Update on the clinical features, treatment and histogenesis of combined hepatocellular-cholangiocarcinoma

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Abstract. Combined hepatocellular-cholangiocarcinoma (CHC) is a rare subtype of primary liver cancer with histological features of both hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC). The clinicopathological features, treatment and histogenesis of CHC remain to be elucidated due to its low morbidity. In total, 26 patients with CHC, 901 patients with HCC and 40 patients with ICC were included in the present retrospective study and their clinicopathological characteristics and prognoses were compared. The treatment patterns of postoperative CHC recurrence were analyzed. Immunohistochemistry for the hepatic progenitor cell (HPC) markers CD90 and epithelial cell adhesion molecule (EpCAM) was performed to investigate the origin of CHC. CHC demonstrated clinicopathological features of both HCC and ICC. Patients with CHC had similar overall survival (OS) and disease-free survival (DFS) rates to those with ICC, but all had significantly lower survival rates compared with those with HCC. Univariate analysis revealed that α-fetoprotein, carbohydrate antigen 19-9, carcinoembryonic antigen, tumor size, macrovascular invasion and lymph node metastasis were risk factors for both OS and DFS. Most patients with recurrent CHC received comprehensive treatment. Immunohistochemistry revealed that patients with CHC exhibited high expression levels of CD90 and EpCAM. In conclusion, compared with patients with HCC and ICC, patients with CHC appear to have intermediate clinical characteristics. Comprehensive postoperative therapy may be a promising strategy for patients with CHC. The high expression level of HPC markers in tumor tissues suggests that CHC may originate from HPCs.

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Abbreviations: CHC, combined hepatocellular-cholangiocarcinoma; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; HPC, hepatic progenitor cell; PLC, primary liver cancer; CEA, carcinoembryonic antigen; AFP, α-fetoprotein; CA19-9, carbohydrate antigen 19-9; OS, overall survival; DFS, disease-free survival; PBS, phosphate-buffered saline; HBV, hepatitis B virus; HCV, hepatitis C virus

Key words: CHC, HCC, ICC, prognosis, HPC

Introduction

Primary liver cancer (PLC) is the sixth most common cancer and fourth leading cause of cancer-related mortality worldwide, with >840,000 novel cases and 780,000 deaths annually (1). There are three subtypes of PLC: Hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC) and combined hepatocellular-cholangiocarcinoma (CHC).

CHC is an uncommon and unique type of PLC with the phenotypic characteristics of both HCC and ICC. The incidence of CHC varies from 2.4 to 14.2% among studies (2-4). This type of carcinoma contains explicit mixed HCC and ICC components. Currently, there is no unified pathological classification of CHC. The first scientific description of CHC was published in a study by Allen and Lisa in 1949 (5), in which this tumor was defined into three types: Type A (double cancer; HCC and ICC located in different parts of the same liver), type B (combined type; HCC and ICC existing in adjacent parts with continuous growth) and type C (mixed type; HCC and ICC components completely combined within the same tumor). In 1985, Goodman *et al* (6) divided CHC into type I (collision), characterized by the coincidence of HCC and ICC in the same patient; type II (transition), characterized by the presence of an identifiable transition between HCC and ICC; and type III (fibrolamellar), similar to the fibrolamellar variants of HCC but with the existence of pseudodoglands secreting mucin. However, in 2010, the World Health Organization classified CHC into two main types: A typical type, which is similar to type C, as explained by Allen and Lisa, and subtypes with stem cell characteristics, which are divided into classic, intermediate and bile duct cell subtypes (7).

However, due to the low prevalence of CHC, its clinicopathological characteristics and prognosis vary among studies. Certain studies have reported that the biological characteristics of CHC are analogous to those of HCC, rather than those of ICC (8-10). However, at the same time, other studies have indicated that CHC is genetically more similar to ICC compared with HCC (11,12). In order to gain a more comprehensive understanding of the clinical pathologies and outcomes of CHC, the present study, which aimed to compare the clinicopathological characteristics and prognosis of CHC with HCC and ICC, and to investigate the clinical prognostic factors in patients with CHC, investigated the clinical characteristics, prognosis and histogenesis of CHC after surgery.

Patients and methods

Patients. The clinical data of patients with PLC who underwent a hepatectomy at Guangxi Medical University Cancer Hospital (Nanning, China) between January 2014 and December 2018 were retrospectively analyzed. R0 margins were observed in all cases. CHC was diagnosed according to the definitions of types B and C according to the classification by Allen and Lisa (5). Meanwhile, the exclusion criteria were as follows: i) Patients who received preoperative chemotherapy, radiotherapy or other non-surgical treatment; and ii) patients with incomplete clinical data.

Patient history was reviewed for clinicopathological information, including sex, age, viral hepatitis B and C status, background liver disease, serum bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), carcinoembryonic antigen (CEA), α-fetoprotein (AFP), carbohydrate antigen 19-9 (CA19-9) and Child-Pugh status (13). Pathological data included tumor number and size, presence of capsules, cirrhosis, Edmondson-Steiner grading (14), satellite lesions, macrovascular and microvascular invasion, and lymph node metastasis. Clinical and pathological features, overall survival (OS) and disease-free survival (DFS) were compared between patients with CHC and those with HCC or ICC. The prognostic factors for OS and DFS, recurrence patterns and management of patients with CHC were also investigated.

Patients were regularly monitored for tumor recurrence or distant metastasis every 3 months in the first 2 years and then every 3-6 months, with measurements of serum tumor markers, liver function tests, chest radiography, color ultrasound, dynamic contrast-enhanced computed tomography or magnetic resonance imaging.

Immunohistochemistry. The present study performed immunohistochemical analysis of CD90 and EpCAM to examine whether CHC arose from the HPCs in the present study. Surgical tissues were fixed in 10% formalin at room temperature for 24 h, embedded in paraffin, cut into 5-μm tissue slices, deparaffinized in xylene and rehydrated using graded alcohol solutions. Antigen retrieval was performed for 5 min at 100˚C in citrate buffer (10 mmol/l; pH 6.0) in a pressure cooker. Endogenous peroxidases were blocked at room temperature by immersing sections in 3% hydrogen peroxide for 20 min. Tissue samples were then incubated at 37˚C for 2 h with a mouse monoclonal antibody against human CD90 (1:200; cat. no. ab92574; Abcam) and a mouse monoclonal antibody against human epithelial cell adhesion molecule (EpCAM; 1:200; cat. no. ab223582; Abcam). The tissue slices were washed with phosphate-buffered saline (PBS), incubated with biotinylated anti-mouse immunoglobulin diluted in PBS for 30 min at room temperature and washed again with PBS. The sections were incubated with anti-HRP conjugate for 10 min, rinsed with PBS and incubated with diaminobenzidine for 10 min. Lastly, the sections were counterstained at room temperature with hematoxylin. A light microscope was used for analysis. For statistical analysis (ImageJ; National Institutes of Health), patients were categorized as positive for these markers if the percentage of cells positive for CD90 or EpCAM was ≥5%.

Statistical analysis. Continuous variables are presented as the mean ± SD or median ± IQR, with normally distributed data as the mean ± SD and non-normally distributed data as the median ± IQR, and categorical variables are presented as the frequency (percentage). The significance of the baseline differences among the three groups was evaluated using analysis of one-way variance with Bonferroni's post hoc test for continuous variables or the Mann-Whitney U test with Bonferroni's post hoc test for non-parametric variables. When multiple comparisons are made among different groups, the risk of Type I errors (false-positives) increases. Therefore, P-value correction was applied to control for this, reducing the P-value threshold by dividing the α level (0.05) by the number of comparisons (n=3), resulting in a new significance threshold of 0.0167. The χ^2 test or Fisher's exact test was used to compare categorical data. Survival analysis was performed using the Kaplan-Meier method and intergroup differences were assessed using the log-rank test. The prognostic risk factors for poor DFS and OS were assessed using univariate and multivariate Cox regression analyses. The SPSS statistical software (version 24.0; IBM Corp.) was used for all analyses and $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinical data. The baseline characteristics of the patients with CHC, HCC and ICC are provided in Table I. There were 22 men (84.6%) in the CHC group, the male:female ratio was 5.5:1 and the mean age was 53.2±9.2 years (range, 34-68 years). There were no significant differences in sex distribution or mean age in the three groups. In total, 13 patients (50.0%) had hepatitis B virus (HBV), while no patient had hepatitis C virus (HCV) infection with CHC, and significantly fewer of these patients had HBV infection compared with those in the HCC group ($P<0.001$). There were no significant differences in serum total bilirubin, ALT, AST and CA19-9 among the CHC, HCC and ICC groups ($P=0.057$). The serum CEA level in the CHC group was significantly higher compared with that in the HCC group ($P=0.001$) but similar to that in the ICC group ($P=0.704$). No significant differences were observed in terms of Child-Pugh status, tumor capsule, tumor number, tumor size, Edmondson-Steiner grading and satellite lesions among the three groups (all $P>0.05$).

In the CHC group, liver cirrhosis was significantly more regular compared with that in the ICC group ($P<0.01$) but was

Table I. Baseline characteristics of patients with CHC, HCC and ICC.

Characteristics	CHC (n=26)	HCC (n=901)	ICC (n=40)	P-value			
				CHC vs. HCC vs. ICC	CHC vs. HCC	CHC vs. ICC	HCC vs. ICC
Sex, n (%)				0.001[a]	0.839	0.080	<0.001[a]
Male	22 (84.6)	775 (86.0)	26 (65.0)				
Female	4 (15.4)	126 (14.0)	14 (35.0)				
Mean age ± SD, years	53.2±9.2	49.8±10.8	55.9±11.1	0.001[a]	0.085	0.264	0.001[a]
Diabetes, n (%)				0.625	0.340	0.356	0.886
No	25 (96.2)	817 (90.7)	36 (90.0)				
Yes	1 (3.8)	84 (9.3)	4 (10.0)				
HBV viral infection, n (%)				<0.001[a]	<0.001[a]	0.843	<0.001[a]
No	13 (50.0)	112 (12.4)	21 (52.5)				
Yes	13 (50.0)	789 (87.6)	19 (47.5)				
HCV viral infection, n (%)				0.691	0.589		0.503
No	26 (100.0)	891 (98.9)	40 (100.0)				
Yes	0 (0.0)	10 (1.1)	0 (0.0)				
Total bilirubin, U/ml[b]	12.3±4.8	15.0±10.0	14.2±18.4	0.001[a]	0.100	0.256	0.001[a]
ALT, U/ml[b]	46.5±54.2	44.6±42.8	32.1±31.2	<0.001[a]	0.098	0.748	<0.001[a]
AST, U/ml[b]	44.3±31.0	52.6±47.0	33.1±16.7	<0.001[a]	0.101	0.284	<0.001
CEA, ng/ml[b]	73.1±234.3	3.0±5.4	33.9±98.6	<0.001[a]	0.001[a]	0.704	0.002[a]
CA19-9, U/ml[b]	111.4±265.8	28.8±53.4	343.1±437.9	<0.001[a]	0.176	0.149	<0.001[a]
AFP, n (%)				<0.001[a]	0.049[a]	0.032[a]	<0.001[a]
≤400 ng/ml	19 (73.1)	483 (53.6)	37 (92.5)				
>400 ng/ml	7 (26.9)	418 (46.4)	3 (7.5)				
Child-Pugh, n (%)				0.223	0.083	0.322	0.945
A	24 (92.3)	880 (97.7)	39 (97.5)				
B	2 (7.7)	21 (2.3)	1 (2.5)				
Liver cirrhosis, n (%)				<0.001[a]	0.021[a]	<0.01[a]	<0.001[a]
No	7 (26.9)	449 (49.8)	33 (82.5)				
Yes	19 (73.1)	452 (50.2)	7 (17.5)				
Tumor capsular, n (%)				0.057	0.241	0.033[a]	0.042[a]
No	4 (15.4)	230 (25.5)	16 (40.0)				
Yes	22 (84.6)	671 (74.5)	24 (60.0)				
Tumor number, n (%)				0.517	0.610	0.318	0.592
Solitary	17 (65.4)	631 (70.0)	31 (77.5)				
Multiple	9 (34.6)	270 (30.0)	9 (22.5)				
Tumor size, n (%)				0.539	0.636	0.305	0.322
≤5 cm	11 (42.3)	340 (37.7)	12 (30.0)				
>5 cm	15 (57.7)	561 (62.3)	28 (70.0)				
Macrovascular invasion, n (%)				0.440	0.442	0.219	0.318
No	20 (76.9)	630 (69.9)	25 (62.5)				
Yes	6 (23.1)	271 (30.1)	15 (37.5)				
Microvascular invasion, n (%)				0.091	0.067	0.491	0.210
No	19 (73.1)	495 (54.9)	26 (65.0)				
Yes	7 (26.9)	406 (45.1)	14 (35.0)				
Edmondson-Steiner grading, n (%)				0.164	0.517	0.105	0.078
I and II	9 (34.6)	369 (41.0)	22 (55.0)				
III and IV	17 (65.4)	532 (59.0)	18 (45.0)				

Table I. Continued.

Characteristics	CHC (n=26)	HCC (n=901)	ICC (n=40)	P-value			
				CHC vs. HCC vs. ICC	CHC vs. HCC	CHC vs. ICC	HCC vs. ICC
Satellite lesions, n (%)				0.971	0.852	0.966	0.872
No	22 (84.6)	774 (85.9)	34 (85.0)				
Yes	4 (15.4)	127 (14.1)	6 (15.0)				
Lymph node metastasis, n (%)				<0.001[a]	<0.001[a]	0.286	<0.001[a]
No	21 (80.8)	889 (98.7)	36 (90.0)				
Yes	5 (19.2)	12 (1.3)	4 (10.0)				
CD34, n (%)				<0.001[a]	0.257	0.020[a]	<0.001[a]
Negative	5 (19.2)	107 (11.9)	19 (47.5)				
Positive	21 (80.8)	794 (88.1)	21 (52.5)				
CK7, n (%)				<0.001[a]	<0.001[a]	0.959	<0.001[a]
Negative	7 (26.9)	697 (77.4)	11 (27.5)				
Positive	19 (73.1)	204 (22.6)	29 (72.5)				
CK19, n (%)				<0.001[a]	<0.001[a]	0.257	<0.001[a]
Negative	2 (7.7)	684 (75.9)	7 (17.5)				
Positive	24 (92.3)	217 (24.1)	33 (82.5)				
Glypican-3, n (%)				<0.001[a]	0.119	<0.001[a]	<0.001[a]
Negative	7 (26.9)	118 (13.1)	32 (80.0)				
Positive	19 (73.1)	783 (86.9)	8 (20.0)				

[a]P<0.05. [b]Data are presented as median ± IQR. CHC, combined hepatocellular cholangiocarcinoma; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma.

similar to that in the HCC group (P=0.021). However, there were no significant differences in macrovascular and microvascular invasion between the CHC group and the HCC and ICC groups (all P>0.05). Lymph node metastasis was significantly more frequent in the CHC group compared with that in the HCC group (P<0.001).

No significant difference was observed in the percentage of CD34 between the CHC group and the HCC and ICC groups. The percentage of patients with glypcian-3 positivity in the CHC group was similar to that in the HCC group (P>0.05) but significantly higher compared with that in the ICC group (P<0.05). By contrast, the distributions of both CK7- and $CK19^+$ patients in the CHC group were similar to those in the ICC group (both P>0.05) but higher compared with those in the HCC group (both P<0.05).

OS and DFS. The median OS time in the CHC group was 30 months and the 1- and 3-year OS rates were 65.4 and 33.9%, respectively. The median OS time in the HCC group was not reached and the 1- and 3-year OS rates were 82.1 and 63.2%, respectively. The median OS time in the ICC group was 30 months and the 1- and 3-year OS rates were 77.5 and 45.4%, respectively. The OS and DFS in the CHC group were significantly lower compared with those in the HCC group (both P<0.05) but not significantly different from those in the ICC group (both P>0.05) (Fig. 1).

Univariate and multivariate analysis. Univariate analysis demonstrated that AFP, CA19-9 and CEA levels, tumor size, macrovascular invasion and lymph node metastasis were significant predictive factors for lower OS and DFS in patients with CHC (both P<0.05) (Table II).

Recurrence patterns of CHC and management after recurrence. The recurrence patterns and management of CHC are summarized in Table III. Tumor recurrence occurred in 20 of the 26 patients with CHC. There were 13 cases of intrahepatic recurrence, 4 cases of extrahepatic recurrence, and 3 cases of both intrahepatic and extrahepatic recurrence. Among the patients with extrahepatic metastases, 3 patients exhibited lymph node metastasis, 2 patients exhibited peritoneal implantation and 2 patients exhibited bone metastases.

However, the management of tumor recurrence differed. In the present study, intrahepatic recurrence was treated with resection (n=2), transcatheter arterial chemoembolization (TACE; n=8), radiofrequency ablation (RFA; n=1) and comprehensive treatment with TACE + radiotherapy + percutaneous ethanol injection (n=1) and TACE + RFA (n=1). Extrahepatic relapses were treated with chemotherapy (n=3) or radiotherapy (n=1). Both intrahepatic and extrahepatic recurrences were comprehensively treated using TACE + chemotherapy + radiotherapy (n=1), TACE + RFA (n=1) or resection + TACE (n=1).

Table II. Univariate and multivariate analyses of prognostic factors in patients with combined hepatocellular cholangiocarcinoma.

	Overall survival			Disease-free survival		
	Univariate analysis	Multivariate analysis		Univariate analysis	Multivariate analysis	
Variables	P-value	HR (95% CI)	P-value	P-value	HR (95% CI)	P-value
Sex	0.455			0.308		
Age (>50 years)	0.374			0.144		
Diabetes	0.284			0.578		
HBV viral infection	0.202			0.599		
Total bilirubin (>21 U/ml)	0.683			0.968		
AFP (>400 ng/ml)	0.029	1.481 (0.363-6.041)	0.584	0.015[a]	2.509 (0.763-8.251)	0.130
CEA (>5 ng/ml)	0.005	3.157 (0.775-12.866)	0.109	0.009[a]	1.645 (0.484-5.592)	0.426
CA19-9 (>5 U/ml)	0.001	4.235 (0.938-19.131)	0.061	0.013[a]	2.350 (0.729-7.576)	0.152
ALT (>40 U/ml)	0.551			0.469		
AST (>40 U/ml)	0.245			0.318		
Child-Pugh	0.817			0.986		
Liver cirrhosis	0.329			0.548		
Tumor capsular	0.927			0.400		
Tumor number	0.738			0.299		
Tumor size (>5 cm)	0.003	3.394 (0.618-18.625)	0.160	0.009[a]	1.762 (0.505-6.148)	0.374
Macrovascular invasion	0.002	4.528 (1.001-20.470)	0.050	0.043[a]	2.765 (0.785-9.741)	0.113
Microvascular invasion	0.193			0.139		
Edmondson-Steiner grading	0.485			0.560		
Satellite lesions	0.103			0.337		
Lymph node metastasis	<0.001	3.818 (0.918-15.879)	0.065	0.006[a]	2.176 (0.587-8.067)	0.245

[a]P<0.05. HR, hazard ratio; CI, confidence interval; AFP, α-fetoprotein; CEA, carcinoembryonic antigen; ALT, alanine transaminase; AST, aspartase transaminase.

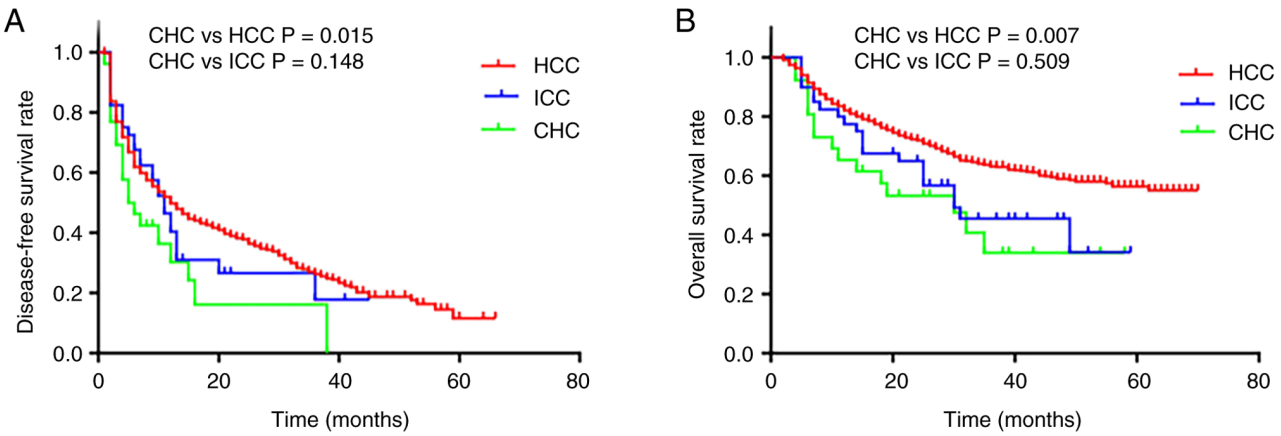

Figure 1. Survival curves. (A) Disease-free survival rate and (B) overall survival rate of patients with CHC, HCC and ICC. CHC, combined hepatocellular-cholangiocarcinoma; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma.

CD90 and EpCAM expression in CHC tissues. All 26 CHC samples were immunohistochemically positive for the classic hepatic progenitor cell (HPC) markers CD90 and EpCAM (Fig. 2). In total, 22/26 patients (84.6%) expressed CD90, 21/26 patients (80.8%) expressed EpCAM and 19/26 patients (73.1%) patients demonstrated simultaneous co-expression of CD90 and EpCAM (Table IV).

Discussion

CHC is an extremely rare type of hepatic malignancy with clinical characteristics and prognoses different from those of HCC and ICC. The incidence of CHC varies widely from 2.4 to 14.2% (2,4). The different rates of CHC may be due to ambiguous definitions and the exclusion of unresectable

Table III. Recurrence patterns and management of recurrence after hepatectomy of combined hepatocellular-cholangiocarcinoma.

Variables	Patients, n (%)
Recurrence	20 (76.9)
Location	
Intrahepatic	13 (65.0)
Extrahepatic	4 (20.0)
Both	3 (15.0)
Extrahepatic metastases	7 (27.0)
Lymph node metastasis	3 (42.8)
Peritoneal implantation	2 (28.6)
Bone metastases	2 (28.6)
Management	
Resection	2 (10.0)
TACE	8 (40.0)
RFA	1 (5.0)
Chemotherapy	3 (15.0)
Radiotherapy	1 (5.0)
Comprehensive treatment	5 (25.0)
TACE + chemotherapy + radiotherapy	1 (5.0)
TACE+ radiotherapy + percutaneous ethanol injection	1 (5.0)
TACE + RFA	2 (10.0)
Resection + TACE	1 (5.0)

TACE, transcatheter arterial chemoembolization; RFA, radiofrequency ablation.

Table IV. Expression levels of CD90 and EpCAM in 26 patients with combined hepatocellular-cholangiocarcinoma.

Variables	n/total n (%)
Positive for CD90	22/26 (84.6)
Positive for EpCAM	21/26 (80.8)
Positive for both CD90 and EpCAM	19/26 (73.1)

EpCAM, epithelial cell adhesion molecule.

cases in different reports. The incidence of CHC in the present study was 2.7%, which falls within the range found in the aforementioned studies.

The clinical and pathological features of CHC compared with those of HCC or ICC vary in different studies. Among the groups, the age at diagnosis, sex ratio and co-occurring cirrhosis were similar in certain reports, as were the rates of viral hepatitis infection (15,16). Furthermore, patients with CHC had less capsule formation compared with those with HCC (8,9). However, studies from Asia have identified that patients with HCC and ICC are younger compared with patients with CHC and that mainly men are affected. In Chu's and Lee's series, the majority of patients with CHC were men, with a high prevalence of HBV infection, suggesting that HBV infection is a major cause of both HCC and CHC (17,18). In the present study, the sex ratios were similar across groups; however, men were the majority in all three groups, and the age and tumor volume were similar in the CHC group compared with those in the HCC and ICC groups. Furthermore, the proportion of HBV infection was lower in CHC compared with that in HCC but similar between CHC and ICC; HCV infection was similar across groups. The percentage of patients with CHC with cirrhosis was higher compared with that of patients with ICC but similar to that of patients with HCC.

An increase in AFP was identified among the groups. Several studies have revealed that a high increase in AFP level (≥400 IU/l) is an independent prognostic factor in CHC (9,16). However, certain reports demonstrated that the level of AFP in CHC was lower compared with that in HCC but had no significant difference (12,19). In the present study, there was no significant difference in the AFP levels between the CHC group and the HCC and ICC groups.

Previous research revealed that serum CEA and CA19-9 levels were higher in patients with CHC compared with those in patients with ICC (19,20). However, the present study pointed out that the serum CEA level in the CHC group was significantly higher compared with that in the HCC group but similar to that in the ICC group, and there was no statistically significant difference in CA19-9 serum level between CHC and HCC, as well as between CHC and ICC.

In addition, the immunohistochemical characteristics of patients with CHC demonstrated intermediate features of HCC and ICC. Both the ICC-specific markers CK7 and CK19, and the HCC-specific marker glypcian-3, were elevated in the patients with CHC. These findings concurred with those reported by Lee *et al* (21).

Several previous studies have consistently reported that the prognosis of CHC is worse compared with that of HCC, whereas that of ICC varies (8,9,20,22). The present study results demonstrated that the OS and DFS of patients with CHC were significantly lower compared with those of patients with HCC, but not significantly different from those of patients with ICC. Factors associated with poor clinical prognosis and tumor recurrence and survival have been reported in several studies, including high levels of AFP, CEA and CA19-9, multiple tumors, large tumor size (>5 cm), satellite lesions, vascular invasion, advanced tumor stage and lymph node metastasis, which may represent more advanced tumor states (17,20,23,24). The present study identified that levels of CEA and lymph node metastases in the CHC group were significantly higher compared with those in the HCC group but similar to those in the ICC group, which also suggested that patients with CHC had intermediate clinical features compared with patients with HCC and ICC.

Few studies have reported on the recurrence of CHC after surgery. A previous study identified that CHC recurred continuously in the liver, whereas other studies demonstrated that extrahepatic recurrence was generally observed in the lymph nodes (9,25,26). The present study determined that the most frequent form of recurrence was intrahepatic metastasis.

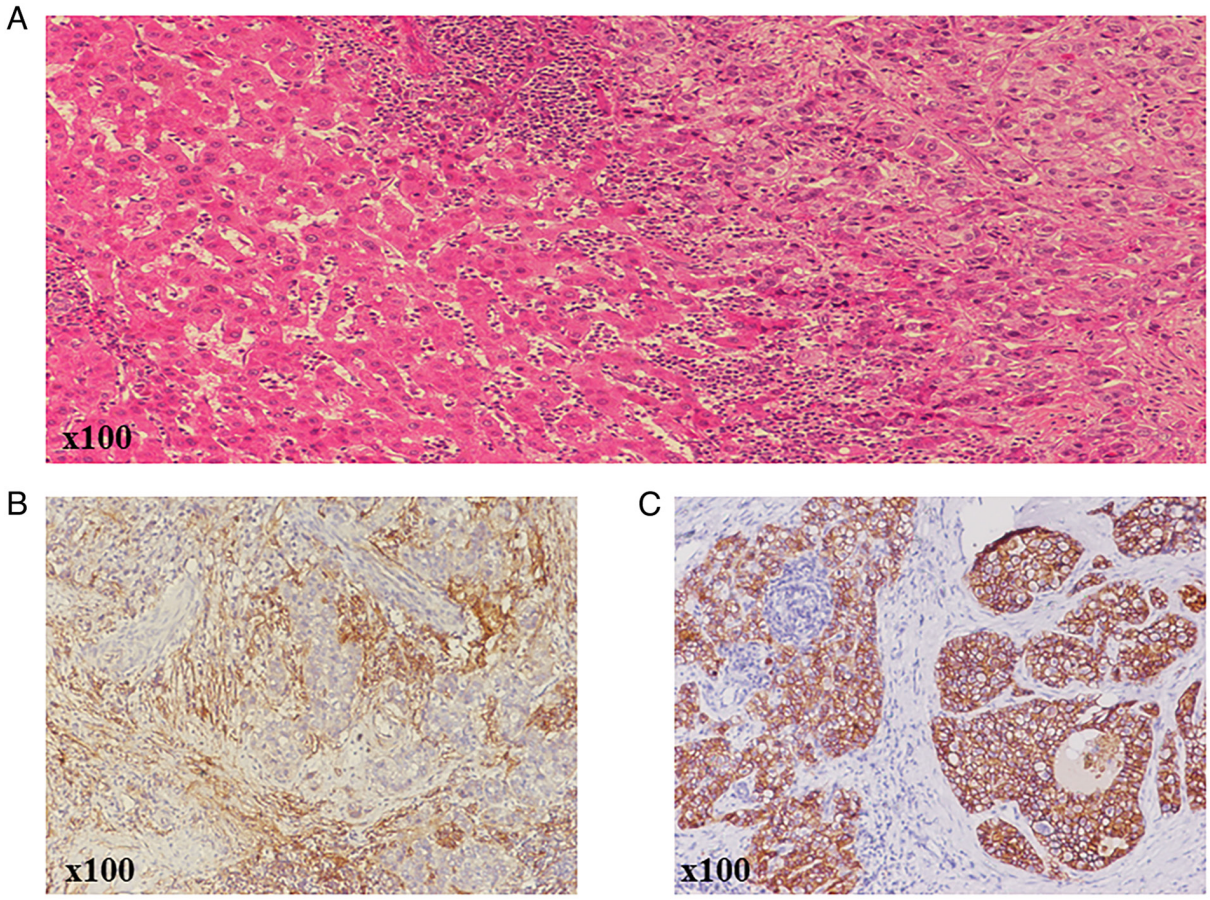

Figure 2. Representative images of CHC tissue from the same patient. (A) Histological findings of the representative CHC (H&E staining; x100 magnification). (B) Positive cytoplasmic staining for CD90 (x100 magnification). (C) Positive membrane staining for epithelial cell adhesion molecule (x100 magnification). CHC, combined hepatocellular-cholangiocarcinoma.

Currently, there are no explicit guidelines for the treatment of recurrent CHC. Most of the 20 patients with recurrence in the present study underwent TACE. However, the outcome after relapse is poor and therefore the prognosis for CHC after surgery is poor due to the high rate of recurrence and ineffective treatment after recurrence. Comprehensive postoperative therapy may be a promising strategy for patients with CHC in the future.

The origin of CHC remains complex and has been a subject of debate. The predominant hypothesis is that it is derived from HPCs, which are intermediate stem cells capable of undergoing bidirectional differentiation into hepatocytes and bile duct epithelial cells. HPCs may serve a key role in human CHC development (27,28).

HPC markers are used to authenticate HPCs. There are a large number of HPC markers, including the classic markers CD90 and EpCAM (29,30). The present study performed immunohistochemical analysis of CD90 and EpCAM to examine whether CHC arose from the HPCs in the present study. All 26 CHC samples revealed positive results for the classical HPC markers CD90 and EpCAM. The present study results indicated that CHC highly expresses HPCs, which is consistent with previous studies suggesting that CHC may be derived from HPCs (7,31); therefore, the present study further suggested that the CHC reported here may originate from the HPCs.

To the best of our knowledge, the present study is the first systematic and comprehensive analysis of the clinical pathologies, origins, risk factors for relapse and survival prognosis, and relapse treatment models of CHC. However, the present study had certain limitations. First, this was a single-center, retrospective study and the number of patients with CHC was relatively small; therefore, further larger multicenter studies are necessary to validate the present study findings. Second, it is difficult to diagnose primary biliary cholangitis, which is considered a risk factor for CHC, and so this was not included in the present study. Lastly, the present study did not routinely test for tumor burden score; therefore, the present study did not evaluate it in the CHC group.

In conclusion, CHC is an extremely rare type of PLC with a poor outcome and intermediate clinical and pathological features between HCC and ICC. CHC also has a high expression level of HPCs, supporting the hypothesis that CHC may originate from HPCs that have the potential to differentiate into hepatocytes and bile duct cells. Most studies have revealed that surgical intervention is a valid treatment for CHC; however, patients with CHC have markedly worse survival outcomes after hepatic resection compared with patients with HCC. Further studies on effective treatment modalities and clinical predictors of CHC are warranted to extend survival in these patients. Further research is necessary to confirm the molecular biology of the histogenesis of inclusive CHC.

Patients with CHC appear to have intermediate clinical features compared with those with HCC and ICC. Comprehensive postoperative therapy may be a promising strategy for patients with CHC. The high expression level of HPC markers in tumor tissues suggested that CHC may originate from HPCs.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

BX and JZho contributed to the conception of the present study and provided feedback on the report. CY, YQ, JZha and RS analyzed data. JH and JC performed the data analyses and wrote the manuscript. All authors have read and approved the final manuscript. BX and JZho confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study protocol was reviewed and approved by the Medical Ethics Committee of Guangxi Medical University Cancer Hospital (Nanning, China; approval no. 6-20-2019). Preoperatively, all patients provided written informed consent for data collection and for research purposes.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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