RESEARCH ARTICLE

Viral Hepatitis B, C Infection and Genotype Distribution among Cholangiocarcinoma Patients in Northeast Thailand

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Abstract

The prevalence of HBV and HCV infection among 295 cholangiocarcinoma (CCA) patients in northeast Thailand was analyzed. Hepatitis B surface antigen (HBsAg) was detected in 8.8% (26/295 cases) and antibodies to HCV (anti-HCV) in 2.7% (8/295 cases) of CCA cases. Screening for HBV DNA was performed in 15 of 26 HBV seropositive cases and genotypes could be determined in all 15. HBV genotypes C and B were detected in 73.3% (11/15 cases) and 26.7% (4/15 cases), respectively. HCV RNA was detected in 87.5% (7/8 cases) of anti-HCV positive cases. Specifically, 57.1% (4/7 cases) were HCV genotype 1a and 42.9% (3/7 cases) were HCV genotype 3a. The prevalence of infection and genotype distribution of both HCV and HBV among CCA in northeast Thailand is comparable to that in the general population, suggesting that HCV and HBV infections are, if at all, not serious risk factors for CCA.

Keywords: Cholangiocarcinoma - HBV - HCV - northeast Thailand

Asian Pacific J Cancer Prev, **13**, 83-87

Introduction

Cholangiocarcinoma (CCA) is a malignancy of the hepatobiliary duct system which originates from the intrahepatic or extrahepatic biliary epithelium. The incidence of CCA varies profoundly worldwide (Shaib and El-Serag, 2004). Southeast Asia has the highest incidence especially in northeast Thailand and Loa PDR, amounting to 113 per 100,000 in men and 50 per 100,000 in women (Sripa and Pairojkul, 2008), while in western countries it has been documented at 0.2 per 100,000 in men and 0.1 per 100,000 in women (Shaib and El-Serag, 2004). There are several established risk factors for CCA, including parasitic infections, primary sclerosing cholangitis, biliary-duct cysts, hepatolithiasis, and toxins. Other less-established, potential risk factors include inflammatory bowel disease (IBD), hepatitis C virus (HCV), hepatitis B virus (HBV), cirrhosis, diabetes, obesity, alcohol, smoking, and host genetic polymorphisms. However, the exact etiology remains undetermined. Persistent inflammation, cellular proliferation, and ultimately, malignant transformation has been considered. One of the risks related to development of CCA is chronic infection. Hepatitis virus associated chronic hepatitis or cirrhosis has been suggested to be involved in the pathogenesis of cholangiocarcinoma. Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infection, which are the most important risk factors of hepatocellular carcinoma (HCC), are also potential risk factors for CCA. Most acute HCV infections are asymptomatic but lead to chronic infection in approximately 85% of cases, who are unable to clear the virus. Chronic HCV infection progresses at a variable rate to cirrhosis in 15-20% of patients over a 10-20 year period, and 2-5% of cirrhosis patients develop hepatocellular carcinoma (HCC). The prevalence of antibodies to HCV in the Thai population varies from 0.89-5 % (Chainuvati et al., 1991; Barusrux et al., 1997; Songsivilai et al., 1997). In the northeast Thailand, HBV and HCV are endemic, while neither HIV nor HTLV-I are predominant. Co-infections by HCV and HIV or HGV have also been found (Barusrux et al., 1997; Barusrux and Urwijitaroon, 2006). High serum concentrations of anti-HCV have previously been reported among 31-40 year old male blood donors in Khon Kaen which represents the central area of northeast Thailand.

According to previous research conducted in various parts of the world, the HBV and HCV prevalence in CCA has varied as summarized in Table 1. Some of the previous data are still controversial due to the limited number of cases, especially in Thailand where CCA is more common than in most other countries. Therefore, this study was aimed at determining prevalence and genotypes of HBV

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Table 1. Previous Reports on HBV and HCV Seropositive in Cholangiocarcinoma Patients

Country	HBV n (%)	HCV n (%)
China	68 (8.8%)[a], 52 (32.7%)[b], 312 (48.4%)[c]	312 (2.9%)[c]
Thailand	55 (9.1%)[d], 30 (16.7%)[e]	30 (0.0%)[e]
Korea	41 (12.5%)[f], 622 (13.5%)[g]	41 (13.8%)[f], 622 (1.9%)[g]
Japan	50 (4.0%)[h]	50 (36.0%)[h]
Italy	26 (11.5%)[i]	26 (23.0%)[i]
US	625 (0.2%)[j]	625 (0.8%)[j], 535 (0.9%)[k], 83 (6.0%)[l]

[a]Liu et al., 2003; [b]Guo et al., 2003; [c]Zhou et al., 2008; [d]Pinyosophon et al., 2002; [e]Songsivilai et al., 1996; [f]Shin et al., 1996; [g]Lee et al., 2008; [h]Yamamoto et al., 2004; [i]Donato et al., 2001; [j]Shaib et al., 2005; [k]Welzel et al., 2007; [l]Shaib et al., 2007

and HCV among CCA patients in northeast Thailand, where both HBV and HCV are endemic. The information will be useful for epidemiology studies and to clarify the contribution of both HBV and HCV to the development of CCA in northeast Thailand.

Materials and Methods

Serum samples

The project was approved by the Ethics Committee, Khon Kaen University (HE450525). A total of 295 cholangiocarcinoma serum samples were randomly collected from -70°C storage at the cholangiocarcinoma center, Khon Kaen University.

Detection of Anti-HCV and HBsAg

Each serum sample was screened for anti-HCV and HBsAg using a commercially available third generation ELISA for anti-HCV (Murex anti-HCV Version 4; Abbott murex, UK) and HBsAg (Murex HBsAG Version 3; Abbott murex, UK), respectively according to the manufacturer's specifications.

Detection and analysis of HCV RNA in anti-HCV seropositive samples

The anti-HCV positive samples were tested for HCV RNA by reverse transcription-PCR using primers specific for both 5'UTR and core regions. Total HCV RNA was extracted from serum by single step guanidine isothyocyanate–phenol–chloroform extraction using TrizolR LS Reagent (Invitrogen, Calif). cDNA was synthesized by OC2(5' CATGGTGCACGGTCACGAG 3') primer targeting the 5'UTR and core regions. Both 5'UTR and core regions were amplified by nested PCR with primer pairs as previously described (Sunanchaikarn et al., 2007). The PCR products were subjected to 2% agarose gel electrophoresis revealing the 260 bp product of 5'UTR and the 405 bp product of the core regions. These PCR products were purified by the Perfectprep Gel Cleanup Kit (Eppendrof, Hmburg, Germany) for nucleotide sequence determination. Direct sequencing was performed based on the dideoxynucleotide chain termination method with the Big Dye terminator V.3.1 cycle sequencing Ready Reaction Kit (ABI, Fostercity, CA). The sequences were classified into genotypes based on highest percent similarity scores in comparison with reference sequences retrieved from databases (GenBank, DDBJ, and EMBL).

Detection and analysis of HBV DNA in HBsAg seropositive samples

The HBsAg positive samples were tested for HBV DNA of the pre S region by nested PCR. The outward primers were preS1F and R3 yielding an 873 bp outer PCR product. The inward primers S1F and R1 yielded the nested 598 bp PCR product of pre S which was subsequently analyzed by 2% agarose gel electrophoresis. This PCR product was purified by the Perfectprep Gel Cleanup Kit (Eppendorf, Hamburg, Germany) for nucleotide sequence determination. Direct sequencing was performed based on the dideoxynucleotide chain termination method with the Big Dye terminator V.3.1 cycle sequencing Ready Reaction Kit (ABI, Fostercity, CA).

Table 2. Serological Study of Viral Hepatitis among 295 Cholangiocarcinoma Patients in Northeastern Thailand

Anti-HCV	HBsAg	Total n (%)	Total Age (year)	Male n (%)	Male Age (year)	Female n (%)	Female Age(year)
+	+	2 (0.7)	46±1.41	1 (0.5)	45	1 (1.0)	47
-	+	24 (8.1)	53.8±9.46	20(10.1)	53.3±9.11	4 (4.1)	56.8±12.12
+	-	6 (2.0)	50.7±5.50	5 (2.5)	49.6±5.41	1 (1.0)	56
-	-	263(89.2)	56.2±10.65	172(86.9)	57.3±10.64	91 (93.8)	55.8±10.07
Total		295	56.4±10.35	198	56.7±10.51	97	55.7±10.03

Table 3. HBV, HCV Viremia and Genotype Distribution among Seropositive Cases of Cholangiocarcinoma Patients Compared with General Population, Thai

	Cholangiocarcinoma n	Cholangiocarcinoma (%)	General population, Thai n	General population, Thai (%)
HBV	295		3,072 (Adult Age >20 yrs)[a]	
Seroprevalence	26	(8.8%)	190	(6.2%)
M	21	(10.6%)	-	
F	5	(5.1%)	-	
HBV genotyping				
PCR positive	15	(57.7%)	147	(60%)[b]
Genotype				
A	-		2	(1.36%)
B	4	(26.7%)	17	(11.56%)
C	11	(73.3%)	128	(87.08%)
HCV	295		5825[c]	
Seroprevalence	8	(2.7%)	125	(2.15%)
M	6	(3.0%)	-	
F	2	(2.0%)	-	
PCR positive	7	(87.5%)	58	(58%)
Genotype			45	(77.6%)
1[a]	4	(57.1%)	3	(6.7%)
1[b]	-		12	(26.7%)
2[a]	-		1	(2.2%)
2[c]	-		1	(2.2%)
3[a]	3	(42.9%)	23	(51.1%)
3[b]	-		1	(2.2%)
6	-		4	(8.9%)

[a]Chongsrisawat et al., 2006; [b]Suwannakarn et al., 2008; [c]Sunanchaikarn et al., 2007

Results

Detection of Anti-HCV and HBsAg

The average age of the 295 cholangiocarcinoma patients in northeast Thailand was 56.4±10.35 years, comprising 56.7±10.51 years in 198 males and 55.7±10.03 years in 97 females. As for antibodies to hepatitis virus, 2.7% (8/295) were anti-HCV and 8.8% (26/295) were HBsAg positive, with 10.6% (21/198) males expressing HBsAg and 3.0% (6/198) displaying anti-HCV in comparison with 5.1% (5/97) females found with HBsAg and 2.0% (2/97) with anti-HCV as summarized in Table 2.

HCV genotyping and HBV genotyping in seropositive samples

HCV viremia was found in 87.5% (7/8 cases) of anti-HCV seropositive samples. Genotype analysis revealed that HCV genotype 1a was the most prevalent (57.1%), followed by 3a (42.9%). Genotype analysis revealed that HBV genotype C was the most prevalent (73.3%), followed by genotype B (26.7%) as shown in Table 3.

Discussion

This study has investigated the prevalence of HBV and HCV antibodies among CCA patients in northeast Thailand. The average age of CCA patients was 56.4±10.35 years. There is no significant age difference between female and male patients (Table 2). CCA can be classified into two major types with respect to location such as intrahepatic cholangiocarcinoma (ICC) and extrahepatic cholangiocarcinoma (ECC) (Patel, 2006). Many reports in the early 2000s have shown a global trend towards increased incidence of ICC and decreased incidence of ECC (Patel, 2002).

Chronic viral hepatitis and liver cirrhosis have been postulated as risk factors for CCA (Torbenson et al., 2007). Based on case control studies, HBV has been reported as a potential risk for ICC in many populations. The risk estimate was reported as 2.3 in 622 cases compared with 2,488 controls in Korea (Lee et al., 2008), 2.7 in 26 cases compared with 824 controls in Italy (Donato et al., 2001), and 8.9 in 312 cases compared with 438 controls in China (Zhou et al., 2008). HCV has also been documented as a potential risk for ICC in many populations. The risk estimate was reported as 4.4 in 535 cases compared with 102,782 controls (Welzel et al., 2007), 5.2 in 625 cases compared with 90,834 controls (Shaib et al., 2005), 7.9 in 83 cases compared with 236 controls (Shaib et al., 2007) in the US, 3.9 in 41 cases compared with 406 controls in Korea (Shin et al., 1996), 6.02 in 50 cases compared with 205 controls in Japan (Yamamoto et al., 2004), and 9.7 in 26 cases compared with 824 controls in Italy (Donato et al., 2001). However, according to various reports, the association between ICC and HCV or HBV is still controversial.

A case-control study in Japan comparing 50 ICC cases with 205 controls showed a strong association between ICC and anti-HCV (risk estimate=6.02), but no significant association between ICC and HBsAg (Yamamoto et al., 2004). A case-control study in US comparing 625 ICC cases with 90,834 controls showed a similarly strong association between ICC and anti-HCV (risk estimate=5.2), whereas HBV infection was not a significant risk factor for ICC (risk estimate=0.8) (Shaib et al 2005). In contrast, a case-control study in China comparing 312 ICC cases with 438 controls showed a strong association between ICC and HBsAg (risk estimate=8.9), but no significant association with anti-HCV (risk estimate=0.93) (Zhou et al., 2008). A case-control study in Korea comparing 622 cases of ICC with 2,488 controls showed a significant association between ICC and HBV (risk estimate=2.3) whereas HCV infection was not a significant risk factor for ICC (Lee et al., 2008).

Previous reports have shown that the potential risk estimate for CCA associated with either HBV (Shaib et al., 2007) or HCV (Shaib et al., 2007, Welzel et al., 2007, El-Serag et al., 2009) was lower in ECC cases compared to ICC cases. Combined, these data support the importance of distinguishing between ICC and ECC. Both incidence and risk factors of each CCA type differ with regard to epidemiology characteristics (Patel, 2006, Gatto et al., 2010). Several reports have shown an association between the presence of HBV/HCV and/or cirrhosis and increased risk of ICC. A case-control study in the US compared 625 cases of ICC with 90,834 controls. Only HCV was significantly associated with ICC. It was unclear if patients with HCV also had been diagnosed with cirrhosis. However, nonspecific cirrhosis was strongly associated with ICC (Shaib et al., 2005). Another case-control study in the US compared 83 ICC and 163 ECC to 236 controls. Both HBV and HCV were significant risk factors for ICC, but neither HCV nor HBV status was a significant risk factor for ECC. Although cirrhosis was not analyzed as a separate variable, 80% of HCV-positive patients had cirrhosis (Shaib et al., 2007).

CCA is a rare malignancy in Western countries, but more common in Asia. Japan and Western nations, including the United States, where HCV is more prevalent, were more likely to show an association between HCV and ICC but not ECC. On the other hand, in countries such as Korea and Thailand where both HBV and CCA are endemic, data have shown HBV but not HCV as a risk factor for ICC (Lee et al., 2008, Songsivilai et al., 1996). Most acute hepatitis virus infections are asymptomatic but with patients who are unable to clear the virus it can lead to chronic infection and progress at a variable rate to cirrhosis. Cirrhosis is the most consistently illustrated risk factor for ICC, but not ECC.

Although northeast Thailand is an endemic area the infection our results have shown a lower rate of HBV (8.8%) and HCV (2.7%) infection. This might be due to the lack of an accurate and consistent CCA classification system. However, the previous cases control study in northeast Thailand (Nakhon Phanom) was reported among 103 age-sex-place of residence matched case-control pairs for HBV and 106 matched pairs for HCV showed 7(7/0) and 2.25(9/4) of odds ratio. The odds ratio for CCA was 4.00 (95%CI: 1.29-16.44) when anti-HCV and HBsAg were combined. The risk for HBsAg and/or anti-HCV positive was still marginally increased with an OR of 4.69

but was not statistically significant (95%CI: 0.98-22.47) after adjustment for anti-OV. Thus HBV and HCV may play role in the development of CCA in northeast Thailand (Srivatanakul et al., 2010).

HBV has been classified into 8 genotypes (A to H) (Kramvis et al., 2005). In this study, HBV genotypes C (73.3%) and B (26.7%) were reported among CCA cases from northeastern Thailand similar to the HBV genotype distribution in Thailand (Tangkijvanich et al., 2005). Unfortunately, patients with genotype C had a higher concentration of HBeAg and exhibited earlier progression to cirrhosis and HCC than those with genotype B. However, there were no differences in the risk of developing HCC and its prognosis between patients with this genotype (Tangkijvanich et al., 2005).

The classification of HCV genotypes in this study has been based on homology of the 5'UTR and core region upon direct sequencing. Only HCV 1a (57.1%) and 3a (42.9%) were found, similar to other studies conducted on Thai blood donors (Apichartpiyakul et al., 1994; Songsiviai et al., 1996; Kanistanon et al., 1997; Hotta et al., 1997; Apichartpiyakul et al., 1999; Hansurabhanon et al., 2002). HCV genotype 3a is closely associated with IVDUs in the United States (Zein et al., 1996). The significantly higher prevalence of HCV genotype 1a observed in this study may be due to the genetic diversity of HCV or particular lifestyle of the subjects, for example, tattooing which is a common practice among northeast males. In addition to differences in geographical distribution, some differences in clinicopathological features of HCV infection have been observed among different types and subtypes (Hotta et al., 1997). Although HCV type 6 variants are common and restricted to Southeast Asian countries (Kanistanon et al., 1997; Apichartpiyakul et al., 1994; Hotta et al., 1997; Apichartpiyakul et al., 1999; Akkarathamrongsin et al., 2010; Akkarathamrongsin et al., 2011). HCV genotype 6 was not associated with any CCA case in this study.

HBV vaccines are currently available to prevent infection but hepatitis C vaccine has not yet been developed. Genotyping of HCV is clinically significant because it is the single most important predictor of response to HCV treatment. Patients infected with HCV genotype 3a display a higher sustained virological response (SVR) rate to interferon treatment than those infected with genotypes 1, 4, 5, and 6. Patients infected with HCV genotypes 1 and 4 do not respond to interferon treatment and these genotypes are associated with more severe liver damage than genotypes 2 and 3 (Manns et al., 2001).

This study provides information on pre-existing HBV and HCV infection and molecular epidemiology which can assist in disease prognosis and treatment strategies for CCA in northeast Thailand. Although HBV and HCV infection is endemic in northeast Thailand, a low rate of HCV and slightly higher one of HBV among CCA patients has been demonstrated in this study similar to previous reports. The association of risk factors with CCA is not entirely clear, as studies have arrived at different conclusions. Additional established risk factors for CCA including parasitic infections, biliary-duct cysts, hepatolithiasis, and primary sclerosing, cholangitis, cirrhosis, obesity, diabetes, alcohol, smoking, and genetic polymorphisms should be included. Moreover, an accurate and consistent CCA classification system should be considered for further investigation of risk factors.

Acknowledgements

This research was partially supported by CHE-TRF (MRG4680180), The Higher Education Research Promotion and National Research University Project of Thailand (HR1155A-55), Thailand Research Fund (DPG5480002), Office of the Commission on Higher Education, Center of Excellence in Clinical Virology, Chulalongkorn University. We would like to thank cholangiocarcinoma center staffs, for assistance with sample management, Miss. Siriporn Jantasorn for sample collecting and some data recording. We wish to acknowledge the support of the Khon Kaen University Publication Clinic, Research and Technology Transfer Affairs, Khon Kaen University, for their assistance. The authors declare no competing financial interests.

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