RESEARCH COMMUNICATION

The Metabolic Syndrome and Risk Factors for Biliary Tract Cancer: A Case-control Study in China

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Abstract

Objectives: Recent data show that the metabolic syndrome may play a role in several cancers, but the etiology for biliary tract cancer is incompletely defined. The present aim was to evaluate risk factors for biliary tract cancer in China. Methods: A case-control study in which cases were biliary tract cancer patients referred to Peking Union Medical College Hospital (PUMCH). Controls were randomly selected from an existing database of healthy individuals at the Health Screening Center of PUMCH. Data on the metabolic syndrome, liver diseases, family history, and history of diabetes and hypertension were collected by retrospective review of the patients' records and health examination reports or by interview. Results: A total of 281 patients (102 intrahepatic cholangiocarcinoma (ICC), 86 extrahepatic cholangiocarcinoma (ECC) and 93 gallbladder carcinoma (GC)) and 835 age- and sex-matched controls were enrolled. HBsAg$^+$/anti-HBc$^+$ (P=0.002), history of diabetes (P=0.000), cholelithiasis (P=0.000), TC (P=0.003), and HDL (P=0.000) were significantly related to ICC. Cholelithiasis (P=0.000), Tri (P=0.001), LDL (P=0.000), diabetes (P=0.000), Apo A (P=0.000) and Apo B (P=0.012) were significantly associated with ECC. Diabetes (P=0.017), cholelithiasis (P=0.000) and Apo A (P=0.000) were strongly inversely correlated with GC. Conclusion: Cholelithiasis, HBV infection and metabolic symptoms may be potential risk factors for the development of biliary tract cancer.

Keywords: Cholangiocarcinoma - gallbladder carcinoma - risk factors - metabolic syndrome

Introduction

Biliary tract cancer are highly fatal cancers of the biliary epithelium, arising within the liver (intrahepatic cholangiocarcinoma; ICC), in the extrahepatic bile ducts (extrahepatic cholangiocarcinoma; ECC) and gallbladder (gallbladder carcinoma; GC). They are accounting for approximately 3% of all gastrointestinal malignancies (Patel, 2006; Gatto et al., 2010; Wijaya and Abdullah, 2011). Recent data show that the incidence and mortality rates of biliary tract cancer have been increasing in several areas worldwide (Sandoh et al., 1997; Hsing et al., 1998; Nijhawan et al., 1999; West et al., 2006; Matsuda and Marugame, 2007; Lepage et al., 2011), especially the incidence of intrahepatic CCA (Patel, 2001; Shaib and El-Serag, 2004; Welzel et al., 2006; Welzel et al., 2007). A Chinese study also indicates that the incidence of biliary tract cancer has rapidly increased in Shanghai from 1972 to 1994 (Hsing et al.,1998), but the reasons for such increase are not clear.

The risk factors for ECC and GC have been reported in several Chinese studies (Zou and Zhang, 2000; Tang et al., 2007; Hsing et al., 2008). However, risk factors for ICC are seldom reported in China (Zhou et al., 2008; Tao et al., 2010), especially the metabolic syndromes for ICC. China is one of the biggest countries, recently

the quality of people's life was improved, and obesity and different metabolic syndromes have become major health problems threatening people's life. Several studies in America and Europe have suggested that obesity and metabolic syndromes were significant associated with biliary tract cancer (Seccia and Cavina, 1989; Moerman and Bueno-de-Mesquita, 1999; Randi et al., 2006; Ben-Menachem, 2007; Tyson and El-Serag; Welzel et al., 2007; Welzel et al. 2007; Hsing et al., 2008; Khan et al., 2008). Furthermore, a recent case-control study in China suggests that some metabolic syndromes are strong risk factors for the development of ECC and GC (Hsing et al., 2008; Shebl et al., 2011). However that study did not include ICC.

The advances in stem cells location and other recent evidences suggest that ICC, ECC and GC are biologically different cancers, giving further support to a number of recent epidemiological studies showing large differences in terms of incidence, mortality and risk factors (Cardinale et al., 2010). In present work, we prefer to perform a detailed evaluation of the relation between metabolic syndromes as well as some other possible risks and biliary tract cancer by subsites in a cohort of men and women in China. The consistent use of a more refined classification in our study would allow a better understanding of risk factors for biliary tract cancer.

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Materials and Methods

Study population and design

We conducted a hospital-based case-control study which including 1116 subjects (281 patients with histologically confirmed biliary cancers and 835 healthy controls) from Peking Union Medical College Hospital (PUMCH), Beijing, China. This hospital is a major diagnosis and treatment hospital for biliary tract cancer in China.

Using the PUMCH Patient Information Database, we compiled a list of all patients who had been diagnosed with biliary cancers (ICC, ECC and GC) between 1998 and 2010. Patients who had undergone a primary attempt at curative resection and whose diagnoses were confirmed by pathological examination were included in this study. Biliary tract cancer was classified as intrahepatic, extrahepatic or the gallbladder carcinoma depending on radiologic findings by computed tomography, magnetic resonance imaging, endoscopic retrograde cholangiopancreatography, or magnetic resonance cholangiopancreatography. Tumors arising in the intrahepatic bile ducts were classified as intrahepatic and in the extrahepatic bile ducts were classified as extrahepatic (including Klatskin's tumors that occur at the bifurcation of the left and right hepatic ducts), those arising in the gallbladder were classified as gallbladder carcinoma.

We performed a manual retrospective review of the patients' records to collect demographic, clinical, and risk factor information. This included a detailed assessment of family history of cancer, personal medical history, hormone and medication intake, and occupational exposure to chemicals. Data collection, including age, sex, demographic data, history of systemic diseases and gastrointestinal surgery, and a complete physical examination were conducted by the doctors before operating. Other related information was collected by interviewing patients or their family members and was recorded by a physician in a structured data collection sheet. Such recording is routinely performed in our gastrointestinal oncology clinic, and the forms have been kept as a part of patient medical records. Age, gender, history of hypertension and diabetes, HBV infection, metabolic syndromes, and previous cholecystectomy date were abstracted.

Using the Statistical Package for Social Science Program (SPSS, version 13, Chicago, IL), controls were randomly selected from an existing database of healthy individuals at the Health Screening Center of PUMCH and were frequency matched to cases by gender and age (± 5 years) at a ratio of 3:1. The database consists of healthy individuals who are genetically unrelated family members, spouses, and friends of patients who had cancer other than gastrointestinal cancers. The Health Screening Center of PUMCH is one of the major centers providing routine physical examinations in Beijing, and those who came for examinations are mostly native residents.

We performed a manual retrospective review of the health examination reports to collect demographic, clinical, and risk factor information for the controls. Data collection, including age, sex, demographic data, history of systemic diseases and gastrointestinal surgery, and a complete physical examination were completed by doctors at the physical examination centers. Other related information was collected and recorded in a structured data collection sheet. This information had been recorded by examining physicians using the same procedure that was used for cases. Controls were interviewed between 2007 and 2010. Eight participants were excluded from the study because of incomplete health examination records and loss to follow-up.

Cholelithiasis (including cholecystolithiasis, choledocholithiasis and hepatolithiasis) in all patients were diagnosed using data from clinical imaging studies (abdominal ultrasound, CT scan, ERCP and MRI) and by reviewing medical records. All patients underwent at least one of the aforementioned imaging studies. For control subjects, possible risk factors were determined based on abdominal ultrasound data and by reviewing health examination reports. All of the controls had previously undergone ultrasound screening for the detection of stones.

Laboratory tests

Blood samples were drawn via venipuncture from all study participants after overnight fasting by clinical nurses for laboratory examination. Serum lipids, including triglyceride (Tri), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), apolipoprotein A (Apo A), and apolipoprotein B (Apo B) were measured using Hitachi Modular analytics system (Roche Modular DPP, Hitachi Ltd, Tokyo, Japan). The presence of HBsAg, and anti-HBc were tested using a second-generation enzyme-linked immunosorbent assay (Abbott Laboratories, North Chicago, IL). Chronic Hepatitis B infection was defined as the presence of both HBsAg and anti-HBc.

Statistical analysis

Analyses of variables were carried out using SPSS. Univariate analyses were performed using Fisher's exact test for categorical variables. Variables with a P value of < 0.05 in the univariate analyses were further adjusted for age and sex and tested in a multiple logistic regression analysis. The model was built using a forward selection process. Variables with a likelihood ratio test P value of < 0.05 were kept in the model and considered statistically significant.

Results

Patient Characteristics

We included 281 biliary tract cancer cases and 835 controls in our analysis. Within the patient group, 102 had ICC (36.3%), 86 had ECC (30.6%) and 93 had GC (33.1%). The ICC patients, ECC patients, GC patients and the control group had a similar mean age (58.7 years, 58.6 years, and 59.7 years, respectively); the mean age of all patients was 59.4 years and there were no significant differences in gender among all groups. In addition, there was no significant difference in the year of diagnosis between ICC, ECC and GC cases. During this study,

Table 1. Risk Factors of Biliary Cancers: Univariate and Multivariate Logistic Regression Analysis Using the Fisher Exact Test

	Controls[1] N=835(%)	ICC Cases N=102(%)	ICC Univariate P value	ICC Multivariate[1] P value	ECC Cases N=86(%)	ECC Univariate P value	ECC Multivariate[2] P value	Controls[2] N=809(%)	GC Cases N=93(%)	GC Univariate P value	GC Multivariate[3] P value
Age											
<50yr	115(13.8)	18(17.6)			11(12.8)			112(13.8)	10(10.8)		
≥50yr	720(86.2)	84(82.4)	0.293		75(79.8)	1.000		697(86.6)	83(89.2)	0.522	
Gender											
Female	427(51.1)	45(44.1)			38(44.2)			413(51.1)	59(63.4)		
Male	408(48.9)	57(55.9)	0.208		48(55.8)	0.257		396(48.9)	34(36.6)	0.028	0.625
HBV											
HBsAg⁻/anti-HBc⁻	419(50.2)	15(14.7)			37(43.0)			401(49.6)	38(40.9)		
HBsAg⁺/anti-HBc⁺	29(3.5)	28(22.5)	0.000	0.002	6(6.9)	0.109		27(3.3)	7(7.5)	0.033	0.092
HBsAg⁻/anti-HBc⁺	387(46.3)	59(57.8)	0.000	0.068	43(50)	0.390		381(47.1)	48(51.6)	0.256	
Diabetes mellitus											
No	766(91.7)	86(84.3)			67(77.9)			743(91.8)	79(84.9)		
Yes	69(8.3)	16(15.7)	0.000	0.000	19(22.1)	0.000	0.000	66(8.2)	14(15.1)	0.034	0.017
Hypertension											
No	622(74.5)	76(73.5)			60(69.8)			603(74.5)	67(72.0)		
Yes	213(25.5)	26(25.5)	1.000		26(30.2)	0.366		206(25.5)	26(28.0)	0.617	
Cholelithiasis											
No	794(95.1)	85(83.3)			60(69.8)			769(95.1)	34(36.6)		
Yes	41(4.9)	17(16.7)	0.000	0.000	26(30.2)	0.000	0.000	40(4.9)	59(63.4)	0.000	0.000
TC(mmol/L)											
<5.70	724(86.7)	77(75.5)			46(53.5)			650(80.3)	75(80.6)		
≥5.70	111(13.3)	25(24.5)	0.004	0.003	40(46.5)	0.000	0.378	159(19.6)	18(19.4)	1.000	
Tri(mmol/L)											
<1.7	674(80.7)	81(79.4)			34(39.5)			650(80.3)	68(73.1)		
≥1.7	161(19.3)	21(20.6)	0.791		52(60.5)	0.000	0.001	159(19.6)	25(26.9)	0.104	
HDL(mmol/L)											
<0.93	67(8.02)	89(87.3)			53(61.6)			65(8.02)	42(45.2)		
≥0.93	768(91.98)	13(12.7)	0.000	0.000	33(38.4)	0.000	0.643	744(91.98)	51(54.8)	0.000	0.141
LDL(mmol/L)											
<3.63	619(74.1)	77(75.5)			46(53.5)			599(74.0)	67(72.0)		
≥3.63	216(25.9)	25(24.5)	0.811		40(46.5)	0.000	0.000	210(25.9)	26(28.0)	0.709	
Apo a(g/L)											
<1	7(0.8)				52(61.2)			7(0.87)	37(39.8)		
≥1	828(99.2)				33(38.8)	0.000	0.000	802(99.1)	56(60.2)	0.000	0.000
Apo b(g/L)											
<1	525(62.9)				35(41.2)			509(62.9)	48(41.2)		
≥1	310(37.1)				50(58.8)	0.000	0.012	300(37.1)	45(58.8)	0.042	0.54

All ORs were estimated by the exact method using SPSS software; [1]Model included age, gender, HBV markers, diabetes mellitus, cholelithiasis, TC and HDL; [2]Models included age, gender, diabetes mellitus, cholelithiasis, TC, HDL, Tri, Apo a and Apo b; [3]Models included age, gender, HBV markers, cholelithiasis, TC, HDL, Apo a and Apo b; ICC, intrahepatic cholangiocarcinoma; ECC, extrahepatic cholangiocarcinoma; GC, gallbladder ca rcinoma Control1 were the all controls, Control2 were those controls who had not accepted cholecystectomy

unlike ECC and GC, most ICC patients fail to test serum Apo A and Apo B, so we can not evaluate the relationship between their plasma level and ICC.

ICC

ICC patients had a significantly higher prevalence of HBsAg⁺/anti-HBc⁺ (22.5% vs. 3.5%), HBsAg⁻/anti-HBc⁺ (57.8% vs.46.3%), history of diabetes (15.7% vs. 8.3%), cholelithiasis (16.7% vs. 4.9%), and higher levels of TC (24.5% vs. 13.3%) than controls, while the rates of HDL (12.7% vs.91.9%) was lower than the case groups. During the multivariate logistic regression analysis, HBsAg⁻/anti-HBc⁺ fail to relate with the development of ICC, but the rest remained significant after adjustment for covariates (Table 1). The estimated ORs of the above variables were adjusted for age and gender. Hypertension, LDL, and Tri were not significantly different between the ICC cases and controls (Table 1).

ECC

HBV markers were not significantly different between the ECC cases and controls (Table 1). However, incidence of cholelithiasis (30.2% vs. 4.9%), previous diabetes (22.1% vs. 8.3%), TC (46.5% vs. 13.3%), Tri (60.5% vs. 19.3%), LDL (46.5% vs. 25.9%), and Apo B (58.8% vs. 37.1%) were significantly higher in ECC patients than the controls, while the rates of HDL (38.4% vs. 91.9%) and Apo A (38.8% vs. 99.2%) were lower than the case groups, while the data for history of hypertension were not statistically different between these two groups (Table 1).

The multivariate logistic regression analysis showed that incidence of cholelithiasis and diabetes, levels of Tri, LDL, Apo B were significantly positively associated with ECC, while the Apo A was strongly inversely correlated with ECC. None of the other risk factors was associated with greater odds of ECC (Table 1).
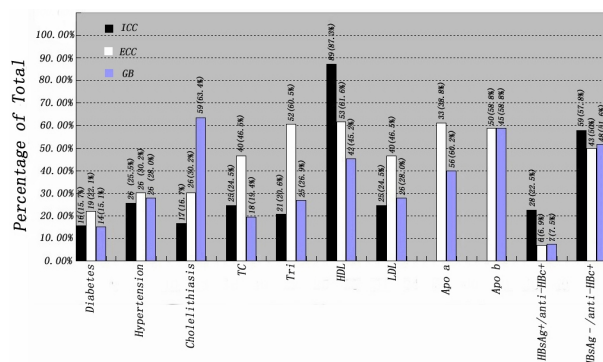
Figure 1. Associations between Biliary Tract Cancer and Risk Factors

GC

HBV markers were not significantly different between the GC cases and controls (Table 1). Incidence of cholelithiasis (63.4% vs. 4.9%), HBsAg+/anti-HBc+ (7.5% vs.3.3%), previous diabetes (15.1% vs. 8.2%), female (63.4% vs. 51.1%) and levels of Apo B (58.8% vs. 37.1%) were significantly higher in GC patients than controls, while the rates of HDL (54.8% vs. 91.98%) and Apo A (60.2% vs. 99.1%) were lower than the control group, and history of hypertension, previous diabetes, LDL, TC and Tri were not different between these two groups (Table 1).

The multivariate logistic regression analysis showed that incidence of cholelithiasis and diabetes were significantly positively associated with GC, while the Apo A was strongly inversely correlated with GC. None of the other risk factors was associated with greater odds of GC (Table 1).

Discussion

To our knowledge, this is the largest Chinese hospital-based case-control study to evaluate metabolic syndromes as risk factors for intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma and gallbladder carcinoma, respective. The results of this study provide the evidence that metabolic syndromes as well as some other medical conditions (cholecystolithiasis and HBV infection) are associated with an increased risk of biliary tract cancer.

The relationship between diabetes and malignancies has long been investigated. A large body of epidemiological evidence have found that diabetes is a significant risk factor for total cancer incidence, notably liver (El-Serag et al., 2006), pancreatic (Ben et al., 2011), colorectal, and probably endometrial (Lee et al., 2011) and prostate (Lee et al., 2011). These cancers have possible underlying mechanisms to do with energy throughput and balance, hyperinsulinemia or insulin resistance, or other hormone sensitivity. However, the relationship between diabetes and biliary tract cancer has still been controversial (Yamamoto et al., 2004; Shaib et al., 2005; Shaib et al., 2007; Welzel et al., 2007; Welzel et al., 2007; Zhou et al., 2008). In present study we found that, compared with non-diabetic individuals, diabetic individuals may have increased risk of developing biliary tract cancer,

including ICC, ECC and GC. Although the exact roles of diabetes and high serum glucose levels in biliary tract cancer are still unclear, several biological mechanisms may have been indicated to explain the potentially causal relationship between diabetes and cancer risk. Insulin resistance and subsequent hyperinsulinemia is a common phenomenon in diabetes patients (Suzuki et al., 2008). As a growth promoter, insulin may up-regulate the production of insulin-like growth factor-1, which may stimulate growth through cellular proliferation and inhibition of apoptosis within the cholangiocytes (Cai et al., 2008). An important role of insulin-like growth factor-1 in the carcinogenesis of cholangiocytes is supported by in vitro and in vivo studies (Alvaro et al., 2006). Another mechanism may be increasing oxidative stress which may cause a permanent proinflammatory state that reduces intracellular antioxidant capacity and predisposes to malignant transformation (Federico et al., 2007). Reactive oxygen species generated by diverse free radicals and oxidants can cause cell deoxyribonucleic acid (DNA) damage by direct oxidation or by interfering with cell DNA repair. It can also form derivatives and alter intracellular homeostasis by reacting with proteins and lipids favoring the accumulation of mutations (Ohshima et al., 2003).

Observational studies in human have shown a positive correlation of dyslipidemia and cancer development. Some experts suggest that suppressed HDL and elevated triglycerides in patients are risk factors for colon (Giovannucci, 2001), breast (Furberg et al., 2004; Michalaki et al., 2005), and prostate cancers (Hammarsten and Hogstedt, 2004). Besides that Wuermli L found hypertriglyceridemia may be a prognostic indicator for prostate cancer according to one recent Swiss case control study that controlled for age, BMI, diabetes mellitus, and statin medication. Taking these studies into account, there seems to be reasonable epidemiological evidence for a causal relationship between lipid metabolism and biliary tract cancer. Recently, the finding of an increased risk of biliary tract cancer after dyslipidemiais is supported by some previous research (Ben-Menachem, 2007; Welzel et al., 2007). Our results are consistent with the results reported by these non-Chinese studies, we first found in China that TC and HDL related to ICC; Tri, LDL, Apo A, Apo B associated with ECC; Apo A connected with GC. Although there is still unclear the exact roles of different lipids playing in biliary tract cancer and the role of each metabolic syndromes was not identical in different biliary tract cancer, we believe that metabolic syndromes palying a key role in the development of biliary tract cancer. Since, the associations between serum lipids and biliary tract cancer were statistically independent of gallstones; pathways other than those related to gallstones may also be involved in biliary carcinogenesis. Inflammation, independent of or in conjunction with gallstones, is a likely mechanism, since proinflammatory cytokines, such as tumor necrosis factor (TNF-α) and interleukins (IL-1, IL-6) have been expressed in gallbladder (Shi et al., 2004) and/or bile duct cancer tissue (Park et al., 1999; Jaiswal et al., 2000), and since most of the identified risk factors for biliary tract cancer, including hyperlipidemia

and diabetes (Shaib et al., 2005; Welzel et al., 2007), are linked to inflammation. Also, increased levels of TC, Tri, and LDL and decreased levels of HDL and Apo A have been associated with increased circulating levels of proinflammatory cytokines, including TNF-α, IL-1, and IL-6 (Feingold et al., 1990; Hardardottir et al., 1994). In addition, high LDL levels are associated with increased oxidized LDL. Oxidized LDL is linked to an increase in reactive oxygen species (Wells et al., 2005; Benitez et al., 2006), causing DNA damage, and inactivating tumor suppressor genes (Feig et al., 1994), all of which have been found to play a role in carcinogenesis, including biliary cancer (Rashid et al., 2002). Together, these data suggest that high serum levels of TC, Tri, and LDL and low level of HDL may play a role in biliary tract carcinogenesis through their lithogenic and inflammatory properties.

In addition, all the ICC, ECC and GC patients in our study have a higher prevalence of cholelithiasis. According to most researchers, chronic mechanical stimuli from the stones, chemical irritation by bile stasis, and infection may result in adenomatous hyperplasia of the epithelium of the bile duct and subsequently in the metaplasia-dysplasia-carcinoma pathway (Hou, 1956; Koga et al., 1985). The two large SEER-Medicare studies showed a strong positive association of biliary tract cancer with choledocholithiasis, with risk estimates ranging from 4 to 64 (Shaib et al., 2005; Welzel et al., 2007). In the Danish, population-based, case-control study conducted by Welzel et al found choledocholithiasis and cholangitis were, again, significantly associated with ICC (Welzel et al., 2007). However, it has been noted in a few publications that diabetes has been independently associated with an increased risk of biliary stones (Festi et al., 2008; Shebl et al., 2010). In the study from China, Shebl et al (Shebl et al., 2010) found that diabetes was associated with a two-fold risk of biliary stones, and about 60% of the effect of diabetes on biliary tract cancer was mediated by gallstones. Given the possibility that the association between cholelithiasis and biliary tract cancer might be confounded by diabetes, we excluded the patients who had diabetes and found that cholelithiasis did not change the association (data not shown), these data indicate that cholelithiasis may be a independent risk factor in bile duct tumorigenesis.

In recent decades, accumulating evidence has defined longstanding pre-existing HBV infection as a strong risk factor for intrahepatic cholangiocarcinoma. Many well conceived case-report studies and one cohort study have demonstrated the association between HBV infection and ICC, with ranges of OR from 2.7 to 9.7 (Cardinale et al., 2010). Our result revealed by multivariate analysis that HBV was independently associated with ICC, which is consistent with the reported findings in China (Zhou et al., 2010) and our previous study (Tao et al., 2010). A US cohort of individuals found HBV nucleic acids in ICC. Furthermore, recent studies show that HBV has been suggested to be involved in the pathogenesis of ICC through the inflammatory process (Tomimatsu et al., 1993; Reeves and DeMatteo, 2000; Liu et al., 2003). These strongly support the notion that HBV can be associated with ICC even in the absence of cirrhosis (Perumal et

al., 2006) and further support the potential role of HBV infection in the pathogenesis of biliary cancers.

So far, there is no evidence which actually supports a role for HBV infection in ECC development. Although HBV can be detected in hepatic bile ducts, most studies demonstrated that they are related only to intrahepatic bile duct carcinoma (Perumal et al., 2006; Lee et al., 2008). In our study, the number of ECC patients and GC positive for HBsAg was higher than that in control groups, but the difference did not attain statistical significance. The detailed mechanism of HBV infection on ECC and GB still needs further investigation.

There were several methodological strengths and limitations in our study. The absence of information about BMI, cigarette smoking, and HCV infection in the controls precluded us from estimating the magnitude of biliary tract cancer risk associated with these factors. Furthermore, our study is hospital-based in a single institution, not a population-based study. This design might have introduced a selection bias because of differential referral patterns. However, those limitations were outweighed by several strengths. First, we do not believe that we introduced an ascertainment bias because of misdiagnosis, given that all patients had pathologically and clinically confirmed biliary tract cancer and all were tested for viral hepatitis. Second, control subjects were healthy individuals selected from genetically unrelated family members, spouses, and friends of non-liver cancer patients at the Health Screening Center of PUMCH. The reasons for control subject participation in this study were not related to the risk factors being studied; therefore these control subjects represented the study base from which our cases were selected. Third, the accuracy of diagnostic confirmation of cancer and risk factors is highly qualitative, given that the data of the detailed behavioral and epidemiologic information is prospectively and systematically collected by providers for all patients at their initial visit to the surgical department of PUMCH.

Acknowledgements

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