ORIGINAL ARTICLE

HER-2 Expression Status in Patients with Cholangiocarcinoma

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Background: Cholangiocarcinoma is the most common primary cancer of liver in North-Eastern region of Thailand. Human epidermal growth factor receptor-2 (HER-2) overexpression is a poor prognostic factor of many types of cancer but there is limited data in cholangiocarcinoma. The present study aimed to investigate prevalence of HER-2 overexpression in cholangiocarcinoma and analyzed its association with overall survival and clinicopathological features.

Materials and Methods: The present study was retrospective cohort analytical single-centered study which included adult patients with cholangiocarcinoma in Srinagarind Hospital, Khon Kaen, Thailand. Archived pathological tissue diagnosed as cholangiocarcinoma were stained for HER-2 using a labeled streptavidin biotin peroxidase method with the polyclonal antibody (Dako A0485) and classified into positive and negative group as, in the present study, 3+ and 0, 1+, 2+ by immunohistochemistry results, respectively. Prevalence of HER-2 overexpression was presented with percentage and 95% confidence interval (95% CI).

Results: A total of 93 cases, of which 71% were male, were included. Mean age was 63 and 62 years in HER-2 positive and negative groups, respectively. The proportion of intrahepatic was more than extrahepatic cholangiocarcinoma (72% vs. 28%). HER-2 positive was revealed 10 cases, as 10.8% (95% CI 5.3% to 18.9%). Most cases were early to locally advance stage (89% vs. 90%) Median overall survival was not reach in HER-2 positive group compared with 30.9 months in negative group (hazard ratio 0.39; 95% CI 0.12 to 1.21; p 0.089).

Conclusion: Among patients with cholangiocarcinoma, 1 of 10 cases had HER-2 overexpression.

Keywords: Cholangiocarcinoma; Prognostic factor; Prognostic marker; HER-2 overexpression; Overall survival; Clinicopathological feature; Biomarker

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Hepatobiliary malignancies are the seventh most common cancer in the world by global cancer statistic 2020[1]. In Thailand, they are the most common cancer in men and the fifth most common cancer in women[2]. In primary hepatobiliary malignancies, cholangiocarcinoma is the second most common cancer behind the hepatocellular carcinoma and the most common primary cancer of liver in North-Eastern region of Thailand[3].

Human epidermal growth factor receptor-2 (HER-2) amplification and overexpression are important driving mutations that contribute to major step of carcinogenesis. It can be evaluated by ethier immunohistochemistry or in situ hybridization. In the field of cholangiocarcinoma, there is no consensus criteria for classifing overexpression status to positive or negative. Apart from that, there are criteria for HER-2 analysis in breast cancer and HER-2 criteria in gastroesophageal adenocarcinoma[4,5]. Before the era of targeted therapy, previous studies showed that HER-2 overexpression or amplification were related to poor prognosis and more aggressiveness of breast cancer[6]. It also was a poor prognostic factor of many types of cancer[7,8].

The prevalence of HER-2 overexpression in cholangiocarcinoma, in the present study which about half of study population were Thai, was 73%[9]. It varied among studies, ranging from 0% to 73% and rarer in Western country (1.4%)[9-20]. This variation may be resulted from diversity of criteria for defining positivity and the association between HER-2 overexpression and liver fluke infestation that was more common in Thailand[9,11,21,22].

As the prognostic biomarker, there was study showed

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that HER-2 overexpressed mostly in well-differentiated cholangiocarcinoma[10]. There was a study, conducted in Japan, analyzed 68 cholangiocarcinoma sample reported that patients with HER-2 overexpression were associated with shorter survival time than others. However, this was a small study and included only patients in Japan[23]. There was no association between HER-2 expression status and various clinicopathologic features in another study[24]. In subgroup of lymph node metastasis in patients with resected extrahepatic cholangiocarcinoma, HER-2 amplification was associated with higher mortality rate (Hazard ratio (HR) 43.6, 95% confidence interval (95% CI) 1.51 to 1219.6) but was not associated with clinicopathological features[25]. Another study, conducted in Thailand, classified cholangiocarcinoma to 4 clusters by integrative clustering of tumor whole genome. They found that survival rate, as secondary outcome, of cluster 1 and cluster 2, which had HER-2 amplification as a part of genetic mutation profile, was lower than cluster 3 and cluster 4, which had no HER-2 amplification[11]. In western population with cholangiocarcinoma, HER-2 overexpression was an independent prognostic factor for mortality (Hazard ratio 3.08, 95% CI 1.23 to 7.28)[26]. However, there was no association with survival in HER-2 positive group in one study[21].

Current data about HER-2 overexpression as a prognostic and predictive marker of cholangiocarcinoma is limited and inconclusive[4]. To conduct study assessing these aspects, the exact prevalence of HER-2 overexpression is required. The major risk factor of cholangiocarcinoma in Thai patients is liver fluke, converserly to other regions that the main mechanism is primary sclerosing cholangitis and there is no large study that investigate survival outcome. So, there might be the different survival outcome in the study conducted in different region. The present study aimed to study about prevalence of HER-2 overexpression and its association of clinicopathological features and survival outcome in patients with cholangiocarcinoma attended in Srinagarind Hospital.

Materials and Methods

The present study was retrospective cohort analytical, single-centered study which included patients aged above 18 years old diagnosed as cholangiocarcinoma by pathologist and attended in Srinagarind hospital, Khon Kaen University, Khon Kaen, Thailand, between 1st January 2015 and 31st May 2019.

All archived pathological tissue diagnosed as cholangiocarcinoma were stained for HER-2 using a labeled streptavidin biotin peroxidase method with the polyclonal antibody (Dako A0485) and classified into HER-2 positive or HER-2 negative by a certified and appropriately blinded pathologist using 2016 HER-2 criteria in gastroesophageal adenocarcinoma from the college of American pathologists, American society of clinical pathology and American society of clinical oncology (CAP-ASCP-ASCO), defined HER-2 positive as strong complete basolateral or lateral membranous reactivity in ≥10% of cancer cells (intensity of staining was 3+). Other results were classified as HER-2 negative as shown as Figure 1.

The primary endpoint was prevalence of HER-2 overexpression in patients with cholangiocarcinoma. The authors included clinical data (including date of last follow-up/death, status at last follow-up, parameters to calculate Charlson's comorbidities index (CCI)[27], Eastern Cooperative Oncology Group (ECOG) performance status, cancer staging and other basic characteristic), laboratory data on the date of admission at the time of diagnosis (including liver function test[28], coagulogram, complete blood count, serum creatinine, serum cancer antigen 19-9 (CA19-9) as there was association with survival outcome after resection[29]), pathological data and treatment received (including chemotherapy, surgical resection and surgical or radiological or body intervention) from Thai civil registry, Srinagarind Hospital's cancer registry, electronic medical record and patient's chart review using case record form.

The sample size was calculated using prevalence of HER-2 overexpression of 0.73[9], desired precision of 0.1 and confidence level of 0.95. The calculated sample size was 76.

Prevalence of HER-2 overexpression in patients with cholangiocarcinoma was presented with percentage and 95% CI. Baseline characteristics of patients were summarized. Estimation of percentage point was used for discrete

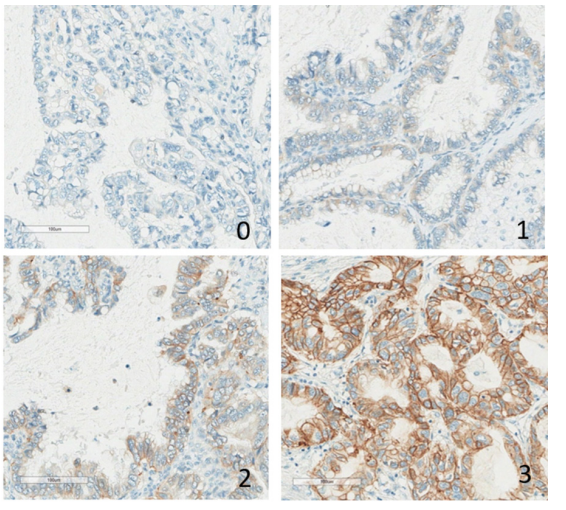

Figure 1. Interpretation of immunohistochemistry of study tissue according to 2016 HER-2 criteria in gastroesophageal adenocarcinoma from the college of American pathologists, American society of clinical pathology and American society of clinical oncology.

variable. Mean with standard deviation (SD) or median with inter-quartile range (IQR) were used for continuous variable. Associations between HER-2 overexpression and clinicopathological features were reported as odds ratio (OR) and 95% CI. The p-value was calculated by Fisher's exact test. Continuous data was tested for normality by Kolmogorov-Smirnov test and analyzed by independent samples t-test and presented as mean difference with 95% CI, p-value and mean with SD or median with IQR. Loss of follow-up or alive until 31st May 2020 were defined as censored. Survival outcome of patients with HER-2 positive group compared with HER-2 negative group was analyzed by Cox regression model and log rank test presented with Kaplan-Meier curve, Overall survival (OS), HR with 95% CI and p-value. Subgroup analysis of survival outcome was conducted with the subgroup of intrahepatic/extrahepatic cholangiocarcinoma and tumor staging. Prespecified analysis using different cut-off of intensity of staining to compare OS was done, analyzed by Cox regression model. All data analyses were performed using IBM SPSS Statistic version 26.

The present study was approved by the Institutional Review Board (HE631276), the Office of Human Research Ethics, Khon Kaen University.

Results

The authors identified 93 cases of cholangiocarcinoma in pathological archives during the period of 1st January 2015 to 31st May 2019, of which 66 cases (71%) were male. 67 cases (72%) were intrahepatic cholangiocarcinoma. 26 cases (28%) were extrahepatic cholangiocarcinoma. Prevalence of HER-2 positive in patients with cholangiocarcinoma in Srinagarind Hospital was 10.8% (95% CI 5.3% to 18.9%) (10 of 93 cases), composed of 9 cases (13.4%) of patients with intrahepatic cholangiocarcinoma and 1 case (3.8%) of patients with extrahepatic cholangiocarcinoma. The prevalence of HER-2 staining intensity 2+ and 1+ were 14.0% (13 cases) and 15.1% (14 cases), respectively and was shown as Figure 2.

The baseline characteristics of overall population was shown in Table 1. The mean age were 63 and 62 years with standard deviation of 6.5 and 7.9 years in HER-2 positive group and HER-2 negative group, respectively. The median points of CCI were 5 in both groups with IQR of 2 and 1 in HER-2 positive group and HER-2 negative group, respectively. In HER-2 positive group, there was higher proportion of pateints who received chemotherapy as a part of treatment (80% compared with 51.8%) and higher proportion of patients with ECOG performance status 0 (100% compared with 61.8%) compared with HER-2 negative group. Most patients were in early or locally advanced stage (8 of 9 (89%) and 71 of 79 (90%) in HER-2

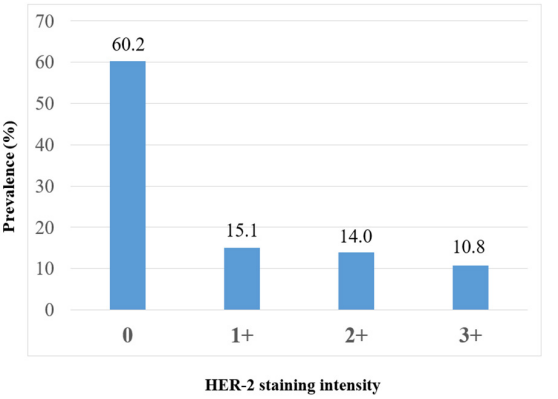

Figure 2. Bar chart demonstrated prevalence of each HER-2 staining intensity; x-axis showed HER-2 staining intensity group; y-axis showed prevalence in each group.

positive and negative group, respectively, were in stage I to III). Median follow-up time were 47 months with IQR of 28 months and 30 months with IQR of 33 months for HER-2 positive group and HER-2 negative group, respectively. At the end of follow-up, 3 cases (30%) of HER-2 positive group and 48 cases (57.8%) of HER-2 negative group have died. All patients were undergone surgical resection. In HER-2 positive group, 8 of 10 cases (80%) received chemotherapy which more than in HER-2 negative group, which 43 of 83 cases (51.8%) received chemotherapy. Most common selected chemotherapy regimen in both adjuvant and palliative settings was cisplatin with 5-fluorouracil, as 4 of 10 patients (40%) in HER-2 positive group and 19 of 45 (42%) patients in HER-2 negative group. Second most common regimen was cisplatin with gemcitabine, as 3 of 10 patients (30%) in HER-2 positive group and 17 of 45 patients (38%) in HER-2 negative group.

Median OS of overall population was 32.0 months (95% CI 16.9 to 47.1 months). HER-2 positive cholangiocarcinoma tended to associate with better survival outcome with the median OS of not reach (NR) compared with 30.9 months in HER-2 negative group without statistically significance, HR 0.39 (95% CI 0.12 to 1.21; p 0.089) (Figure 3). Subgroup analysis of survival outcome showed that even in subgroup of intrahepatic or extrahepatic cholangiocarcinoma, there was no significant association between HER-2 positive and OS, HR 0.49 (95% CI 0.15 to 1.59; p 0.234) and 0.04 (95% CI 0.00 to 237.10; p 0.477), respectively. Subgroup analysis by cancer staging showed HER-2 positive tended to have positive effect on survival outcome without statistically significance in patients with stage I, II and III with HR of 0.48 (95% CI 0.06 to 3.76; p 0.486), 0.40 (95% CI 0.00 to 3664.66; p 0.581) and 0.41 (95% CI 0.06 to 3.02; p 0.380), respectively. In prespecified analysis, defining HER-2 staining 2+ or 1+ as HER-2 positive, there was no significant

Table 1. Baseline characteristic of overall population classified by Human epidermal growth factor receptor-2 (HER-2) immunohistochemistry results (defined positive as 3+ in staining intensity) presented as count and percentage by total number within column unless specified otherwise

Characteristics	HER-2 positive (3+) (n=10)	HER-2 Negative (0, 1+, 2+) (n=83)	All patients (n=93)
Sex, male	9 (90.0%)	57 (68.7%)	66 (71.0%)
Age (years) [1]	63, 6.5	62, 7.9	62, 8.2
Body mass index (kg/m^2) [2]	22.9, 3.75	22.4, 3.89	22.1, 4.00
Charlson's comorbidities index [2]	5, 2	5, 1	5, 1
Cancer staging			
Stage I	5 of 9 (55.6%)	26 of 79 (23.9%)	31 of 88 (35.2%)
Stage II	1 of 9 (11.1%)	10 of 79 (12.7%)	11 of 88 (12.5%)
Stage III	2 of 9 (22.2%)	35 of 79 (44.3%)	37 of 88 (42.0%)
Stage IV	1 of 9 (11.1%)	8 of 79 (10.1%)	9 of 88 (10.2%)
ECOG			
ECOG 0	4 of 4 (100%)	21 of 34 (61.8%)	25 of 38 (65.8%)
ECOG 1	0	12 of 34 (35.3%)	12 of 38 (31.6%)
ECOG 2	0	0	0
ECOG 3	0	1 of 34 (2.9%)	1 of 38 (2.6%)
ECOG 4	0	0	
Blood chemistry			
Albumin (g/dL) [2]	4.0, 0.5	4.2, 0.8	4.1, 0.8
Directed bilirubin (mg/dL) [2]	0.2, 0.5	0.2, 1.1	0.2, 0.5
Alanine aminotransferase (U/L) [2]	33, 44	36, 51	36, 47
Aspartate aminotransferase (U/L) [2]	32, 19	37, 41	37, 40
Alkaline phosphatase (U/L) [2]	116, 140	141, 134	136, 112
International normalized ratio [2]	1.02, 0.14	1.03, 0.14	1.02, 0.13
Hemoglobin (g/dL) [1]	12.5, 1.39	12.4, 1.57	12.4, 1.49
Cancer antigen 19-9 (U/mL) [2]	11.4, 81.3	36.3, 111.2	35.2, 111.8
Intrahepatic type	9 (90.0%)	58 (69.9%)	67 (72.0%)
Extrahepatic type	1 (10.0%)	25 (30.1%)	26 (27.9%)
Perihilar	1 (10.0%)	21 (25.3%)	22 (23.7%)
Distal	0	4 (4.8%)	4 (4.3%)
Treatment received			
Chemotherapy	8 (80%)	43 (51.8%)	51 (54.8%)
Number of cycles (times) [2]	6, 4	2, 6	3, 6
Surgery	10 (100%)	83 (100%)	93 (100%)
Other surgical/radiological intervention	4 (40.0%)	30 (36.1%)	34 (36.5%)

[1] Mean with standard deviation, [2] Median with interquartile range

ECOG=Eastern Cooperative Oncology Group performance status

association between HER-2 positive and OS, HR 0.79 (95% CI 0.56 to 1.12; p 0.183) and 0.81 (95% CI 0.46 to 1.43; p 0.464), respectively.

The present study could not demonstrate association between HER-2 positive and any clinicopathological features (Table 2).

Discussion

Prevalence of HER-2 positive in patients with cholangiocarcinoma was 10.8%. This was corresponded with study in Thailand that assessed HER-2 amplification with the prevalence of 8.5%[11]. The incidence of liver fluke infestation, that associated with HER-2 amplification, was decreased overtime due to National Public Health Development plan, liver fluke control program and other liver fluke control policy, developed since 1987[11,30,31]. So, this may address why another study, which about half of study population were Thai and was conducted in 1989, showed prevalence was 73%[9]. The prevalence of HER-2 overexpression in patient with cholangiocarcinoma varied among studies, ranging from 0% to 73% and was rarer in Western country (1.4%)[9-20]. This variation may be resulted from: (a) including cytoplasmic HER-2 overexpression in HER-2 positive group by some studies, (b) variation

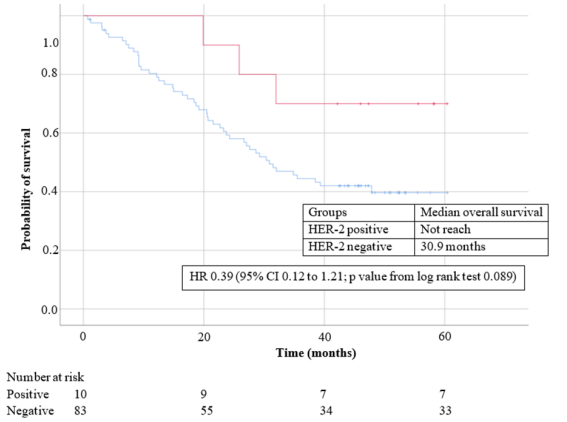

Figure 3. Kaplan-Meier Curve demonstrated probability of survival (y-axis) and time (months; x-axis) in patient with cholangiocarcinoma according to HER-2 positive (red line) or HER-2 negative (blue line) with Hazard ratio (HR), 95% confidence interval (95% CI) and p-value.

of criteria for defining positivity, (c) some guidelines changed definition for interpretation in updated versions e.g. United Kingdom guideline that reverted to criteria of >10% cells staining for HER2 instead of the >30% cut-off used in previous guideline, and (d) the association between HER-2 overexpression and liver fluke infestation that was more common in Thailand[9,11,18,21,32]. For example, the study using weakly positive for membrane staining as the cut-off for positive interpretation showed that prevalence of HER-2 positive was 67%[18], and another study using 2+ intensity, defined as moderately membrane staining without requirement of amount of stained tumor cell, for positive interpretation showed that prevalence was 65%[17]. Whereas the study using breast cancer criteria for HER-2 classification showed lower prevalence (4.5%) than others[15]. In recently published study from our organization, the scoring system and criteria for classifing overexpression of HER-2 were different from this study causing difference in prevalence of HER-2 overexpression (29.5%)[20]. In the present study there was higher proportion of HER-2 positive in intrahepathic than extrahepatic cholangiocarcinoma, 13.4% and 3.8% respectively. Result was inconsistence with previous tumor genome study, found that HER-2 alterations were occurred predominantly in extrahepatic cholangiocarcinoma (11% compared with 3% in intrahepatic cholangiocarcinoma)[33]. Few subjects with extrahepatic cholagiocarcinoma included in the authors' study, due to relatively higher incidence of intrahepatic cholangiocarcinoma in North-Eastern region of Thailand, may contribute to this inconsistence[3]. Further study is required.

The authors chose immunohistochemistry to assess overexpression of HER-2 rather than in situ hybridization because it was a method that assess final product expressed in central dogma. However, there was correlation between HER-2 overexpression defined by both methods[15]. As there was no consensus criteria to defined HER-2 positive in cholangiocarcinoma, the authors used 2016 HER-2 criteria in gastroesophageal adenocarcinoma from CAP-ASCP-ASCO. The reason was that malignancies originated from organs other than breast tended to have less intensity of HER-2 immunostaining pattern.

We found that HER-2 positive in patients diagnosed as cholangiocarcinoma had no significant association with survival outcome compared with HER-2 negative with median OS of NR and 30.9 months, respectively (HR 0.39; 95% CI 0.12 to 1.21; p 0.089). This finding was inconsistent with some of previous studies that reported HER-2 as poor prognostic factor[11,23,25,26]. In one study, patients with resected extrahepatic cholangiocarcinoma with HER-2 amplification was associated with higher mortality rate only in subgroup of lymph node metastasis[25]. Several reasons may account for this finding: (a) the present study mostly included resectable cholangiocarcinoma, this resectability may also associate with lower mortality rate compared with others[34-38], (b) there was higher proportion of patients with ECOG performance status 0 in HER-2 positive group that may positively affect OS[39], (c) the authors proposed that there may be other undiscovered prognostic factors, (d) in the present study, patients in HER-2 positive group had higher proportion of receiving chemotherapy that improved survival outcome in resectable cholangiocarcinoma[37,40], (e) limited number of study population causing low power to detect significance of OS, and (f) the authors did not collect data on residual tumor classification of surgical margin that can affect the primary outcome, that was the limitation of the present study. In another recently published study from our organization, analyzed HER-2 overexpression and survival outcome as secondary outcome, showed HER-2 had no significant effect on survival outcome that correlated with the authors' study[20]. Another limitation was no incorporation of in situ hybridization with immunohistochemistry testing in the present study. Therefore, there must be cautious that, in this study, HER-2 negative means HER-2 immunohistochemistry status of 0, 1+ and 2+.

Median overall survival for all study patients was 32 months that was more than previous study with median overall survival of 12 months in stage I and II disease[34]. Improvement of screening program in Thailand was proposed.

In the present study, most common selected chemotherapy regimen (adjuvant and palliative settings) was cisplatin with 5-fluorouracil and second most common was cisplatin with gemcitabine due to there was no standard regimen for adjuvant setting and our institution conducted clinical trial assessing adjuvant chemotherapy of cisplatin with gemcitabine.

Table 2. Association between HER-2 positive and clinicopathological features presented as count, percentage by total number within row and statistical interference unless specified otherwise

Clinicopathological features (n)	HER-2 Positive	Statistical interference	p-value
Tumor differentiation			
Poorly/moderately (10)	2 (20.0%)	OR 2.29 (95% CI 0.49 to 8.44)	0.307 [1]
Well (71)	7 (9.9%)		
Portal vein invasion			
Yes (25)	1 (4.0%)	OR 0.30 (95% CI 0.04 to 2.27)	0.278 [1]
No (68)	9 (13.2%)		
Hepatic vessel invasion			
Yes (17)	0	-	0.199 [1]
No (76)	10 (13.2%)		
Perineural invasion			
Yes (10)	2 (20.0%)	OR 2.08 (95% CI 0.51 to 8.45)	0.292 [1]
No (83)	8 (9.6%)		
Lymphovascular invasion			
Yes (12)	2 (16.7%)	OR 1.69 (95% CI 0.41 to 7.02)	0.613 [1]
No (81)	8 (9.9%)		
Lymph node metastasis			
Yes (35)	1 (2.9%)	OR 0.18 (95% CI 0.02 to 1.39)	0.084 [1]
No (58)	9 (15.5%)		
Stage IV disease			
Yes (9)	1 (11.1%)	OR 1.03 (95% CI 0.15 to 7.19)	1.000 [1]
No (83)	9 (10.8%)		
Classification			
Intrahepatic (67)	9 (13.4%)	OR 3.88 (95% CI 0.47 to 32.27)	0.273 [1]
Extrahepatic (26)	1 (3.8%)		
Tumor size (cm) (67)	4.5, 3.8 [2]	Mean difference -0.4 (95% CI -2.3 to 1.4)	0.626 [3]
Age (years) (93)	63, 6.5 [4]	Mean difference 1.3 (95% CI -3.9 to 6.5)	0.610 [5]

OR=Odds ratio; 95% CI=95% confidence interval

[1] The *p*-value (2-tailed) by Fisher exact test; [2] Median with interquartile range (IQR), compared with median of 4.8 cm. and IQR of 4.4 cm; [3] The *p*-value (2-tailed) by independent sample test (equal variances not assumed); [4] Mean with standard deviation, compared with mean of 62 years and standard deviation of 7.9; [5] The *p*-value (2-tailed) by independent sample test (equal variances assumed)

The strength of this study is that almost all of cases in the present study has exact time-to-event and follow-up period. Another strength is that we used whole-slide staining instead of tumor microarray technique.

In current world-wide evidence, it is inconclusive to establish HER-2 overexpression as negative or positive prognostic biomarker. Longer follow-up time and larger sample size were suggested. The results of the present study will be used for further prognostic study and for further predictive study for HER-2 targeted therapy. The results of the present study will be used for further prognostic study and for further study assessing predictive effect of HER-2 for HER-2 targeted therapy in cholangiocarcinoma.

Conclusion

Among patients with cholangiocarcinoma, 1 of 10 cases had HER-2 overexpression. The present study cannot demonstrate association between HER-2 positive and survival outcome in patients with cholangiocarcinoma. However, from the present study, HER-2 positive group received effective treatment with adjuvant chemotherapy tended to have good survival outcome.

What is already known on this topic?

Human epidermal growth factor receptor-2 (HER-2) overexpression is a poor prognostic factor of many types of cancer but there is limited data in cholangiocarcinoma.

What this study adds?

The results of this study revealed that 1 of 10 cases of cholangiocarcinoma patients had HER-2 overexpression and tended to have good survival outcome when they received adjuvant chemotherapy.

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Conflicts of interest

The authors declare no conflict of interests.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209-49.
2. National Cancer Institute (NCI), Thailand. Hospital-based cancer registry 2015. Bangkok: NCI, Thailand; 2017.
3. Banales JM, Cardinale V, Carpino G, Marzioni M, Andersen JB, Invernizzi P, et al. Expert consensus document: Cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA). Nat Rev Gastroenterol Hepatol 2016;13:261-80.
4. Galdy S, Lamarca A, McNamara MG, Hubner RA, Cella CA, Fazio N, et al. HER2/HER3 pathway in biliary tract malignancies; systematic review and meta-analysis: a potential therapeutic target? Cancer Metastasis Rev 2017;36:141-57.
5. Bartley AN, Washington MK, Colasacco C, Ventura CB, Ismaila N, Benson AB 3rd, et al. HER2 testing and clinical decision making in gastroesophageal adenocarcinoma: Guideline from the College of American Pathologists, American Society for Clinical Pathology, and the American Society of Clinical Oncology. J Clin Oncol 2017;35:446-64.
6. Dawood S, Broglio K, Buzdar AU, Hortobagyi GN, Giordano SH. Prognosis of women with metastatic breast cancer by HER2 status and trastuzumab treatment: an institutional-based review. J Clin Oncol 2010;28:92-8.
7. Takenaka M, Hanagiri T, Shinohara S, Kuwata T, Chikaishi Y, Oka S, et al. The prognostic significance of HER2 overexpression in non-small cell lung cancer. Anticancer Res 2011;31:4631-6.
8. Gravalos C, Jimeno A. HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target. Ann Oncol 2008;19:1523-9.
9. Voravud N, Foster CS, Gilbertson JA, Sikora K, Waxman J. Oncogene expression in cholangiocarcinoma and in normal hepatic development. Hum Pathol 1989;20:1163-8.
10. Yoshikawa D, Ojima H, Iwasaki M, Hiraoka N, Kosuge T, Kasai S, et al. Clinicopathological and prognostic significance of EGFR, VEGF, and HER2 expression in cholangiocarcinoma. Br J Cancer 2008;98:418-25.
11. Jusakul A, Cutcutache I, Yong CH, Lim JQ, Huang MN, Padmanabhan N, et al. Whole-genome and epigenomic landscapes of etiologically distinct subtypes of cholangiocarcinoma. Cancer Discov 2017;7:1116-35.
12. Shamekh R, Rosa M, Sayegh Z, Ghayouri M, Kim R, Malafa MP, et al. Expression of Her-2/neu in extrahepatic cholangiocarcinoma. Pathol Lab Med Int 2017;9:9-14.
13. Collier JD, Guo K, Mathew J, May FE, Bennett MK, Corbett IP, et al. c-erbB-2 oncogene expression in hepatocellular carcinoma and cholangiocarcinoma. J Hepatol 1992;14:377-80.
14. Albrecht T, Rausch M, Rössler S, Albrecht M, Braun JD, Geissler V, et al. HER2 gene (ERBB2) amplification is a rare event in non-liver-fluke associated cholangiocarcinogenesis. BMC Cancer 2019;19:1191.
15. Yang X, Wang W, Wang C, Wang L, Yang M, Qi M, et al. Characterization of EGFR family gene aberrations in cholangiocarcinoma. Oncol Rep 2014;32:700-8.
16. Nakazawa K, Dobashi Y, Suzuki S, Fujii H, Takeda Y, Ooi A. Amplification and overexpression of c-erbB-2, epidermal growth factor receptor, and c-met in biliary tract cancers. J Pathol 2005;206:356-65.
17. Ogo Y, Nio Y, Yano S, Toga T, Koike M, Hashimoto K, et al. Immunohistochemical expression of HER-1 and HER-2 in extrahepatic biliary carcinoma. Anticancer Res 2006;26:763-70.
18. Brunt EM, Swanson PE. Immunoreactivity for c-erbB-2 oncopeptide in benign and malignant diseases of the liver. Am J Clin Pathol 1992;97(5 Suppl 1):S53-61.
19. Andersen JB, Thorgeirsson SS. A perspective on molecular therapy in cholangiocarcinoma: present status and future directions. Hepat Oncol 2014;1:143-57.
20. Titapun A, Techasen A, Sa-Ngiamwibool P, Sithithaworn P, Luvira V, Srisuk T, et al. Serum IgG as a marker for Opisthorchis viverrini-associated cholangiocarcinoma correlated with HER2 overexpression. Int J Gen Med 2020;13:1271-83.
21. Shafizadeh N, Grenert JP, Sahai V, Kakar S. Epidermal growth factor receptor and HER-2/neu status by immunohistochemistry and fluorescence in situ hybridization in adenocarcinomas of the biliary tree and gallbladder. Hum Pathol 2010;41:485-92.
22. Banales JM, Marin JJG, Lamarca A, Rodrigues PM, Khan SA, Roberts LR, et al. Cholangiocarcinoma 2020: the next horizon in mechanisms and management. Nat Rev Gastroenterol Hepatol 2020;17:557-88.
23. Motojima K, Komuta K, Hiasa A, Tsuribune T, Hashimoto T, Tsunoda T, et al. Evaluation of immunoreactivity to erbB-2 protein as a marker of prognosis in bile duct carcinoma. Nihon Geka Gakkai Zasshi 1992;93:952-5.
24. Ukita Y, Kato M, Terada T. Gene amplification and mRNA and protein overexpression of c-erbB-2 (HER-2/neu) in human intrahepatic cholangiocarcinoma as detected by fluorescence in situ hybridization, in situ

hybridization, and immunohistochemistry. J Hepatol 2002;36:780-5.
25. Kim HJ, Yoo TW, Park DI, Park JH, Cho YK, Sohn CI, et al. Gene amplification and protein overexpression of HER-2/neu in human extrahepatic cholangiocarcinoma as detected by chromogenic in situ hybridization and immunohistochemistry: its prognostic implication in node-positive patients. Ann Oncol 2007;18:892-7.
26. Fernandes VTO, De Barros E. Silva MJ, Begnami MD, Saito A. Prognosis of HER2 expression in cholangiocarcinoma when evaluated using gastric cancer methodology of immunohistochemistry. J Clin Oncol 2015;33:e15203.
27. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987;40:373-83.
28. Park J, Kim MH, Kim KP, Park DH, Moon SH, Song TJ, et al. Natural history and prognostic factors of advanced cholangiocarcinoma without surgery, chemotherapy, or radiotherapy: A large-scale observational study. Gut Liver 2009;3:298-305.
29. Briggs CD, Neal CP, Mann CD, Steward WP, Manson MM, Berry DP. Prognostic molecular markers in cholangiocarcinoma: a systematic review. Eur J Cancer 2009;45:33-47.
30. Bureau of Health Promotion, Department of Health, Ministry of Health, Thailand. Development and primary health care within the 6th national economic and social development plan (1987-1991). Nonthaburi: Bureau of Health Promotion; 1987.
31. Jongsuksuntigul P, Imsomboon T. Opisthorchiasis control in Thailand. Acta Trop 2003;88:229-32.
32. Rakha EA, Pinder SE, Bartlett JM, Ibrahim M, Starczynski J, Carder PJ, et al. Updated UK Recommendations for HER2 assessment in breast cancer. J Clin Pathol 2015;68:93-9.
33. Javle M, Bekaii-Saab T, Jain A, Wang Y, Kelley RK, Wang K, et al. Biliary cancer: Utility of next-generation sequencing for clinical management. Cancer 2016;122:3838-47.
34. Kamsa-Ard S, Luvira V, Suwanrungruang K, Kamsa-Ard S, Luvira V, Santong C, et al. Cholangiocarcinoma trends, incidence, and relative survival in Khon Kaen, Thailand from 1989 through 2013: A population-based cancer registry study. J Epidemiol 2019;29:197-204.
35. Dhanasekaran R, Hemming AW, Zendejas I, George T, Nelson DR, Soldevila-Pico C, et al. Treatment outcomes and prognostic factors of intrahepatic cholangiocarcinoma. Oncol Rep 2013;29:1259-67.
36. Fernández-Ruiz M, Guerra-Vales JM, Colina-Ruizdelgado F. Comorbidity negatively influences prognosis in patients with extrahepatic cholangiocarcinoma. World J Gastroenterol 2009;15:5279-86.
37. Wirasorn K, Ngamprasertchai T, Chindaprasirt J, Sookprasert A, Khantikaew N, Pakkhem A, et al. Prognostic factors in resectable cholangiocarcinoma patients: Carcinoembryonic antigen, lymph node, surgical margin and chemotherapy. World J Gastrointest Oncol 2013;5:81-7.
38. Chinchilla-López P, Aguilar-Olivos NE, García-Gómez J, Hernández-Alejandro KK, Chablé-Montero F, Motola-Kuba D, et al. Prevalence, risk factors, and survival of patients with intrahepatic cholangiocarcinoma. Ann Hepatol 2017;16:565-8.
39. Peixoto RD, Renouf D, Lim H. A population based analysis of prognostic factors in advanced biliary tract cancer. J Gastrointest Oncol 2014;5:428-32.
40. Wirasorn K, Ngamprasertchai T, Khuntikeo N, Pakkhem A, Ungarereevittaya P, Chindaprasirt J, et al. Adjuvant chemotherapy in resectable cholangiocarcinoma patients. J Gastroenterol Hepatol 2013;28:1885-91.