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Hepatitis B virus-associated intrahepatic cholangiocarcinoma and hepatocellular carcinoma may hold common disease process for carcinogenesis

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ABSTRACT

Aims: To evaluate potential risk factors for intrahepatic cholangiocarcinoma (ICC) and analyse clinicopathologic characteristics of ICC patients with seropositive hepatitis B surface antigen (HBsAg).

Methods: A retrospective case–control study was conducted. Cases were 317 ICC patients referred to the Eastern Hepatobiliary Surgery Hospital in China between 2003 and 2006. Controls were 634 healthy individuals. Adjusted odds ratios (ORs) were calculated in logistic regression analysis. Among 317 consecutively enrolled ICC patients, 154 patients were seropositive HBsAg (48.6%). We compared clinicopathologic characteristics of these patients (group I) with ICC patients seronegative for HBsAg (group II; $n = 163$) and compared the age and sex distributions of patients in group I with randomly selected hepatitis B virus (HBV)-associated hepatocellular carcinoma (HCC) (group III; $n = 1140$).

Results: Compared with the controls, ICC patients had a high prevalence of seropositive HBsAg, cirrhosis, hepatolithiasis and hepatic schistosomiasis. Compared with seronegative-HBsAg ICC patients, seropositive-HBsAg ICC patients were younger, more frequently male and had a higher proportion of abnormal aminotransferase and serum alpha-fetoprotein (AFP) level, histological inflammation and cirrhosis, right-lobe focus, poor tumour differentiation, tumour encapsulation and microvascular invasion; had a lower proportion of abnormal serum carbohydrate antigen 19-9 (CA19-9) level and lymphatic metastasis. The age and sex distribution profiles were nearly identical between seropositive-HBsAg ICC patients and HBV-associated HCC patients.

Conclusions: The HBV infection, cirrhosis, hepatolithiasis and hepatic schistosomiasis may be potential risk factors for ICC. HBV-associated ICC shares many clinicopathological similarities with HBV-associated HCC. The result indicated HBV-associated ICC and HBV-associated HCC may hold common disease process for carcinogenesis.

1. Introduction

Intrahepatic cholangiocarcinoma (ICC), a malignant tumour arising from peripheral intrahepatic bile duct epithelium, occurs more rarely than hepatocellular carcinoma (HCC). HCC accounts for more than 90% of all primary liver cancers in the hepatitis B virus (HBV) endemic area, while ICC accounts for approximately 5% of primary liver cancers. Chronic HBV

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infection is the predominant risk factor for HCC in eastern Asia and sub-Saharan Africa; chronic hepatitis C viral (HCV) infection, in western countries and Japan.[1] The aetiology and carcinogenesis of ICC remain unclear. Although several risk factors have been reported as potential risk factors for ICC, such as liver fluke infestation,[2,3] primary sclerosing cholangitis (PSC),[4,5] hepatolithiasis[6] and chronic HBV or HCV infection,[7] most occur in the absence of known aetiological factors.[8,9] Notably, China is an HBV-endemic area and HBV is the predominant cause of HCC. HBV infection may also be a main risk factor for the development of ICC in the Chinese population. However, to date, possible risk factors for ICC development have still not been addressed.

Recently, some researchers have suggested that some cancers may originate from cancer stem cells, which may form via the carcinogenesis of normal stem cells.[10–12] It has been suggested that hepatocytes and cholangiocytes arise from the same pool of hepatic precursor cells, also called oval cells. Carcinogenesis of such hepatic precursor cells may cause ICC.[12] Though several environmental carcinogenesis models of cholangiocarcinoma, including infestation with liver flukes, especially the species *Opisthorchis viverrini* and *Clonorchis senensis*, have been established in animals, neither an aetiological correlation nor any preclinical models associating viral hepatitis with ICC are available.[13]

The goal of this study was to investigate possible risk factors for ICC. To better understand the carcinogenesis of ICC, we analysed the clinicopathologic characteristics of ICC patients with hepatitis B surface antigen (HBsAg) seropositivity.

2. Materials and methods

2.1. Study subjects

The study cases included 317 ICC patients who received surgical dissection at the Eastern Hepatobiliary Surgery Hospital of the Second Military Medical University (Shanghai, China) from January 2003 to December 2006. The diagnosis of ICC was confirmed by pathology.

The healthy controls consisted of 634 healthy people who had visited the Eastern Hepatobiliary Surgery Hospital of the Second Military Medical University for a routine checkup. They were matched to the ICC cases for sex and age (±4 years). The controls were recruited during the same study period as the cases.

To further clarify the characteristics of ICC with seropositive HBsAg, 1140 HBV-associated HCC patients (945 men and 195 women, male-to-female ratio, 4.8:1) who underwent hepatectomy for HCC during the same period in the same hospital were randomly selected for the comparison. The project was approved by the local Ethics Committee.

2.2. Liver cirrhosis

The criteria for the diagnosis of cirrhosis were as follows: (1) clinical manifestations of chronic hepatitis with portal hypertension and/or hepatic decompensation, laboratory tests and hepatic ultrasonography. Characteristic signs included portal hypertension (varices, thrombocytopenia or splenomegaly) and/or signs of hepatic decompensation (jaundice, prolonged prothrombin time and ascites), (2) the histology of the liver parenchyma confirmed cirrhosis.

2.3. Laboratory tests

Blood samples were tested for HBsAg and antibodies against the hepatitis C virus (anti-HCV) using enzyme-linked immunosorbent assays (Abbott Laboratories, North Chicago, IL, USA).

2.4. Statistical methods

Descriptive analyses of the variables were carried out using SPSS 16.0 (SPSS Inc, Chicago, IL, USA). Univariate analyses were performed using the chi-squared test for categorical variables and the independent-samples *T*-test for discrete variables.

Variables with a p-value of <0.05 were considered statistically significant. For each risk factor, odds ratios (ORs) of ICC and their 95% confidence intervals (95% CIs) were computed as estimates of the relative risks by unconditional logistic regression analysis, using the maximum-likelihood estimates.

3. Results

3.1. Demographic and baseline features of cases and controls

A total of 317 patients (223 men and 94 women, male-to-female ratio, 2.37:1) diagnosed with ICC were enrolled in the present study. The mean age was 53.05 ± 10.53 years (range 21–78). Most ICC developed during the fourth to seventh decade, with a peak at 54 years of age (Fig. 1).

Table 1 shows the distribution between controls and ICC cases according to age and gender. No statistical differences in gender and mean age were found between the two groups (ICC cases, controls), suggesting that pairing was effective.

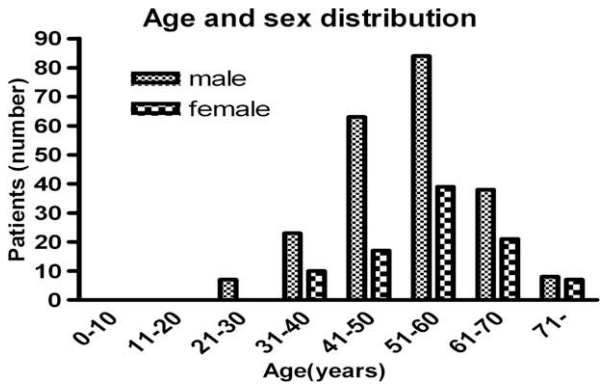

Fig. 1 – Age and sex distribution of intrahepatic cholangiocarcinoma (ICC) between 2003 and 2006 at the Eastern Hepatobiliary Surgery Hospital in China. The majority of ICC developed during the fourth to seventh decade, with a peak at 54 years of age.

Table 1 – Demographic characteristics of cases with ICC and control subjects.

Variable	ICC cases (n = 317)	Controls cases (n = 634)	p Value
Mean age (M ± SD years)	53.05 ± 10.53	52.91 ± 10.48	0.84
Gender			
Male	223	446	1.00
Female	94	188	

ICC: intrahepatic cholangiocarcinoma; M ± SD: mean ± standard deviation.

3.2. *Risk factors*

The distribution of risk factors in each of the categories is summarised in Table 2. HBsAg positivity (48.6%) in ICC patients was significantly different from controls (6.6%, $p < 0.001$). ICC patients also had a higher prevalence of cirrhosis (30.0% versus 1.4%, $p < 0.001$), hepatolithiasis (7.8 versus 1.1%, $p < 0.001$), choledocholithiasis (6.3% versus 1.4%, $p < 0.001$) and liver schistosomiasis (5.0% versus 0.6%, $p < 0.001$). The incidence of cholecystolithiasis was not significantly different between the two groups. It is worth mentioning that the HCV that is prevalent in Japan and some western countries has been proven to be a significant cause of ICC. In our series, only one case of ICC had HCV.

3.3. *Multiple logistic regression analyses*

Using a logistic regression model adjusted for demographics (age, sex), we calculated adjusted ORs for the different risk factors ($p < 0.05$). This analysis showed that, after adjustment, HBsAg seropositivity, cirrhosis, hepatolithiasis and liver schistosomiasis still remained statistically significant in the multivariate analyses (Table 3). Although univariate analysis showed that choledocholithiasis was significantly different between the two groups ($p < 0.001$), multivariate analyses confirmed that the difference was not related to ICC ($p = 0.063$).

3.4. *Clinicopathologic features of ICC patients with seropositive HBsAg*

For the analyses between ICC patients with seropositive HBsAg (group I) and ICC patients with seronegative HBsAg (group II), the following clinical variables were investigated: age, gender, risk factors (hepatolithiasis and hepatic schistosomiasis), total bilirubin (TBIL) (>20 μmol/L versus ⩽20 μmol/L), alanine aminotransferase (ALT) (>42 U/L versus ⩽42 U/L), aspartate aminotransferase (AST) (>37 U/L versus ⩽37 U/L), r-glutamyltransferase (r-GT) (>64 U/L versus ⩽64 U/L), alkaline phosphatase (ALP) (>119 U/L versus ⩽119 U/L), alpha-fetoprotein (AFP) (>20 ng/mL versus ⩽20 μg/L and >400 μg/L versus ⩽400 μg/L) and carbohydrate antigen 19-9 (CA19-9, >37 U/mL versus ⩽37 U/mL). Tumour number ($n < 2$ versus $n \geqslant 2$), location (multiple tumours in same lobe versus in different lobe), tumour size (main tumour or the largest one), capsule (present versus no capsule), tumour differentiation (according to the World Health Organisation (WHO) classification of tumour: well, moderately or poorly differentiated), portal vein invasion (invasion versus no invasion) and microscopic satellite lesion (tiny nodule present around the main tumour versus no satellite). The following variables were significantly different between group I and group II according to univariate analysis: age, gender, hepatolithiasis, ALT, AST, AFP, CA19-9, tumour location, tumour differentiation, microvascular invasion, lymphatic metastasis and histological CK19 (cytokeratin 19) (Table 4).

To further clarify the characteristics of ICC with seropositive HBsAg, the age and sex distribution profiles were compared between ICC patients with seropositive HBsAg (group I) or seronegative HBsAg (group II) and HBV-associated HCC (group III) (Fig. 2). The age and sex distribution profiles were significantly different between ICC patients with seropositive HBsAg and seronegative HBsAg. The mean age of ICC patients

Table 2 – Comparison of the prevalence of risk factors between ICC cases and controls.

Risk factors	Controls (n = 634)		ICC cases (n = 317)		p Value
	n	%	n	%	
Seropositive HBsAg	42	6.6	154	48.6	<0.001
Cirrhosis					
HBV-associated cirrhosis	6	1	84	26.5	<0.001
Alcohol-associated cirrhosis	2	0.3	6	1.9	0.027
Cirrhosis from other causes	1	0.2	5	1.6	0.035
Total	9	1.4	95	30.0	<0.001
Cholelithiasis					
Hepatolithiasis	7	1.1	25	7.8	<0.001
Choledocholithiasis	9	1.4	20	6.3	<0.001
Cholecystolithiasis	56	8.8	32	10.1	0.572
Total	64	10.1	54	17.0	
Liver schistosomiasis	4	0.6	16	5.0	<0.001

ICC: intrahepatic cholangiocarcinoma; HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus.

Table 3 – Multiple logistic regression analysis examining the association between risk factor and ICC.

	P value	ORs	95% CI	
			Lower	Upper
HBsAg seropositivity	<0.001	9.669	6.329	14.770
HBV-associated cirrhosis	<0.001	13.030	5.270	32.219
Alcoholic-associated cirrhosis	0.001	15.365	3.062	77.102
Cirrhosis from other causes	0.014	16.544	1.769	154.766
Hepatolithiasis	<0.001	11.020	4.238	28.657
Choledocholithiasis	0.063	2.676	0.949	7.551
Hepatic schistosomiasis	<0.001	11.063	3.368	36.337

ICC: intrahepatic cholangiocarcinoma; HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; ORs: odds ratios; 95% CI: 95% confidence interval.

Table 4 – Comparison of clinical features of ICC patients between positive HBsAg and negative HBsAg groups.

	HBsAg		*p* Value
	+ (*n* = 154)	− (*n* = 163)	
Gender (M/F)	127/27	96/67	<0.001
Mean age (M ± SD years)	49.33 ± 10.06	56.56 ± 9.76	<0.001
Hepatolithiasis (%)	2 (1.30)	23 (14.11)	<0.001
Hepatic schistosomiasis (%)	7 (4.55)	9 (5.52)	0.692
ALT (>42 U/L) (%)	60 (38.96)	44 (26.99)	0.023
AST (>37 U/L) (%)	71 (46.10)	44 (26.99)	<0.001
TBIL (>20 μmol/L) (%)	32 (20.78)	37 (22.70)	0.679
r-GT (>64 U/L) (%)	95 (61.69)	100 (61.35)	0.951
ALP(>119 U/L) (%)	68 (44.16)	81 (49.69)	0.324
AFP (>20 μg/L) (%)	58 (37.66)	22 (13.50)	<0.001
AFP (>400 μg/L) (%)	20 (12.99)	4 (2.45)	<0.001
CA19-9 (>37 U/mL) (%)	68 (44.16)	99 (60.74)	0.003
Tumour location (%)			<0.001
Left lobe	44 (28.57)	81 (49.69%)	
Right lobe	107 (69.48%)	78 (47.85%)	
Both lobes	3 (1.95%)	4 (2.45%)	
Tumour size (cm)	6.82 ± 3.40	6.56 ± 3.50	0.499
Tumour number (%)			0.940
1	143(92.85)	151(92.64)	
⩾2	11(7.14)	12(7.36)	
Histological inflammation (%)	52(33.77)	10(6.13)	<0.001
Cirrhosis (%)	77(50.00)	18(11.04)	<0.001
Capsule formation (%)	30(19.48)	10(6.13)	<0.001
Tumour differentiation (%)			0.007
Well	3(1.95)	6(3.68)	
Moderate	96(62.34)	124(76.07)	
Poor	55(35.71)	33(20.25)	
Microvascular invasion (%)	33(20.25)	18(11.04)	0.012
Perineural infiltration (%)	0(<0.01)	4(2.45)	0.050
Portal vein invasion (%)	15(9.74)	18(11.04)	0.704
Lymphatic metastasis (%)	16(10.39)	38(23.31)	0.002
Microscopic satellite lesion (%)	41(26.62)	35(22.73)	0.113
Immunohistochemical examinations			
CK 18 positive staining (%)	150(97.40)	157(96.32)	0.581
CK 19 positive staining (%)	145(88.96)	162(99.38)	0.008

ICC: intrahepatic cholangiocarcinoma; HBsAg: hepatitis B surface antigen; M: male; F: female; TBIL: total bilirubin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; and AFP: alpha-fetoprotein; ALP: alkaline phosphatase; r-GT: r-glutamyltransferase; CA 19-9: carbohydrate antigen 19-9; CK: cytokeratin.

with seropositive HBsAg (49.33 ± 10.06 years) was 7 years younger than that of ICC patients without viral hepatitis B (56.56 ± 9.76 years). It is interesting to note that the age and sex distribution profiles were nearly identical between ICC patients with seropositive HBsAg and HBV-associated HCC patients.

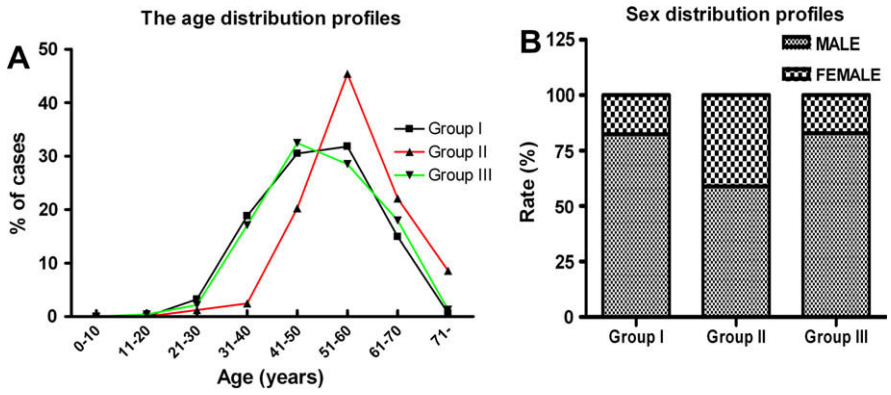

Fig. 2 – Comparison of disease incidence based on age (A) and sex (B) distribution in ICC (intrahepatic cholangiocarcinoma) patients with seropositive HBsAg (hepatitis B surface antigen) (group I), ICC patients with seronegative HBsAg (group II) and HCC (hepatocellular carcinoma) patients-associated with HBV (hepatitis B virus) (group III). The age and sex distribution profiles were significantly diverse between ICC patients with seropositive HBsAg and seronegative HBsAg. It is interesting that the age and sex distribution profiles were nearly identical between ICC patients with seropositive HBsAg and HBV-associated HCC patients.

4. Discussion

In the present study, we not only demonstrated that HBV, cirrhosis, hepatolithiasis and hepatic schistosomiasis were independent risk factors for ICC in the Chinese population, but we also analysed clinicopathologic characteristics of HBV-associated ICC patients and concluded that HBV-associated ICC and HBV-associated HCC may share a common disease process for carcinogenesis-through a similar long-term inflammatory carcinogenic process.

Most ICC developed during the fourth to seventh decade (53.05 ± 10.53 years), with the highest peak at 54 years of age (Fig. 1). The mean age of ICC patients with viral hepatitis B (49.33 ± 10.06 years) were 7 years younger than that of ICC patients without viral hepatitis B (56.56 ± 9.76 years). The age distribution profiles were significantly different between ICC patients with seropositive HBsAg and those with seronegative HBsAg. Notably, the age and sex distribution profiles were nearly identical between ICC patients with seropositive HBsAg and HBV-associated HCC patients (Fig. 2). Our findings are consistent with those reported by Lee and colleagues, recently.[14]

Men exhibit a higher prevalence of hepatocellular carcinoma than do women; the ratio of affected men to women varies between 2:1 and 4:1.[15] In the present work, the ratio of men to women among ICC patients with seropositive HBsAg was 4.7:1, which is higher than that in ICC patients with seronegative HBsAg (1.4:1) and nearly identical to HCC-related HBV (4.8:1). This result suggests that ICC patients with seropositive HBsAg and HBV-associated HCC patients share a similar characteristic of sex distribution.

It is well known that HBV is strongly associated with HCC.[15] The inflammatory immune response to viral antigens induces hepatocyte damage, followed by the regeneration of hepatocytes and the development of fibrosis and cirrhosis, which are important features in the pathogenesis of hepatocellular carcinoma.[16] Hepatitis viral infection and cirrhosis are generally considered to be unrelated to the mechanism of ICC carcinogenesis.[17] However, recent evidence suggests a possible aetiological role for HBV infection in ICC.[18] Our study also demonstrated that the incidence of HBV infection in ICC patients was significantly higher than that in non-cancer individuals (48.6% versus 6.6%), and that chronic HBV infection was independently the most important risk factor for ICC in the Chinese population. Poor liver function, HBV-associated cirrhosis and histological inflammation were also significantly higher in ICC patients with seropositive HBsAg than in ICC patients with seronegative HBsAg. The present results indicate that ICC with seropositive HBsAg and HBV-associated HCC have similar aetiological and histological characteristics and may share a common disease process for carcinogenesis-through a similar long-term inflammatory carcinogenic process.

AFP, a 70-kDa glycoprotein, is normally produced during foetal development by the liver and yolk sac.[19] The protein levels drop off rapidly after birth, and by the second year of life, only trace amounts are detectable in the serum. AFP is increased in the majority of patients with HCC and is useful in the diagnosis and follow-up of cases. Studies suggest that in patients with suspected HCC on clinical grounds, AFP levels >400 μg/L should strongly confirm the presence of HCC via a tissue diagnosis.[20,21] Recently, some researchers have suggested that some cancers may originate from cancer stem cells, which may form via the carcinogenesis of normal stem cells.[10,11,22] It has been suggested that hepatocytes and cholangiocytes arise from the same pool of hepatic precursor cells, also called oval cells. Carcinogenesis of such hepatic precursor cells may cause ICC.[12] Hepatic progenitor cells were also shown to strongly express AFP mRNA and to produce AFP during differentiation.[23–25] Compared with seronegative ICC patients, ICC patients with seropositive HBsAg exhibited a higher incidence of AFP > 20 μg/L (37.66% versus 13.50%) or AFP > 400 μg/L (12.99% versus 2.45%). Our data may indicate that one mechanism for the development of ICC involves the neoplastic transformation of oval cells and that the oval cell precursor retains its ability to produce AFP through the process of malignant transformation.

Lymph node metastasis and perineural invasion are common events in ICC, while they occur rarely in HCC.[26] The data obtained from our study showed that the lymph node metastasis rate and perineural invasion in ICC patients with seropositive HBsAg were lower than in ICC patients with seronegative HBsAg. On the other hand, capsule formation and microvascular invasion in HCC patients are more common than in ICC patients. Our data also showed that capsule formation and vascular invasion in ICC patients with seropositive HBsAg were more common than in ICC patients with seronegative HBsAg. ICC is notoriously associated with poor prognosis, mainly due to frequent lymphatic involvement, periductal invasion, poorly encapsulated tumours, or difficult early diagnosis compared with that for HCC. These characteristics expressed more obviously in seronegative-HBsAg ICC than in seropositive-HBsAg ICC. The results may indicate seropositive-HBsAg ICC patients have a favourable prognosis compared with seronegative-HBsAg ICC patients. Given a higher incidence of microvascular invasion and poor tumour differentiation in seropositive-HBsAg ICC patients compared to seronegative-HBsAg ICC patients, the real prognosis difference between them is still unclear. We plan to further investigate the differences between them in a future study. In addition, our results also demonstrated that ICC patients with seropositive HBsAg had a higher proportion of right-lobe focus. Our understanding is that intrahepatic duct stones mainly occur in the left lobe and that hepatolithiasis mainly presented in ICC patients with seronegative HBsAg.

In conclusion, we have confirmed that HBV infection, cirrhosis (mainly HBV-related), hepatolithiasis and choledocholithiasis are leading risk factors for the development of ICC in the Chinese population. HBV-associated ICC shares many clinicopathological similarities with HBV-associated HCC. We conclude that HBV-associated ICC and HBV-associated HCC may share a common disease process for carcinogenesis-through a similar long-term inflammatory carcinogenic process and, possibly, both arose from the hepatic progenitor cells.

Conflict of interest statement

None declared.

REFERENCES

1. Llovet JM, Bruix J. Novel advancements in the management of hepatocellular carcinoma in 2008. *J Hepatol* 2008;**48**(Suppl.):S20–37.
2. Watanapa P, Watanapa WB. Liver fluke-associated cholangiocarcinoma. *Br J Surg* 2002;**89**:962–70.
3. Hughes NR, Pairojkul C, Royce SG, Clouston A, Bhathal PS. Liver fluke-associated and sporadic cholangiocarcinoma: an immunohistochemical study of bile duct, peribiliary gland and tumour cell phenotypes. *J Clin Pathol* 2006;**59**:1073–8.
4. Chalasani N, Baluyut A, Ismail A, et al. Cholangiocarcinoma in patients with primary sclerosing cholangitis: a multicenter case–control study. *Hepatology* 2000;**31**:247–8.
5. Shaib Y, El-Serag HB. The epidemiology of cholangiocarcinoma. *Semin Liver Dis* 2004;**24**:115–25.
6. Shaib YH, El-Serag HB, Davila JA, Morgan R, McGlynn KA. Risk factors of intrahepatic cholangiocarcinoma in the United States: a case–control study. *Gastroenterology* 2005;**128**:620–6.
7. Shaib YH, El-Serag HB, Nooka AK, et al. Risk factors of intrahepatic cholangiocarcinoma and extrahepatic cholangiocarinoma: a hospital-based case–control study. *Am J Gastroenterol* 2007;**102**:1016–21.
8. Ben-Menachem T. Risk factors for cholangiocarcinoma. *Eur J Gastroenterol Hepatol* 2007;**19**(8):615–7.
9. Blechacz BR, Gores GJ. Cholangiocarcinoma. *Clin Liver dis* 2008;**12**:131–50.
10. Singh SK, Hawkins C, Clarke ID, et al. Identification of human brain tumour initiating cells. *Nature* 2004;**432**:396–401.
11. Singh SK, Clarke ID, Terasaki M, et al. Identification of a cancer stem cell in human brain tumors. *Cancer Res* 2003;**63**:5821–8.
12. Wilkens L, Bredt M, Flemming P, et al. Differentiation of multicentric origin from intra-organ metastatic spread of hepatocellular carcinomas by comparative genomic hybridization. *J Pathol* 2000;**192**:43–51.
13. Blechacz B, Gores GJ. Cholangiocarcinoma: advances in pathogenesis, diagnosis, and treatment. *Hepatology* 2008;**48**:308–21.
14. Lee CH, Chang CJ, Lin YJ, et al. Viral hepatitis-associated intrahepatic cholangiocarcinoma shares common disease processes with hepatocellular carcinoma. *Br J cancer* 2009;**100**:1765–70.
15. Wands J. Hepatocellular carcinoma and sex. *N Engl J Med* 2007;**357**:1974–6.
16. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007;**132**:2557–76.
17. Kuper H, Ye W, Broome U, et al. The risk of liver and bile duct cancer in patients with chronic viral hepatitis, alcoholism, or cirrhosis. *Hepatology* 2001;**34**:714–8.
18. El-Serag HB, Engels EA, Landgren O, et al. Risk of hepatobiliary and pancreatic cancers after hepatitis C virus infection: a population-based study of US veterans. *Hepatology* 2009;**49**:116–23.
19. Gitlin D, Perricelli A, Gitlin GM. Synthesis of-fetoprotein by liver, yolk sac, and gastrointestinal tract of the human conceptus. *Cancer Res* 1972;**32**:979–82.
20. Bruce MG, Bruden D, McMahon BJ, et al. Clinical significance of elevated alpha-fetoprotein in Alaskan Native patients with chronic hepatitis C. *J Viral Hepat* 2008;**15**:179–87.
21. Murugavel KG, Mathews S, Jayanthi V, et al. Alpha-fetoprotein as a tumour marker in hepatocellular carcinoma: investigations in south Indian subjects with hepatotropic virus and aflatoxin etiologies. *Int J Infect Dis* 2008;**12**:e71–6.
22. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 2003;**100**:3983–8.
23. Ishikawa K, Sasaki A, Haraguchi N, Yoshikawa Y, Mori M. A vase of an α-fetoprotein-producing intrahepatic cholangiocarcinoma suggests probable cancer stem cell origin. *Oncologist* 2007;**12**:320–4.
24. Dabeva MD, Shafritz DA. Activation, proliferation, and differentiation of progenitor cells into hepatocytes in the D-galactosamine model of liver regeneration. *Am J Pathol* 1993;**143**:1606–20.
25. Shiojiri N, Lemire JM, Fausto N. Cell lineage and oval cell progenitors in rat liver development. *Cancer Res* 1991;**51**:2611–20.
26. Shirabe K, Shimada M, Harimoto N, et al. Intrahepatic cholangiocarcinoma: its mode of spreading and therapeutic modalities. *Surgery* 2002;**131**:S159–64.