RESEARCH COMMUNICATION

Hepatitis Viruses and Risk of Cholangiocarcinoma in Northeast Thailand

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Abstract

Liver cancer is the most common cancer in males in Thailand and the third in females. A high incidence of cholangiocarcinoma (CCA) is estimated in the northeast of Thailand. Chronic infection with *Opisthorchis viverrini* (OV) is the major risk factor for development of CCA. It has been demonstrated that HCV infection is a risk factor for CCA in non - endemic area of OV infection. We examined the association of HBV and HCV and risk of CCA in the northeast Thailand. All cases of CCA were recruited between 1999 and 2001 from Nakhon Phanom provincial hospital and all community hospitals in the province. One control per case was selected, matched by sex, age (±5 years) and residence. 106 case-control pairs were obtained. Anti-OV, HBsAg, and Anti HCV were determined by ELISA. Among 103 age-sex-place of residence matched case-control pairs, there were 7, 0, 0, 96 pairs for anti-HCV (+) case vs. (-) control, (+) case vs. (+) control, (-) case vs. (+) control and (-) case vs. (-) control combinations (OR=7/0). Among 106 matched pairs, there were 9, 2, 4, 91 pairs for the similar four combinations of HBsAg (OR=2.25 (95% CI: 0.63-10.00)). If the subject had anti-HCV and/or HBsAg, the OR for CCA was 4.00 (95% CI: 1.29-16.44). Even after adjustment for anti-OV, risk for HBsAg and/or anti-HCV positive was still marginally increased with an OR of 4.69 although not reaching statistical significance (95% CI: 0.98-22.47). Hepatitis B and C virus infection may also play role in the development of CCA in northeast Thailand.

Keywords: Hepatitis virus - risk factor - cholangiocarcinoma

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Introduction

The incidence of liver cancer varies widely throughout the world, with high rates in sub-Saharan Africa, eastern and southeastern Asia, and Melanesia and a low incidence in northern and Western Europe and the Americas (Ferlay et al., 2004). Primary cancers of the liver in adults are of two main histological types: hepatocellalar carcinoma (HCC) and cholangiocarcinoma (CCA). More than 80% of liver cancer cases are HCC. CCA is relatively rare in most populations and accounts for an estimated 15% of liver cancer worldwide but second among primary cancer of the liver. However, in part of Southeast Asia, CCA occurs more frequently; it is responsible for more than 60% of liver tumors in the northeastern Thailand. The variation in geographical distribution of this cancer is marked. It accounts for a considerable fraction in Indochina countries such as Thailand, the endemic area of *Opisthorchis viverrini* (OV), and Far East Asia, the endemic area of *Clonorchis sinensis* (Parkin et al., 1993). A relatively high percentage of CCA is also seen in Hong Kong and Italy. The ratio of male : female in CCA ranges from 1.1:1 to 2.2:1 (Parkin et al., 1997) An increasing trend for incidence and mortality of CCA in non-endemic areas of liver fluke was noted in all regions of the world (Khan et al., 2002, Patel et al., 2002, Shaib et al., 2004). Though the advance in diagnostic tools, coding, and classification might also underlie this trend (Sharp et al., 2001), an increase in incidence of this cancer following more and longer exposure to intrahepatic bile duct inflammatory diseases might virtually exist. It has been demonstrated that the interaction between nitrosamines and OV infection may play an important role in the development of CCA in the northeast of Thailand (Srivatanakul et al., 1991a). Ministry of Public Health of Thailand has national program for the control of the liver fluke infection. The prevalence rate of OV infection was more than 50% between 1984 and 1987 in highly endemic areas in the northeast of Thailand. Following the region wide control program started in 1989, the annual positive rates had subsequently decreased to 9.4% in the year 2001 (Jongsuksuntigul and Imsomboon, 2003). The incidence of liver and bile duct cancer in Thailand is still the most common cancer in males and CCA is still very high incidence in the northeast of Thailand (Khuhaprema et al., 2010). CCA appears to be related to hepatitis vial

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infection, especially HCV, and could be detected at an early stage, similar to HCC, by following up cases of chronic hepatitis and cirrhosis (Hsing et al., 2007). HCV-related cirrhosis is major risk factor for CCA in Japanese patients (Kobayashi et al., 2000). It has been demonstrated that HCV infection is a risk factor for CCA in non-endemic area of OV infection (Shaib et al., 2005, Yamamoto et al., 2005). We examined the association of HBV and HCV and risk of CCA in the northeast Thailand.

Materials and Methods

Study Subjects

All cases of cholangiocarcinoma were recruited between September 1999 and 2001 at all community hospitals in Nakhon Phanom Province and Nakhon Phanom Provincial Hospital (Honjo et al., 2005). Criteria for inclusion cases of cholangiocarcinoma diagnosed either by histology, or with typical findings on ultrasound examination with an elevated titre (≥40units/ml) of CA 19-9 and a normal level of alpha-fetoprotein (AFP< 20ng/ml). One control per case was selected, matched by sex, age (± years) and also by residence. Controls had no ultrasonographic findings suggestive of cholangiocarcinoma or hepatocellular carcinoma, normal CA 19-9 and AFP level. A total of 106 case-control pairs were obtained.

Table 1. Distribution of 103 Case-Control Pairs According to Anti-HCV Antibody

Anti-HCV of control	Anti-HCV of case	
	Negative	Positive
Negative	96	7
Positive	0	0

Odds ratio=7/0 infinity 95% CI=1.44-infinity p=0.02

Table 2. Distribution of 106 Case-Control Pairs Accordion to HBsAg

HBsAg of control	HBsAg of case	
	Negative	Positive
Negative	91	9
Positive	4	2

Odds ratio=2.25 95% CI=0.63-10.00 p=0.27

Table 3. Distrlbutlon of 103 Case-Control Pairs According to Anti-HCV Antibody and HBsAg

Anti-HCV or HBsAg of control	Anti-HCV or HBsAg of case	
	Negative	Positive
Negative*	81	16
Positive	4	2

*Negative for both anti-HCV and HBsAg; Odds ratio=4.00 95% CI=1.29-16.44 P=0.01

Data and Specimen Collection

Cases and controls were interviewed before any therapeutic intervention using a questionnaire including dietary habits, smoking (never, occasional (less than one cigarette per day), ex-regular (stopped smoking as regular smoker at least one year ago) and regular smoker (at least one cigarette per day)) and alcohol drinking (never, occasional (less than once a week), ex-regular (stopped drinking as regular drinker at least one year ago) and regular drinker (more than once a week)). Specimens of blood and faeces were obtained. All plasma specimens were kept at -80C until used.

Determination of serum markers for antibodies to Opisthorchis viverrini and hepatitis virus infection

OV-specific IgG was determined by indirect ELISA (Sripa and Kaeskes, 2000) and we used a cut-off value at 0.200 optical density for OV-positive subjects.

The plasma specimens were examined for Hepatitis B surface antigen (HBs Ag) by enzyme-linked immunoassay and antibody to hepatitis C virus (anti-HCV) by Abbott/ AxSYM HCV version 3.0.

Statistical Analysis

Risk for cholangiocarcinoma was evaluated as an odds ratio due to positivity of serum HBsAg employing subjects with absence of the serum HBsAg as the reference group. Risk from the serum anti-HCV antibody was calculated similarly. A conditional logistic regression model was used to calculate the odds ratios keeping matched case-control pairs. Because the OV infection has been shown to be associated with the risk for cholangiocarcinoma (Parkin et al., 1991; Honjo et al., 2005), an adjusted odds ratio was also calculated with an allowance for anti-OV antibody (≥0.200 vs. <0.200).

All the statistical analyses were performed with a statistical package, Stata 10.0 (StataCorp, 2007), and statistical significance was defined as $p<0.05$ unless indicated otherwise.

Results

Demographic characteristics of cases and controls were shown in Honjo et al., 2005.

The results of matched - pair analysis of anti - HCV and HBsAg positive and risk of CCA are shown in Table 1 and 2. Among 103 age-sex-place of residence matched case - control pairs, there were 7, 0, 0, 96 pairs for anti-HCV (+) case vs. (-) control, (+) case vs. (+) control, (-) case vs. (+) control and (-) case vs. (-) control combinations (OR=7/0, 95% CI: 1.44 - infinity, p=0.02) Among 106

Table 4. Relations of Anti-OV Antibody And Marker for Hepatitis Virus Infectlon to Risk of Cholanglocarcinoma Among 100 Palrs

Variable	Category	Case	Control	Adjusted	95% CI		p value
				OR	LL	UL	
Anti-OV Ab (ELISA)	<0.200	48	93	1.00	Reference		
	≥0.200	52	7	25.04	5.81	107.88	<0.01
arti-HCV or HBsAg*	Negative	83	94	1.00	Reference		
	Positive	17	6	4.69	0.98	22.47	0.05

*Adjusted for Anti-OV Ab (ELISA)

matched pairs, there were 9, 2, 4, 91 pairs for the similar four combinations of HBsAg (OR=2.25, 95% CI: 0.63-10.00, p=0.27). When adjusted for anti-OV status, the OR due to HBsAg was 3.53 (95%CI: 0.65-19.27, p=0.15) based on 103 out of the 106 pairs. With further adjustment for smoking and alcohol, the OR due to HBsAg positivity was 2.93 (95% CI=0.47-18.2, p=0.25) based on data from 101 pairs. If the subject has anti-HCV and/or HBsAg, the OR for CCA was 4.00 (95% CI: 1.29-16.44, p=0.01) using subjects negative for both of the two serum markers as reference (Table 3). After adjustment for anti-OV status (Table 4), risk for HBsAg positivity and/or for anti-HCV positivity was still increased with OR of 4.69 although not statistically significant (95% CI: 0.98-22.47, p=0.05) on 100 pairs. With further adjustment for smoking and alcohol, the OR due to HBsAg positivity and/or for anti-HCV positivity was 3.48 (95% CI=0.63-19.2, p=0.15) based on data from 98 pairs.

Discussion

The world wide incidence of cholangiocarcinoma has risen over the past three decades. There is marked geographic variability in the prevalence of the disease, due in large part to regional environmental risk factors. In the United States, approximately 15% of primary liver cancer will be intrahepatic cholangiocarcinoma (ICC) (Jemal et al., 2005). Studies using the SEER data have shown a marked increase in the incidence of ICC in the United States (Patel, 2001; Shaib et al., 2004). The recent increase was noted to affect mostly older people ICC is known to be associated with disorders of the biliary tract, especially primary sclerosing cholangitis, and with inflammatory bowel diseases (Welzel et al., 2007). Hepatitis B virus (HBV) (Zhou et al., 2008) and hepatitis C virus (HCV) infections, as well as liver cirrhosis, regardless of etiology, have been examined as potential risk factors for ICC in countries other than the United States (Shin et al., 1996; Sorensen et al., 1998; Kobayashi et al., 2000; Donato et al., 2001; Yamamoto et al., 2005; Shaib et al., 2007; Weizel et al., 2007). In addition, several studies have suggested that diabetes mellitus also increases the risk of primary liver cancer both HCC and ICC (Adami et al., 1996). The largest US population - based case - control study to examine risk factors for ICC (Shaib et al., 2005) suggest that HCV infection, but not HBV infection, is a potentially strong risk factor for ICC. In addition, the presence of chronic and advanced liver disease of any etiology, HIV infection, diabetes, and smoking were significant risk factors for ICC.

In England and Wales there has also been a steady rise in the mortality rate from intrahepatic cholangiocarcinoma between 1968 and 1998 (Taylor-Robinson et al., 2001). There were increases in the incidence of primary liver cancer, particularly intrahepatic bile duct cancer over the last three decades of the 20th century in England and Wales (West et al., 2006). There has been a marked global increase in mortality from intrahepatic, but not extrahepatic, biliary tract malignancies (Patel, 2002; Shaib et al., 2004). In Crete, between 1992 and 2000, there has been a steadily rising incidence of both intra and extrahepatic cholangiocarcinoma (Mouzas et al., 2002). It is unclear why the incidence of this disease is rising. Furthermore, the incidence is also rising in developing countries where these technologies are not readily available. There is significant geographic variation in the incidence of cholangiocarcinoma with the highest incidence in northern Cambodia, Laos and Thailand. The high incidence in East Asia is likely due to regional risk factor s such as hepatolithiasis and liver fluke infection. A strong association exists between primary sclerosing cholangitis (PSC) and cholangiocarcinoma (Burak et al., 2004). Other abnormalities of biliary anatomy that are etiological factors for cholangiocarcinoma include choledochal cysts and Caroli's disease, a congenital condition characterized by multiple biliary and cysts. Choledochal cysts are associated with a 10% risk of developing cholangiocarcinoma (Chapman, 1999). Gallstones are not a risk factor for cholangiocarcinoma, but hepatolithiasis is strongly associated with this tumor in Asia (Kubo et al., 1995, Lee et al., 2002) and this association was also reported in a patient in France (Lesurte et al., 2002). Hepatolithiasisis common in East Asia with a prevalence as high as 20% in Taiwan (Su et al., 1997). Many toxic and environmental factors are implicated in the pathogenesis of cholangiocarcinoma. Thorium dioxide suspended as a colloid was used as a contrast agent from 1928 to 1965 and marketed under the name Thorotrast. Thorotrast is strongly associated with an increased incidence of cholangiocarcinoma (Rubel and Ishak, 1982, Shin et al., 1996). There is an increased prevalence of cholangiocarcinoma in Russian workers at the Mayak nuclear facility who were exposed to inhaled plutonium and gamma radiation (Gilbert et al., 2000).

Liver flukes are also strongly associated with this disease. The strong association is with the species Opisthorchis viverrini, while Clonorchis sinensis is also clearly a factor in the pathogenesis of this disease (Parkin et al., 1991, Parkin et al., 1993). Potential risk factors for CCA were investigated in a case-control study among inhabitants of northeast Thailand, infection with Opisthorchis viverrini the most important risk factor and at least two-thirds of CCA cases can be attributed to this cause but there was no association with HBV infection (Parkin et al., 1991). The most important risk factor which has been identified for HCC in Thailand is HBV infection (Srivatanakul et al., 1991b). In the present study, our results suggest that HBV infection and especially HCV infection may also play role in the development of CCA in the northeast Thailand. OV infection is still the major risk factor. Further studies are needed to explore the role of HBV and HCV infection in the non-endemic area of OV infection in the other regions of Thailand.

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