RESEARCH ARTICLE

Evaluation of Postoperative Adjuvant Chemotherapy for Intrahepatic Cholangiocarcinoma Patients undergoing R1 and R2 Resections

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Abstract

Surgical resection is the gold standard treatment and is considered the only potential cure for cholangiocarcinoma (CCA). However, most of the patients present at a late stage of disease and positive margins are frequently encountered. Therefore, adjuvant therapeutic modalities, such as chemotherapy and/or radiotherapy are needed to improve the survival time of CCA patients. In this study, we analyzed retrospectively the clinical features, overall survival and efficacy with postoperative adjuvant chemotherapy for 171 intrahepatic CCA patients. All those with histologically proved intrahepatic CCA diagnosed during 1998-2002, at Srinagarind Hospital, Faculty of Medicine, Khon Kean University, Thailand, were included in this study. All patients were considered to have resectable tumors with curative intent, 114 patients received postoperative adjuvant chemotherapy with 5-fluorouracil/mitomycin C, of which only 54 patients were given the full 6 cycle treatment. Mass forming type CCA was the major type found in our series. The predictive clinicopathological factors which influenced an unfavorable outcome were tumor size >4 cm, multiple masses, mass forming and periductal gross type, histology with poor differentiation, involvement of serosa, vasculature or diaphragm, advanced tumor stage and positive surgical margin. On the other hand, R0 resection, skeletonization of hepatoduodenal ligaments and complete postoperative adjuvant chemotherapy were predictive of a favorable outcome. Multivariate analysis Cox proportional hazards models revealed that sex, tumor size, serosa involvement, surgical margin status, skeletonization and postoperative adjuvant chemotherapy were independently associated with long term survival post-surgery. Regardless of the surgical margin status, patients who received complete postoperative adjuvant chemotherapy had a significant survival advantage.

Keywords: Bile duct - survival - prognosis - adjuvant chemotherapy - 5-fluorouracil - treatment

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Introduction

Cholangiocarcinoma (CCA) generally arises as a result of malignant transformation of bile duct epithelial cells lining the intrahepatic and extrahepatic bile ducts. CCA is a devastating cancer that is increasing both worldwide incidence and mortality rate (Khan et al., 2002; Tayor-Robinson et al., 2001; Sirica, 2005) and is accounting for 3% of all gastrointestinal malignancies (Aljiffry et al., 2009). CCA is found frequently in some Southeast Asian countries but the very high prevalence of this cancer has been reported in the Northeast Thailand (Sripa and Pairojkul, 2008). The epidemiology and experimental evidences implicate chronic inflammation from the carcinogenic liver fluke: *Opisthorchis viverrini* (OV), which is endemic in this region, as a major risk factor of CCA (Thamavit et al., 1978). Intrahepatic CCA account for 40-60% of all CCA and typically arise at the biliary bifurcation (Burke et al., 1998;Jarnagin et al., 2001), whereas hilar CCA are often referred to as Klastkin tumors (Klatskin, 1965).

In general, CCA present at an advanced disease stage owing to the absence of early symptoms and effective screening tests. These tumors consider as slow growing tumors which is difficult to make curative resection because its anatomical location and longitudinal extent along the bile duct (Burke et al., 1998). Surgical resection is the gold standard treatment which can provide the only potential cure, with the determinants of resectability including the extent of spread within biliary trees, amount of hepatic parenchyma involved, vascular involvement, lobar atrophy, and metastatic disease (Yachimski and Pratt,

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2008). Resection with negative margins offers increased long-term survival but the anatomical location of the tumor, the extent of perineural, and per-vascular invasion at the time of resection often make curative resection difficult (Hemandez et al., 2008). Recently, a prolonged survival and decreased recurrence rate have been shown with more extended surgical procedures, including major hepatectomy, pancreatoduodenectomy, extended lymphoadenectomy, and vascular resection to obtain negative margins (R0 resection) (Kosuge et al., 1999). In addition, R0 resection with adequate margins is an important prognostic factor affecting survival (Jarnagin et al., 2001). Regardless of aggressive resection, patients are frequently left with positive margins (R1 or R2 resections) because of the difficulties to estimate the proximal extent of the microscopic disease by pre-operative imaging. Therefore, surgery alone is not a sufficient treatment for CCA, especially for the advance stage. Adjuvant therapeutic modalities, including chemotherapy or radiotherapy are needed to obtain long term survival. In this study, we retrospectively analyzed the treatment of 171 histologically proved CCA patients and aimed to evaluate the effectiveness of postoperative adjuvant chemotherapy for CCA patients especially patients with R1 and R2 resection.

Materials and Methods

We conducted the study at Srinagarind Hospital, Khon Kean University, Khon Kaen, Thailand. Informed consent was obtained from every subjects and the study protocol has been approved by the Human Research Ethics Committee, Khon Kaen University (HE471214). Clinicopathological features of 171 patients including age, sex, tumor location, histological grading, and pTNM stage, and chemotherapeutic treatment were evaluated by reviewing the medical charts and pathological records.

Based on the patients database with hepatobiliary/ liver surgery from January 1, 1998 to December 31, 2002, we identified 171 patients with intrahepatic CCA including 126 mass forming, 19 periductal infiltrating and 26 intraductal growth type which had been evaluated by pathological findings. All patients were reviewed at multi-disciplinary tumor conferences with hepatobiliary surgeons and were offered resection when deemed appropriate. Metastatic disease and extra-hepatic extension were excluded. Tumors with occlusion of the main portal vein or invasion of the vessels supplying the hepatic remnant were considered unresectable. Patients with prohibitive medical co-morbidities or insufficient hepatic reserve were not resected. Hepatic lobar atrophy was not a strict contraindication, but the contralateral portal vein and hepatic artery must be free of disease. Patients considered to have resectable tumors were taken to the operating room with curative intent and underwent extrahepatic bile duct excision with anatomical hepatic resection. Proximal and distal bile duct margins were routinely submitted to pathological department for evaluating margin free. A negative margin (R0) was defined as macro- and microscopically tumor free, whereas those having microscopically positive margin was defined as R1. R2 resection was defined as positive margin in both macro- and microscopic examinations. Patients were treated with 5-Fluorouracil (5-FU) based regimen: 1,000 mg/m^2 5-FU for 4 consecutive days and 10 mg/m^2 mitomycin C as a single dose on the first day (as one cycle/ month). The regimens either 5-FU alone or combination of 5-FU and mitomycin C was given for 6 cycles. However, the chemotherapy was terminated when there were recurrent disease, unacceptable treatment toxicity, or request by patients. Full 6 cycles of chemotherapy is considered as the complete course of treatment, whereas patients having taken less than 6 cycles were defined as incompletely treated.

Statistical analysis

We followed each patient from the day of cancer diagnosis until emigration, death, or June 23, 2009. We excluded the patients with pre-operative death (mortality within 30 days post-operation). Survival was determined using the Kaplan-Meier analysis and compared among groups using the log-rank test. Cox regression was used to determine independent predictors of outcomes. Statistic analyses were done using SPSS statistical software version 16.0.1 (SPSS Inc., Chicago, IL) and STATA version 8. $P<0.05$ was considered statistically significant.

Results

Patients demographics and Tumor characteristics

Intrahepatic CCA patients (N=171) were histologically diagnosed and underwent surgical resection in the 1998-2002 period. The mean age at diagnosis was 56 years (ranged 47-65 years); there were 116 (67.8%) men and 55 (33.2%) women with male:female=2.1:1. Of 171 intrahepatic CCA, 73.7% were mass forming (MF); 15.2% were intraductal growth (IG) type and 11.1% were periductal infiltrated (PI) type. The majority of patients (56.1%) possessed single mass and 75.4% harbored tumor size larger than 4 cm. According to AJCC staging classification, 19 (11%) patients were in the early stage (I and II) and 152 (89%) patients were in the late stage (IIIA, IIIB, IIIC and IV). Serosa, vascular and diaphragm involvement were observed in 81.9%, 84.8% and 34.5% respectively. The intended 6 cycles of postoperative adjuvant chemotherapy were administered to the 114 intrahepatic CCA patients. However, only 54 patients could received the full 6 cycle treatment and 60 patients discontinued the treatment before completion (Table 1).

Survival

Median overall survival was 227 days (ranged 218-335 days). The 1, 3 and 5-year overall survival rates were 35.7% (61/171), 19.3% (33/171) and 12.9% (22/171), respectively (Figure 1). Univariate analysis identified the predictive clinicopathological factors of overall survival which influenced the post operative survival including large tumor size (>4 cm), multiple masses, gross morphology with MF and PI type, histology with poorly differentiation, involvement of serosa, vasculature or diaphragm, and advanced tumor stage (Table 1). In addition, we found that the surgical margin status with

R0, and skeletonization of hepatoduodenal ligament were significantly associated with better survival of CCA patients (Table 2). All these significant predictive factors of overall survival were included in the multivariate analysis Cox proportional hazards models. Sex (P=0.001), tumor size (P=0.017), serosal involvement (P=0.045), surgical margin status (P<0.001), skeletonization (P=0.010) and postoperative adjuvant chemotherapy (P=0.002) were independently associated with long term survival (Table 3).

Table 1. Characteristics of Intrahepatic CCA Patients and Factors Influenced Overall Survival (n=171)

Variables		No	%	Hazard ratio	95%CI	P
Age:	<56 years	85	49.7	1		
	>56 years	86	50.3	1.23	0.89 - 1.68	0.21
Sex:	Female	55	33.2	1		
	Male	116	67.8	1.37	0.98 - 1.93	0.069
Tumor size:	<4 cm	42	24.6	1		
	>4 cm	129	75.4	2.23	1.52 - 3.27	<0.05
Tumor number:	Single mass	96	56.1	1		
	Multiple Mass	67	39.2	1.46	1.05 - 2.03	0.022
	No Mass	8	4.7	0.56	0.24 - 1.28	0.171
Gross morphology:						
	Mass forming	126	73.7	1		
	Periductal infiltrating	19	11.1	0.79	0.48 - 1.31	0.369
	Intraductal Growth	26	15.2	0.31	0.19 - 0.52	<0.05
Histology type:	Well differentiation	125	73.1	1		
	Moderately differentiation	26	15.2	1.97	1.27 - 3.07	0.003
	Poorly differentiation	20	11.7	1.49	0.92 - 2.44	0.105
TNM stage:	I	13	7.6	1		
	II	6	3.5	3.42	1.13- 10.29	0.029
	III A	1	0.6	4.66	2.13- 10.17	<0.05
	III B	42	24.6			
	III C	69	40.4			
	IV	40	23.4	9.13	3.96 - 21.02	<0.05
Serosa involvement:						
	Absence	31	18.1	1		
	Presence	141	81.9	2.35	1.52 - 3.63	<0.05
Vascular involvement:						
	Absence	26	15.2	1		
	Presence	145	84.8	3.25	2.00 - 5.27	<0.05
Diaphragm involvement:						
	Absence + no data	112	65.5	1		
	Presence	59	34.5	1.94	1.38 - 2.73	<0.05

Table 2. Benefit Outcome of Surgical Result and Adjuvant Chemotherapy

Variable		No.	%	Hazard ratio	P	95% CI
Surgery	R0 resection	30	17.5	1		
	R1 resection	40	23.4	3.64	<0.05	2.04 - 6.51
	R2 resection	101	59.1	5.56	<0.05	3.27 - 9.48
Skeletonization of hepatoduodenal ligament						
	No	105	61.4	1		
	Yes	66	38.6	0.52	<0.05	0.37 - 0.73
Chemotherapy	Complete	54	31.6	1		
	Incomplete	60	35.08	1.88	0.002	1.27 - 2.78
	No Treatment	57	33.33	1.75	0.006	1.17 - 2.62

Table 3. Multivariate Survival Analysis of Prognostic Factors for Intrahepatic CCA Patients

Variable	Overall Survival (P)	Multivariate analysis (P)	Adjusted Hazard ratio (95% CI)
Age (<56 vs >56)	0.2087	0.695	
Sex (Female vs Male)	0.0672	0.001	1.91 (1.29 - 2.82)
Tumor size (<4 vs >4 cm)	<0.001	0.017	1.84 (1.11 - 3.05)
Tumor number (Single vs. Multiple mass)	0.0085	0.534	
Gross morphology (MF vs. PI and IG)	<0.001	0.442	
Histology type (WD vs. MD and PD)	0.0021	0.273	
TNM stage (I /II vs. III /IV)	<0.001	0.966	
Serosa involvement (Presence vs. Absence)	<0.001	0.045	0.50 (0.25 - 0.98)
Vascular involvement (Presence vs. Absence)	<0.001	0.252	
Diaphragm involvement (Presence vs. Absence)	<0.001	0.45	
Surgery (R0 vs. R1)	<0.001	<0.001	3.68 (1.86 - 7.31)
(R0 vs. R2)	<0.001	<0.001	6.59 (3.29 - 3.23)
Skeletonization of hepatoduodenal ligament (Yes vs. No)	<0.001	0.01	0.63 (0.44 - 0.89)
Postoperative adjuvant chemotherapy (Complete vs. Incomplete + No treatment)	0.0007	0.002	0.56 (0.39 - 0.82)

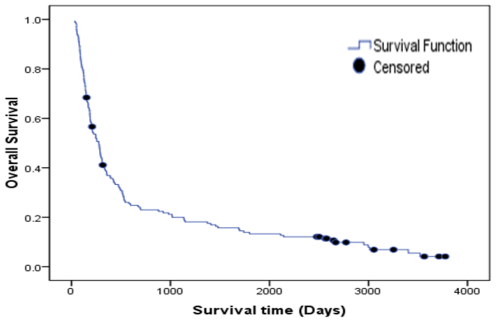

Figure 1. Survival of 171 Intraheparic CCA was Determined using the Kaplan-Meier Analysis and Compared using theLog-Rank Test

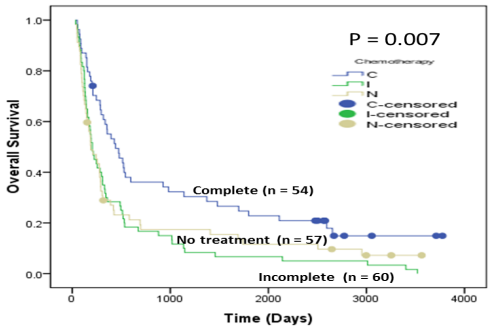

Figure 2. Survival of CCA Patients According to the Postoperative Adjuvant Chemotherapy. CCA patients who received complete chemotherapy showed significantly improvement of overall survival comparing with those who had incomplete or no postoperative adjuvant treatment (P=0.007)

Aggressive surgical resection was considered a better choice of curative treatment in CCA so far. However, not all patients who underwent this treatment had satisfactory with long term survival, especially patients who had R1 and R2 surgical margins (Table 3). In our series, there were 171 CCA patients who underwent aggressive surgical resection with different surgical margins as follows; 17.5% (30/171) R0, 23.4% (40/171) R1 and 59.1% (101/171) R2 resections. We found that 54 (31.6%) of 171 patients, who had received complete 6 cycles of postoperative adjuvant chemotherapy showed significantly longer overall survival comparing with those 117 (68.4%) patients who had incomplete 6 cycles or no postoperative adjuvant treatment (P=0.007) (Figure 2).

Since surgical margin status was proven to be an independent prognostic factor of CCA patients. Patients with R0 resection had a better prognosis than patients with R1 and R2 resections with the median survival of 2,111 days comparing to 305 days and 169 days, respectively (Figure 3A). Therefore, we next investigated the effectiveness of postoperative adjuvant chemotherapy for CCA patients. Regardless of the surgical margin status, patients who received complete 6 cycles of postoperative adjuvant chemotherapy (n=54) had a significant survival advantage with increasing median survival time to 2,653 days, 316 days and 354 days for R0, R1 and R2 patients, respectively (Figure 3B). Since the postoperative

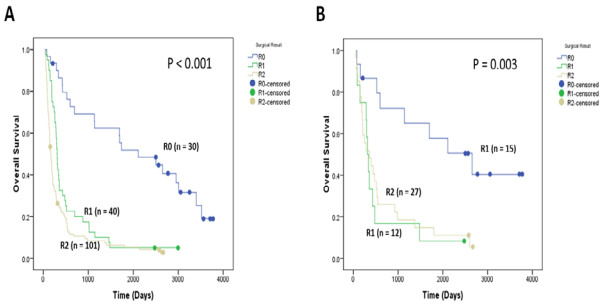

Figure 3. Survival of CCA Patients According to Surgical Margin. A) Patients with R0 resection had a better prognosis than patients with R1 and R2 resections. B) Regardless to the surgical margin status, patients who received complete chemotherapy (n=54) had a significant survival advantage than those who had incomplete or had no postoperative adjuvant treatment

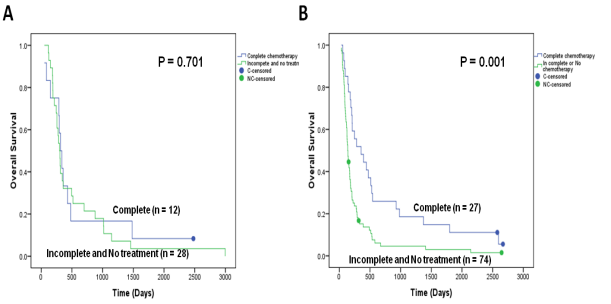

Figure 4. Survival of CCA Patients According to Post-Operative Chemotherapy Treatment. Patients with (A) R1 resection; (B) R2 resection

Table 4. Characteristics of Patients with R1 and R2 Resections and Adjuvant Chemotherapy (n =141)

Variables			Adjuvant Chemotherapy		
		No.	Yes (n=94)	No (n=47)	P
Age	<56 years	67	46	21	0.633
	>56 years	74	48	26	
Sex	Female	46	31	15	0.899
	Male	95	63	32	
Tumor size	<4 cm	29	24	5	0.039
	>4 cm	112	70	42	
Tumor number	Single mass	75	51	24	0.534
	Multiple Mass	64	41	23	
	No Mass	2	2	0	
Gross morphology	Mass forming	111	73	38	0.165
	Periductal infiltrating	19	11	8	
	Intraductal Growth	11	10	1	
Histology type	Well differentiation	100	66	34	0.189
	Moderately differentiation	23	13	10	
	Poorly differentiation	18	15	3	
Distant Metastasis	Absence	102	67	35	0.69
	Presence	39	27	12	
TNM stage	I	1	0	1	0.399
	II	2	2	0	
	III A	0	0	0	
	III B	32	19	13	
	III C	66	46	20	
	IV	40	27	13	
Serosa involvement	Absence	13	12	1	0.04
	Presence	128	82	46	
Vascular involvement	Absence	9	7	2	0.465
	Presence	132	87	45	
Diaphragm involvement	Absent + Not indicate	84	56	28	0.236
	Present	57	38	19	
Surgery	R1 resection	40	30	10	0.187
	R2 resection	101	64	37	
Skeletonization of hepatoduodenal ligament					
	No	93	58	35	0.132
	Yes	48	36	12	

treatment was not obvious in patients with R1 and R2 surgical margins, we further evaluated the effectiveness of postoperative adjuvant chemotherapy in these patients. Of 141 CCA patients with R1 and R2 resections, 94 (66.6%) patients received postoperative adjuvant chemotherapy. There were no significant differences in age, sex, tumor number, gross and histological morphology, staging, vascular and diaphragm involvement between patients with or without postoperative adjuvant chemotherapy. However, patients without adjuvant treatment (n=47) had significantly larger tumor size (>4 cm) and the higher frerquency of the presence of serosal involvement (Table 4). We found that the increment of survival time in R2 patients was appreciably related to fully complete 6 cycles of postoperative adjuvant chemotherapy (median survival=354 days) comparing with incomplete and no treatments (median survival=137 days) (P=0.001) (Figures 4A-B). However, we could not observe any increment of survival time after postoperative adjuvant chemotherapy in CCA patients with R1 resection.

Discussion

This study is our 5 years experience of the treatment for 171 intrahepatic CCA patients with and without postoperative adjuvant chemotherapy. To our knowledge, this is the largest scale retrospective survey for the evaluation of the efficacy of treatment for liver fluke-related intrahepatic CCA patients. A significant survival advantage was obtained in patients who underwent an aggressive surgical resection with curative intent and postoperative adjuvant chemotherapy.

CCA is a major liver cancer found in the northeastern region of Thailand. The incidence of CCA in Khon Kaen province is highest in the country and in the world. Due to lacking of symptom and of specific tumor markers at the early stage, the diagnosis is usually made only at the late stage and hence palliative treatment is often the treatment of choice. Even though CCA at the advanced stage is commonly unresectable, tumor resection with curative intent is the only potential cure for this cancer. Because of the high incidence of locoregional recurrence after surgery, several investigators have recommended an adjuvant radiation therapy (Todoroki et al., 2000; Gerhards et al., 2003; Sagawa et al., 2005; Cheng et al., 2007) or chemo-radiation therapy (Hughes et al., 2007; Krishnan et al., 2008; Nelson et al., 2009) after surgical resection of CCA. Recent reports on postoperative adjuvant treatment for biliary cancer demonstrated that either selected adjuvant radiotherapy or chemo-radiotherapy after surgery showed a survival benefit comparing with surgery alone (Gerhards et al., 2003; Sagawa et al., 2005; Cheng et al., 2007; Hughes et al., 2007).

In general, clinical features of CCA patients in this study were similar with those reported earlier in 1990-1999 for Thai CCA patients (Vatanasapt et al., 1990; Green et al.,1991; Uttaravichien et al.,1999), with mean age of 56 years and male preference. The majority of intrahepatic CCA is MF type and almost all of the patients presented at a late advanced stage. A better prognosis for CCA patients with early tumor stages and R0 resection was observed in

many reports (Jarnagin et al., 2001; Cannon et al., 2012; Otani et al., 2012) and is also true for this study (Table 1, 2). This reflects a crucial need for early diagnosis by specific non-invasive tumor markers in combination with advanced ultrasonography for CCA patients.

Our study demonstrated a significantly longer overall survival in the patients with R0 resection as compared to those patients with R1 or R2 resection (P<0.001). This observation is in agreement with the previous study which showed that R0 resection with adequate margin is an important prognostic factor affecting the survival (Jarnagin et al., 2001). However, only 17.5% of patients in our series had R0 resection. This may be due to the advanced stages of the cancers already at the time of diagnosis. Therefore, the necessity of an early detection should be emphasized again here to improve the treatment of CCA.

We also showed that a significant survival advantage can be obtained in patients who underwent an aggressive surgical resection (with curative intent) followed by postoperative adjuvant chemotherapy compared to those having received aggressive surgery without postoperative adjuvant therapy. Survival benefit in patients with R2 resection was found after complete 6 cycles of postoperative adjuvant chemotherapy (P=0.001). The similar outcomes can be expected for patients with non-liver fluke-related CCA. Significant survival advantage conferred to CCA patients with R1 or R2 resection who had radiotherapy after operation was reported (Cheng et al., 2007). Adjuvant chemotherapy alone or with radiotherapy have been shown to improve patients' survival after surgical resection in several cancers, e.g., pancreatic cancer (Regine et al, 2007; Murakami et al., 2009a; Oettle et al., 2009), and hilar cholangiocarcinoma (Murakami, 2009).

In conclusion, a significant improvement in survival after aggressive resection with postoperative adjuvant chemotherapy (5-FU, and mitomycin-C) for advanced CCA was observed. The effective treatment of postoperative adjuvant chemotherapy was also demonstrated to be an independent prognostic factor for CCA patients with positive surgical margin by improving their survival benefits. We, therefore, suggest that to improve the outcome of treatment, a complete treatment of postoperative adjuvant chemotherapy should be offered for CCA patients who undergo surgery with curative intent.

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