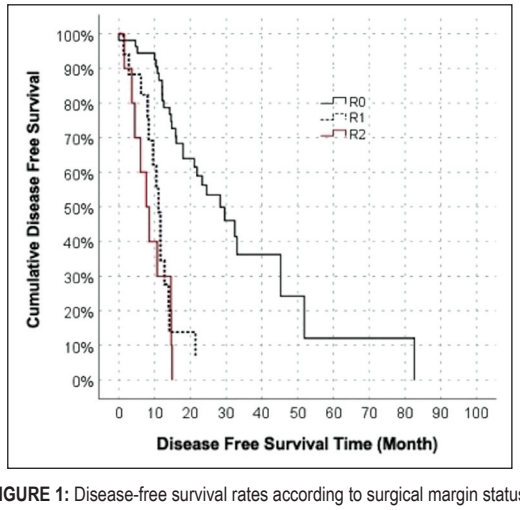

FIGURE 1: Disease-free survival rates according to surgical margin status.

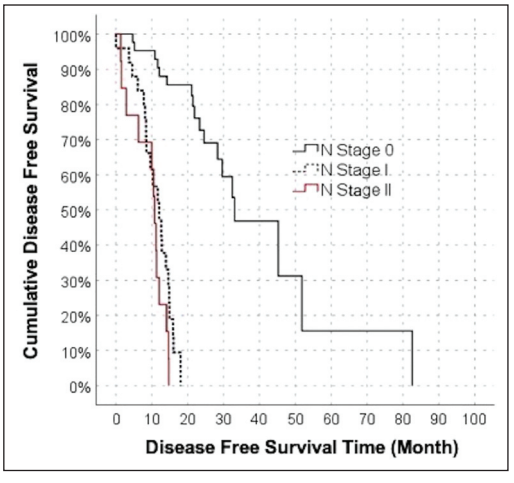

FIGURE 2: Disease-free survival rates according to N stage.

OS was shorter in patients with poorer ECOG PS, later stage at diagnosis, positive surgical margins, higher T and N stages, hepatic and abdominal lymph node metastases, involved metastatic site count, CK20 positive staining, higher preoperative CA 19-9 levels, LVI, PNI and adjuvant RT in univariate analysis (Table 3). Surgical margins (HR: 1.91, 95% CI: 1.22-2.99, p=0.004) as well as the N stage (HR: 4.00, 95% CI: 2.31-6.90, p<0.001) were independent factors for OS. R2 and R1 resected patients had

TABLE 3: Univariate and multivariate analyses of overall survival.

	Univariate analysis					Multivariate analysis				
	HR	95% CI			p value	HR	95% CI			p value
Age	1.03	1.00	-	1.06	0.071					
Sex	0.63	0.34	-	1.15	0.131					
ECOG PS	1.54	1.02	-	2.33	***0.040***					
Primary tumor location	1.28	0.90	-	1.84	0.170					
Stage at diagnosis	3.11	1.89	-	5.12	***0.000***					
Surgical Margin	3.16	2.13	-	4.70	***0.000***	1.91	1.22	-	2.99	***0.004***
T stage	2.00	1.39	-	2.89	***0.000***					
N stage	5.00	3.08	-	8.11	***0.000***	4.00	2.31	-	6.90	***0.000***
Positive CK7 staining	23.36	0.17	-	3,215	0.210					
Positive CK20 staining	2.53	1.31	-	4.91	*0.006*					
Preoperative CA 19-9 level	1.00	1.00	-	1.00	*0.001*					
Lymphovascular invasion	1.89	1.00	-	3.55	*0.049*					
Perineural invasion	2.80	1.47	-	5.36	*0.002*					
Adjuvant radiotherapy	3.74	1.98	-	7.07	*0.000*					
Adjuvant chemotherapy regimen	0.93	0.73	-	1.19	0.577					
Metastatic site										
Liver	3.49	1.81	-	6.71	***0.000***					
Lung	1.04	0.37	-	2.93	0.941					
Bone	0.46	0.06	-	3.35	0.440					
Pleura	1.00	0.24	-	4.28	0.996					
Mediastinal LN	1.95	0.47	-	8.14	0.361					
Intraabdominal LN	2.94	1.57	-	5.53	*0.001*					
Peritoneum	1.76	0.94	-	3.27	0.076					
Metastatic site count	2.04	1.46	-	2.86	***0.000***					

Cox Regression (Forward LR); HR: Hazard ratio; CI: Confidence interval; ECOG PS: Eastern Cooperative Oncology Group performance score; Cytokeratin 7; CK20: Cytokeratin 20; CA 19-9: Carbohydrate antigen 19-9.

shorter OS than R0 resected patients. (median OS: 17.6, 21.2, and 48.1 months respectively, p=0.011). No OS difference was obtained between R1 and R2 resected patients (p=0.49) (Figure 3). Patients with N1 and N2 disease had poorer OS as compared with node-negative disease (mOS: 21.2, 17.7, and 54.7 months respectively, p<0.001). mOS did not differ between N1 and N2 disease (p=0.059) (Figure 4). CK20 staining status was not found to be an independent factor for OS (p>0.05).

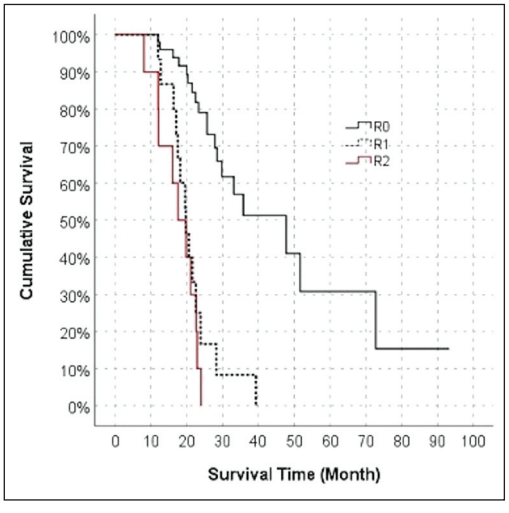

FIGURE 3: Overall survival rates according to surgical margin status.

DISCUSSION

We aimed to assess the association of CK20 expression and pathologic factors with clinical parameters and their impact on the prognosis of resected BTC.

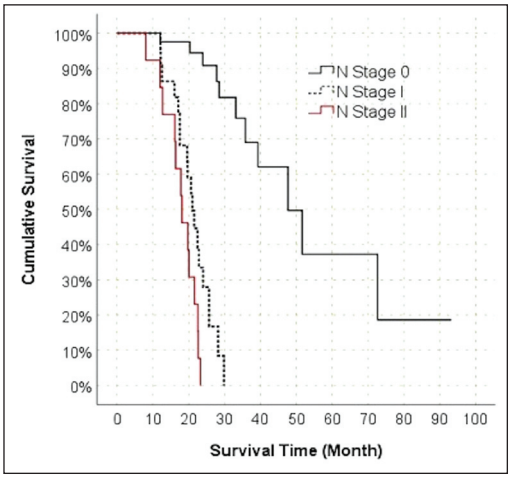

FIGURE 4: Overall survival rates according to N stage.

In the past literature, many studies have described the prognostic effect of CK20 expression. Morini et al. showed that CK20 expressed in the ampulla of Vater carcinomas had aggressive behavior and a poorer survival rate.[25] In another study, CK20 positivity was correlated with poorer outcomes in R0 resected pancreatic carcinoma patients, although the underlying mechanism was largely unknown.[17] An in vitro study by Min et al. demonstrated that peroxisome proliferator-activated receptor CK20 expression induces the metastatic potential of breast cancer cells.[26] The authors stated that CK20, as an intermediate filament, positively affects integrin-mediated signaling and may have a critical role in tumor cell adhesion and migration.

In contrast, various studies conducted on lung and ovarian cancers did not express the prognostic significance of CK20 expression.[19] In our study, patients with CK20 expression had shorter OS (mOS: 32.6 vs. 19.7 months). However, CK20 expression was not found to be an independent factor for survival. Several previous studies have reported that the OS rates for GBCs are greater than that of CCs.[27] Our study results revealed that the CK20 positivity rate was 27% for GBC and 22% for CCs, respectively; therefore, indicating that the presence of primary tumor locations may have decreased the survival difference.

Many studies have also reported that extended surgical approaches and R0 resection of BTCs are mandatory for long-term survival.[28,29] Murakami et al. showed that R0 resection of ICCs and ECCs is correlated with increased OS.[30] Another study by Balachandran et al. demonstrated the same survival outcomes for operated GBC patients as observed in the literature.[31] Our study results reflected that median DFS and OS were increased nearly threefold in R0 resected patients, in line with the previous studies.

Lymph node metastasis is commonly seen in BTCs. A registry study with 18,606 patients in Japan reported a lymph node metastasis rate of 18.7% for GBC, 22.7% for perihilar CC, and 28.1% for distal CC.[32] This study also showed significantly decreased survival rates with lymph node metastasis for each group of BTCs. Many previous studies reported similar survival results for lymph node metastasis which was confirmed by our study in terms of both OS and DFS.[33-35]

PNI is the process of neoplastic invasion of nerves and has been shown to increase recurrence rates in many types of cancers.[36-39] Our results indicated that though PNI was associated with shorter DFS, it could not be shown as an independent factor for OS and was consistent with findings in the past literature. Since 76.5% of PNI (+) patients received standard chemotherapy (cisplatin plus gemcitabine) for advanced disease stage, while only 60% of the PNI (-) group could receive this regimen hence, this may have reduced the difference in OS.[40]

Our study demonstrated that lymph node metastasis, positive surgical margins, and PNI were found to be satisfactory prognostic factors for survival outcomes, whereas CK20 expression was not considered as a valid prognostic component. It is also important to recognize that since our study has a retrospective design, it has few drawbacks that come with this form of research. Moreover, diverse prospective studies with larger patient groups are needed to confirm the validation of these prognostic factors in BTCs.

CONCLUSION

In conclusion, PNI, surgical margin status and lymph node metastasis were found to be prognostic factors in resected BTCs, while CK20 expression was not. Further prospective studies are needed to reveal the value and clinical application of these prognostic factors.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

***Idea/Concept:** İbrahim Çil, Abdilkerim Oyman, İlker Nihat Ökten; **Design:** İbrahim Çil, Abdilkerim Oyman, Melike Özçelik; **Control/Supervision:** Melike Özçelik, Mesut Yılmaz; **Data Collection and/or Processing:** İbrahim Çil, Selver Işık, Murat Ayhan, Mesut Yılmaz, Melike Özçelik; **Analysis and/or Interpretation:** İbrahim Çil, Melike Özçelik; **Literature Review:** İbrahim Çil, Abdilkerim Oyman; **Writing the Article:** İbrahim Çil, İlker Nihat Ökten; **Critical Review:** Melike Özçelik, Mesut Yılmaz; **References and Fundings:** İbrahim Çil, Melike Özçelik; **Materials:** İbrahim Çil, İlker Nihat Ökten, Abdilkerim Oyman.*

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