Enhancers and attenuators of risk associations of chronic hepatitis B virus infection with hepatocellular carcinoma in type 2 diabetes

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Abstract

Chronic hepatitis B virus (HBV) infection promotes hepatocellular carcinoma (HCC) risk. In type 2 diabetes (T2D), use of insulin and statins was associated with reduced cancer risk while co-presence of low LDL cholesterol (LDLC <2.8 mmol/l) plus low triglyceride (TG; <1.7 mmol/l) increased cancer risk. There is experimental evidence showing that insulin insufficiency might promote HCC. In this study, we examined whether this lipid subphenotype and use of insulin or statins might modify the promoting effect of chronic HBV infection (indicated by the presence of hepatitis B surface antigen) on HCC. We analyzed data of 1319 T2D patients enrolled into the Hong Kong Diabetes Registry from December 1996 to January 2005 and followed up to 2005. Additive interaction was estimated using relative excess risk due to interaction and attributable proportion due to interaction. During 5782 person-years of follow-up, 1.74% ($n=23$) of patients developed HCC (incidence, 3.98; 95% confidence interval, 2.36–5.60/1000 person-years). HbA1c $\geq 7.0\%$ and the lipid phenotype (LDLC <2.8 mmol/l plus TG <1.7 mmol/l) increased the hazard ratios (HRs) of chronic HBV infection for HCC from 3.74 to 74.96 and from 11.01 to 89.82 respectively with significant interactions. Use of insulin or statins decreased the HRs from 37.51 to 5.87 and from 64.94 to 16.99 respectively with significant interactions (all P values <0.05). These findings support our hypothesis that hyperglycemia and co-presence of low LDLC plus low TG might enhance, while insulin or statin usage might attenuate the promoting effect of chronic HBV infection on HCC in T2D.

Key Words

- HBsAg
- liver cancer
- low LDL cholesterol
- low triglyceride
- hyperglycemia
- additive interaction
- HbA1c

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Introduction

Diabetes is a global epidemic with rapid increase in Asia (Chan *et al.* 2009). In China, 9.7% of adult men and women had diabetes in 2007–2008 (Yang *et al.* 2010b). Apart from lifestyle changes, many developing areas are

undergoing rapid transition from communicable to noncommunicable disease. In China, 7% of people have chronic hepatitis B virus (HBV) infection as indicated by positive hepatitis B surface antigen (HBsAg) (Wang & Jia 2011).

The latter is a major promoter of hepatocellular carcinoma (HCC), which is one of the most prevalent cancers with poor prognosis (Burrell *et al.* 2012, Forner *et al.* 2012). Diabetes is associated with increased risk of a spectrum of cancers (Yang *et al.* 2012b) with a linear relationship between HbA1c and all-site cancer risk (Yang *et al.* 2010a). In most surveys, among site-specific cancers, HCC ranks second in terms of strength of association with diabetes (El-Serag *et al.* 2006), only preceded by pancreatic cancer (Everhart & Wright 1995).

Using the Hong Kong Diabetes Registry, we reported a series of drug-subphenotype interactions, based on which we put forth an integrated hypothesis, supported by results from mechanistic studies, to explain the biological links between type 2 diabetes (T2D) and cancer (Yang *et al.* 2012b). These included i) downregulation of the adenosine 5'-monophosphate-activated protein kinase (AMPK) pathway suggested by the attenuated cancer risk in metformin users with low HDL cholesterol (HDL <1.0 mmol/l; Yang *et al.* 2011b); ii) activation of the renin-angiotensin system (RAS) and low-grade inflammation suggested by the attenuated cancer risk in RAS inhibitor users with high white blood cell count (WBC $\geq 8.2 \times 10^9/l$; Yang *et al.* 2010c); iii) activation of the RAS and dysregulation of cholesterol metabolism suggested by the attenuated cancer risk in statin users with co-presence of low LDL cholesterol (LDLC; <2.8 mmol/l) plus albuminuria (Yang *et al.* 2009a) with possible cross talks between the RAS and lipid metabolism (Yang *et al.* 2009b); and iv) insulin insufficiency with dysregulation of the insulin-like growth factor 1 (IGF1) and the cholesterol synthesis pathway via the sterol regulatory binding protein 1 (SREBP1), suggested by a synergistic effect of triglyceride (TG) <1.7 mmol/l and LDLC <2.8 mmol/l for cancer (Yang *et al.* 2012a) and attenuated cancer risk in statin users with TG <1.7 mmol/l (Yang *et al.* 2011a).

In an experimental study reported by Japanese workers, compared with insulin-sufficient tumor-prone mice, insulin-deficient and hyperglycemic animals had an increased number and size of liver tumors with reduced apoptosis. Intriguingly, insulin therapy reduced the number and size of liver tumors with increased apoptosis (Yamasaki *et al.* 2010). These findings strongly suggested that either hyperglycemia or lack of insulin or both might play a causal role in promoting HCC development in diabetes. In a large cohort of HBV carriers with an accrual of 1021 HCC events during 328 946 person-years of follow-up, statin use was associated with reduced risk of HCC in a dose-effect manner with 66% risk reduction in subjects using high-dose statins (Tsan *et al.* 2012).

Results from these two studies corroborated our hypothesis regarding the role of insulin insufficiency and dysregulated lipid metabolism in linking diabetes and cancer including HCC.

As HBV infection is a well-established risk factor for HCC (De Mitri *et al.* 2010), we further tested this hypothesis by examining i) the enhancing effects of insulin insufficiency reflected by HbA1c $\geq 7\%$ and co-presence of TG <1.7 mmol/l plus LDLC <2.8 mmol/l and ii) attenuating effects of use of insulin and statins, on the risk association between HBV infection and HCC, if any.

Patients and methods

Patients

The Hong Kong Diabetes Registry was established at the Prince of Wales Hospital affiliated to the Chinese University of Hong Kong in 1995 as a quality improvement program. It enrolled 30–50 ambulatory diabetic patients weekly, referred from general practitioners as well as community- and hospital-based clinics. Among the enrolled patients, 10% or fewer had hospital admissions within 6–8 weeks before the assessment. At enrollment, a 4-h assessment of complications and risk factors was performed on an outpatient basis using standard procedures modified from the European DiabCare protocol (Piwernetz *et al.* 1993). Once a diabetic subject had undergone this comprehensive assessment, they were considered to have entered this study cohort and would be observed until death.

Hong Kong maintains a highly subsidized health care system. The Hospital Authority (HA) is the governing body of all public-funded hospitals and outpatient clinics, which provides 95% of the total hospital bed-days and 80% of the outpatient visits (Yang *et al.* 2008b). All patients admitted to HA hospitals or followed at HA hospital clinics were dispensed medications on site. These drug-dispensing data were computerized in the HA Central Computer System since 1 December 1996. We retrieved the dispensing data of drugs of interest from 1 December to 30 July 2005, the censoring date of this study. Discharge diagnoses of hospital admissions and mortality during this period were retrieved from the HA Central Computer System to define clinical endpoints. These databases were successfully matched by a unique identification number, the Hong Kong Identity Card number, which is compulsory for all residents in Hong Kong, and used by all government departments and major organizations. Ethical approval was obtained

from the Chinese University of Hong Kong Clinical Research Ethics Committee and informed consent was obtained from all patients for research purposes.

From December 1996 to January 2005, 7387 diabetic patients were enrolled in this registry. We sequentially excluded 323 patients with T1D defined as acute presentation with ketoacidosis, heavy ketonuria (>3+), or continuous requirement of insulin within 1 year of diagnosis (Laakso & Pyorala 1985) and five with uncertain T1D status, 45 with non-Chinese or unknown nationality, and 175 patients with history of cancer or on cancer treatment documented at enrollment. In addition, 732 patients with missing values on variables used in the analysis were excluded. In the remaining 6107 patients, 1319 patients had HBsAg measurement data and were included in the analysis.

The endpoint

A team of trained personnel at the HA routinely coded all hospital discharge principal diagnoses according to the International Classification of Diseases, Ninth Revision (ICD9). ICD9 codes were used to identify first admissions due to HCC, defined as having a first HCC during the follow-up period (Code 155).

Clinical and laboratory measurements

At enrollment, patients underwent a 4-h comprehensive screening for complications and other risk factors after at least 8 h of fasting without taking any medication (Yang *et al.* 2008b). BMI was measured in subjects wearing light clothing and without shoes. Sitting blood pressure (BP) was measured after at least 5 min of rest using Dinamapp machine. The abbreviated Modification of Diet in Renal Disease formula recalibrated for Chinese (Ma *et al.* 2006) was used to estimate glomerular filtration rate (eGFR) expressed in ml/min per 1.73 m²: $eGFR = 186 \times (SCR \times 0.011)^{-1.154} \times (age)^{-0.203} \times (0.742 \text{ if female or } 1 \text{ if male}) \times 1.233$, where SCR was serum creatinine expressed as $\mu\text{mol/l}$ (original mg/dl converted to $\mu\text{mol/l}$) and 1.233 is the adjusting coefficient for Chinese. Plasma glucose was measured by a hexokinase method (Hitachi 911 automated analyzer, Boehringer Mannheim, Mannheim, Germany). HbA1c was measured by an automated ion-exchange chromatographic method (Bio-Rad Diamat HbA1c analyzer, Bio-Rad Laboratory with reference range: 5.1–6.4%). Both interassay and intra-assay coefficients of variation (CV) for HbA1c were $\leq 3.1\%$ at values below 6.5%. Total cholesterol, TG, and HDLC were

measured by enzymatic methods on a Hitachi 911 automated analyzer (Boehringer Mannheim) using reagent kits supplied by the manufacturer of the analyzer. LDLC was calculated by Friedewald's equation (Friedewald *et al.* 1972). Spot urinary creatinine (Jaffe's kinetic method) and albumin (immunoturbidimetry method) were also measured on the Hitachi 911 analyzer using reagent kits supplied by the manufacturer. The interassay CV were 12.0 and 2.3% for urinary albumin concentrations of 8.0 and 68.8 mg/l, respectively. The lowest detection limit was 3.0 mg/l. Albuminuria was defined as urinary albumin to creatinine ratio $\geq 2.5 \text{ mg/mmol}$ in male and $\geq 3.5 \text{ mg/mmol}$ in female. HBsAg was tested using commercially available ELISA kits (Abbott GmbH Diagnostika).

Statistical analyses

The Statistical Analysis System (release 9.10) was used to perform the statistical analysis (SAS Institute, Inc., Cary, NC, USA). Cox proportional hazard analysis was used to obtain hazard ratio (HR) and 95% confidence interval (95% CI) of predictors for HCC. Follow-up time was calculated as the period from enrollment to the date of first admission for HCC, death, or 30th July 2005, whichever came first.

We defined HbA1c $\geq 7.0\%$ as suboptimal glycemic control (American Diabetes Association 2012) and tested its additive interaction with chronic HBV infection, defined by the presence of HBsAg, on risk of HCC. Our previous analysis showed that co-presence of LDLC $< 2.80 \text{ mmol/l}$ and TG $< 1.70 \text{ mmol/l}$ (Yang *et al.* 2012a) and that of LDLC $< 2.8 \text{ mmol/l}$ and albuminuria (Yang *et al.* 2009a) had synergistic effects on cancer risk and thus were coded as two new variables (yes/no). We also tested whether these interactions would modify the effect size of HBV infection on the risk of HCC. Relative excess risk due to interaction (RERI) and attributable proportion due to interaction (AP) (Andersson *et al.* 2005, Li & Chambless 2007) were used to estimate additive interaction of HBV infection with other factors. RERI was the excess risk due to interaction relative to the risk without exposure. AP was referred to the attributable proportion of disease due to interaction in persons with both exposures. RERI > 0 or AP > 0 indicated a significant additive interaction. A significant interaction between a factor and HBV infection for HCC suggests that the factor enhances the effect of HBV infection on HCC risk. For use of insulin and statins, we coded nonuse of these drugs as risk factors. Thus, a significant additive interaction between HBV infection and nonuse of a drug suggests that use of the drug might attenuate the effect of HBV infection on HCC risk.

Table 1 Clinical and biochemical characteristics of the study cohort stratified according to availability of HBsAg status.

	HBsAg status not available (n=4784)	HBsAg status available (n=1319)	
	Median (25th–75th) or % (n)	Median (25th–75th) or % (n)	P value ^a
Baseline variables			
Age (years)	57 (47–67)	58 (48–68)	0.1960
Duration of diabetes (years)	6 (2–11)	7 (2–12)	0.1888
Gender (male)	43.6% (2087)	54.7% (721)	<0.0001
Smoking status			<0.0001
Ex-smoker	14.2% (679)	19.5% (257)	
Current smoker	14.8% (707)	16.5% (218)	
Alcohol intake			<0.0001
Ex-drinker	11.2% (535)	15.9% (210)	
Current drinker	7.5% (358)	6.8% (89)	
BMI (kg/m ²)	24.7 (22.4–27.3)	25.0 (22.7–27.9)	0.0019
Systolic BP (mmHg)	134 (121–148)	136 (124–151)	0.0029
Diastolic BP (mmHg)	75 (68–82)	75 (68–80)	0.8432
HbA1c (%)	7.2 (6.3–8.3)	7.5 (6.5–8.7)	<0.0001
HDLC (mmol/l)	1.27 (1.07–1.51)	1.29 (1.07–1.52)	0.0034
LDLC (mmol/l)	3.12 (2.50–3.80)	3.10 (2.47–3.80)	0.0376
Triglyceride (mmol/l)	1.31 (0.93–1.91)	1.40 (1.00–2.09)	0.0015
ACR (mg/mmol)	1.83 (0.73–8.28)	4.37 (1.02–47.97)	<0.0001
eGFR (ml/min per 1.73 m ²)	104.6 (85.5–125.5)	95.7 (69.3–117.9)	<0.0001
Medications during follow-up ^b			
Statins	35.0% (1672)	49.0% (646)	<0.0001
ACEIs/ARBs	55.0% (2631)	68.8% (970)	<0.0001
Insulin	33.3% (1591)	56.3% (742)	<0.0001
Metformin	76.5% (3660)	68.3% (901)	<0.0001
Gliclazide/glibenclamide	65.1% (3212)	60.4% (797)	0.0019
Rosiglitazone/pioglitazone	6.7% (321)	8.95% (118)	0.0054
Events during follow-up			
Hepatocellular carcinoma	0.33% (16)	1.74% (23)	<0.0001
Death	6.3% (302)	15.4% (203)	<0.0001
Cirrhosis			
At baseline	0.0% (1)	0.1% (1)	0.3856
During follow-up	0.4% (18)	1.6% (21)	<0.0001
Either of them	0.4% (19)	1.7% (22)	<0.0001

LDLC, LDL cholesterol; HDLC, HDL cholesterol; BP, blood pressure; ACR, spot urine albumin:creatinine ratio; eGFR, estimated glomerular filtration rate; ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; HBsAg, hepatitis B surface antigen.

^aKolmogorov–Smirnov two-sample test or χ^2 test was used to test differences in distribution between the two groups.

^bDrug use during follow-up was defined as drug use in the period from enrollment to the first liver cancer event, death, or 30 July 2005, whichever came first.

A two-step scheme was used to adjust for covariates. First, we used a minimum adjustment scheme that included only age, sex, BMI (<24.0 and ≥ 27.6 kg/m² due to nonlinear risk association; Yang *et al.* 2008a), use of alcohol and tobacco, and cirrhosis at enrollment or during follow-up. Secondly, we adjusted for HbA1c (Yang *et al.* 2010a,b), systolic BP and risk factors in T2D for cancer that showed nonlinear relationships with cancer. These risk factors included LDLC-related risk indicators (LDLC <2.80 mmol/l plus TG <1.7 mmol/l, LDLC <2.80 mmol/l plus albuminuria, LDLC ≥ 3.80 mmol/l; Yang *et al.* 2012b) and HDL-cholesterol-related risk indicators (HDL <1.0 and ≥ 1.30 mmol/l; Yang *et al.* 2011b). In addition, we adjusted for use of insulin (Yang *et al.* 2010a), metformin (Yang *et al.* 2011b), RAS inhibitors (Yang *et al.* 2009b,

2010c), gliclazide/glibenclamide (Yang *et al.* 2010d), and rosiglitazone/pioglitazone during follow-up (Yang *et al.* 2012c), which was defined as use of the drug from enrollment to the earliest date of HCC, death, or 30 July 2005, whichever came first.

In our review of major biases of pharmaco-epidemiological analysis in nonclinical trial setting, we demonstrated that time-fixed Cox model analysis with exclusion of prevalent users and adjustment for drug use indication was free of major biases and could reproduce the effect of a drug as reported in clinical trials (Yang *et al.* 2012d). Thus, when we tested the additive interaction of HBV infection with nonuse of drugs, we excluded subjects ever exposed to the drug during the 2.5 years before enrollment (Yang *et al.* 2011b). Propensity scores for use of

Table 2 Clinical and biochemical characteristics of the study cohort stratified by the status of chronic HBV infection indicated by positive HBsAg.

	HBsAg negative (n = 1115)	HBsAg positive (n = 204)	
	Median (25th–75th) or % (n)	Median (25th–75th) or % (n)	P value
Baseline variables			
Age (years)	59 (48–69)	52 (46–62)	<0.0001
Duration of diabetes (years)	7 (2–11)	5 (1–10)	0.0233
Gender (male)	52.0% (580)	69.1% (141)	<0.0001
Smoking status			0.1942
Ex-smoker	19.4% (216)	20.1% (41)	
Current smoker	15.8% (176)	20.6% (42)	
Alcohol intake			0.2855
Ex-drinker	16.5% (184)	12.8% (26)	
Current drinker	6.5% (72)	8.3% (17)	
BMI (kg/m ²)	25.2 (22.8–28.0)	24.1 (22.1–27.3)	0.0026
Systolic BP (mmHg)	137 (125–152)	131 (121–142)	<0.0001
Diastolic BP (mmHg)	75 (68–82)	73 (67–80)	0.1290
HbA1c (%)	7.4 (6.5–8.7)	7.6 (6.3–8.9)	0.7806
HbA1c ≥ 7.0%	62.6% (698)	62.3% (127)	0.9252
HDLC (mmol/l)	1.23 (1.03–1.49)	1.29 (1.07–1.52)	0.1106
LDLC (mmol/l)	3.12 (2.50–3.80)	2.81 (2.28–3.50)	0.0003
Triglyceride (mmol/l)	1.48 (1.05–2.15)	1.11 (0.81–1.48)	<0.0001
LDLC < 2.8 mmol/l plus triglyceride < 1.7 mmol/l	29.2% (326)	43.6% (89)	<0.0001
ACR (mg/mmol)	5.48 (1.15–60.79)	1.84 (0.57–8.85)	<0.0001
LDLC < 2.8 mmol/l plus albuminuria	19.6% (218)	19.1% (39)	0.8856
eGFR (ml/min per 1.73 m ²)	93.6 (65.7–117.5)	102.4 (86.0–121.0)	0.0002
Medications during follow-up ^a			
Statins	52.9% (590)	27.5% (148)	<0.0001
ACEIs/ARBs	71.5% (797)	53.9% (110)	<0.0001
Insulin	56.9% (643)	52.9% (108)	0.2994
Metformin	68.6% (765)	66.7% (136)	0.5834
Gliclazide or glibenclamide	61.1% (681)	56.9% (116)	0.2578
Rosiglitazone or pioglitazone	9.8% (109)	4.4% (9)	0.0136
Events during follow-up			
Hepatocellular carcinoma	0.72% (8)	7.4% (15)	<0.0001
Death	16.1% (180)	11.3% (23)	0.0764
Cirrhosis			
At baseline	0% (0)	0.1% (1)	0.1547
During follow-up	0.9% (10)	5.4% (11)	<0.0001
Either of them	0.9% (10)	5.8% (12)	<0.0001

HBsAg, hepatitis B surface antigen; LDLC, LDL cholesterol; HDLC, HDL cholesterol; BP, blood pressure; ACR, spot urine albumin:creatinine ratio; eGFR, estimated glomerular filtration rate; ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; HBV, hepatitis B virus.

^aDrug use during follow-up was defined as drug use in the period from enrollment to the first hepatocellular carcinoma event, death, or 30 July 2005, whichever came first.

the two drugs were calculated, using a logistic regression procedure with stepwise selection of variables at enrollment ($P < 0.30$ for entry and stay; Yang *et al.* 2012d). The two propensity scores were used in the second adjustment scheme in both analyses. This study considered P values below 0.05 to be statistically significant.

Results

Clinical characteristics

Patients with HBsAg assay results had worse glucose and lipid control and renal function and were more likely to

have cirrhosis than those without HBsAg status. The former were more likely to use insulin, statins, and angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers but less likely to use oral antidiabetes drugs including metformin. They were more likely to develop HCC and die (Table 1).

Patients with positive HBsAg had a median age of 52 (25th–75th percentile: 46–62) years. During 5782 years of follow-up, 1.74% ($n = 23$) developed HCC with an annual incidence of 3.98 (95% CI, 2.36–5.60)/1000 person-years. Patients with positive HBsAg status were younger, had lower LDLC and TG, and less likely to use statins and

Table 3 HRs of carrier status of HBsAg for the risk of HCC in relation to HbA1c in type 2 diabetes.

Exposures	HCC (%)	HR	95% CI of HR	P value
Independent models				
Model 1				
HBsAg positive vs negative	15 (7.35%)	9.17	3.47–24.28	<0.0001
HbA1c ≥ 7.0 vs $< 7.0\%$	18 (2.18%)	1.73	0.63–4.73	0.2884
LDLC < 2.80 plus TG < 1.70 mmol/l yes vs no	15 (4.73%)	3.92	1.55–9.95	0.0040
Cirrhosis at enrollment or during follow-up	3 (13.64%)	2.15	0.50–9.22	0.3035
Model 2				
HBsAg positive vs negative	15 (7.35%)	11.36	3.74–34.46	<0.0001
HbA1c ≥ 7.0 vs $< 7.0\%$	18 (2.18%)	8.73	1.75–43.60	0.0083
LDLC < 2.80 plus TG < 1.70 mmol/l yes vs no	15 (4.73%)	8.74	2.36–32.37	0.0012
Cirrhosis at enrollment or during follow-up	3 (13.64%)	3.16	0.71–14.05	0.1311
Interactive models for HBsAg positive and HbA1c $\geq 7.0\%$				
Model 1				
HBsAg positive and HbA1c $< 7.0\%$	2 (2.60%)	5.12	0.81–32.30	0.0826
HBsAg negative and HbA1c $\geq 7.0\%$	5 (0.72%)	0.90	0.21–3.79	0.8878
HBsAg positive and HbA1c $\geq 7.0\%$	13 (10.24%)	16.00	4.27–59.89	<0.0001
HBsAg negative and HbA1c $< 7.0\%$	3 (0.72%)		Reference	
Model 2				
HBsAg positive and HbA1c $< 7.0\%$	2 (2.60%)	3.74	0.56–25.06	0.1736
HBsAg negative and HbA1c $\geq 7.0\%$	5 (0.72%)	4.25	0.70–25.74	0.1158
HBsAg positive and HbA1c $\geq 7.0\%$	13 (10.24%)	74.96	10.79–520.71	<.0001
HBsAg negative and HbA1c $< 7.0\%$	3 (0.72%)		Reference	
Interactive models for HBsAg positive and LDLC < 2.8 plus TG < 1.7 mmol/l				
Model 1				
HBsAg positive and LDLC < 2.8 plus TG < 1.7 mmol/l=no	4 (3.28%)	10.75	2.58–44.73	0.0011
HBsAg negative and LDLC < 2.8 plus TG < 1.7 mmol/l=yes	4 (1.70%)	4.43	1.08–18.09	0.0382
HBsAg positive and LDLC < 2.8 plus TG < 1.7 mmol/l=yes	11 (13.41%)	39.11	11.39–134.77	<0.0001
HBsAg negative and LDLC < 2.8 plus TG < 1.7 mmol/l=no	4 (0.75%)		Reference	
Model 2				
HBsAg positive and LDLC < 2.8 plus TG < 1.7 mmol/l=no	4 (3.28%)	11.01	2.37–51.14	0.0022
HBsAg negative and LDLC < 2.8 plus TG < 1.7 mmol/l=yes	4 (1.70%)	8.17	1.44–46.29	0.0177
HBsAg positive and LDLC < 2.8 plus TG < 1.7 mmol/l=yes	11 (13.41%)	89.82	17.60–458.35	<0.0001
HBsAg negative and LDLC < 2.8 plus TG < 1.7 mmol/l=no	4 (0.75%)		Reference	

HR, hazard ratio; CI, confidence interval; TG, triglyceride; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma. Model 1: adjusted for age sex, intake of tobacco (ex-smoker and current smoker) and alcohol (ex-drinker and current drinker), BMI (< 24 and ≥ 27.6 kg/m²), and cirrhosis. Model 2: further adjusted for LDLC-related risk indicators (LDLC < 2.80 mmol/l plus albuminuria or plus TG < 1.70 mmol/l and LDLC ≥ 3.80 mmol/l where appropriate), HDLC (< 1.0 and ≥ 1.3 mmol/l), systolic BP, as well as use of statins, RAS inhibitors (ACEIs or ARBs), insulin, metformin, glibenclamide/gliclazide, and rosiglitazone/pioglitazone during follow-up period.

more likely to develop HCC than those with negative HBsAg status (Table 2).

Enhancers of the effect of chronic HBV infection on HCC

Chronic HBV infection, HbA1c $\geq 7\%$, and co-presence of low LDLC plus low TG were independently associated with higher HRs for HCC (Table 3). On multivariable analysis, HbA1c $\geq 7\%$ greatly increased the HR of positive HBsAg for HCC from 3.74 to 74.96 (Table 3) with significant additive interaction (AP: 0.91; Table 4). Similarly, co-presence of low LDLC plus low TG greatly increased the HR of HBsAg for HCC from 11.01 to 89.82 (Table 3) with significant additive interaction (AP: 0.80; Table 4). Figure 1A and B showed the adjusted cumulative incidences of HCC stratified by various combinations of chronic HBV infection, HbA1c $\geq 7\%$, or co-presence of low LDLC plus low TG.

Attenuators of the effect of HBV infection on HCC

In multivariable analysis, use of insulin attenuated the HR of HBsAg for HCC from 37.51 to 5.87 (Table 5) with significant additive interaction (AP: 0.80; Table 4). Similarly, use of statins attenuated the HR of HBsAg for HCC from 64.94 to 16.99 (Table 5) with significant additive interaction (AP: 0.67; Table 4). Figure 1C and D showed the adjusted cumulative incidences of HCC stratified by various combinations of HBV infection, insulin, or statins use.

Additional analysis on cirrhosis status

We compared the clinical profile of patients with cirrhosis and those without. Patients with cirrhosis had higher HDLC and were less likely to be treated with statins and metformin but more likely to be treated with insulin in multivariable analysis (data not shown).

Table 4 Additive interactions of HBsAg carrier status with enhancers and alleviators for HCC in type 2 diabetes.

Measures of additive interaction	Estimate	95% CI	P value*
Interaction between HBsAg positive and HbA1c $\geq 7.0\%$			
Model 1			
RERI	10.98	−5.70 to 27.66	0.1698
AP	0.69	0.20 to 1.18	0.0062
Model 2			
RERI	67.97	−68.87 to 204.82	0.3303
AP	0.91	0.78 to 1.04	<0.0001
Interaction between HBsAg positive and LDLC < 2.8 mmol plus TG < 1.7 mmol/l			
Model 1			
RERI	24.93	−13.12 to 62.99	0.1991
AP	0.64	0.26 to 1.02	0.0010
Model 2			
RERI	71.64	−21.82 to 165.11	0.1330
AP	0.80	0.61 to 0.98	<0.0001
Interaction between HBsAg positive and nonuse of insulin			
Model 1			
RERI	21.43	−21.22 to 64.08	0.3247
AP	0.69	0.22 to 1.16	0.0038
Model 2			
RERI	29.94	−42.64 to 102.51	0.4187
AP	0.80	0.42 to 1.18	<0.0001
Interaction between HBsAg positive and nonuse of statins			
Model 1			
RERI	39.09	−51.40 to 129.67	0.3972
AP	0.68	0.15 to 1.21	0.0115
Model 2			
RERI	43.67	−79.86 to 167.21	0.4884
AP	0.67	0.04 to 1.31	0.0384

RERI, relative excess risk due to interaction; AP, attributable proportion due to interaction; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma. Models 1 and 2 were adjusted for the same covariates as in Tables 3 and 5. *Statistically significant with RERI > 0 or AP > 0 indicating additive interaction.

Discussion

In this prospective cohort of patients with T2D, we reported for the first time that suboptimal glycemic control (HbA1c $\geq 7\%$) and co-presence of low LDLC plus low TG markedly enhanced the effect of HBV infection on the risk of HCC while use of insulin or statins attenuated the effect, even after adjustment for cirrhosis. HBV infection is a well-established risk factor for HCC, although the underlying molecular mechanisms and pathways remain to be defined (Pan et al. 2011). Recent studies have established that diabetes is strongly associated with increased risk of HCC (El-Serag et al. 2006). In an observational study, the authors reported a synergistic effect between diabetes and HBV infection on the risk of incident HCC (Ko et al. 2012). In this study, suboptimal glycemic control markedly enhanced

the risk association between chronic HBV infection and HCC while use of insulin attenuated the risk. These findings reaffirmed the report from a Japanese study where the researchers showed a marked increase in the number and size of liver tumors in insulin-deficient and hyperglycemic animals that was attenuated by insulin (Yamasaki et al. 2010).

In connection with these novel findings, we had reported a synergistic effect of LDLC < 2.80 mmol/l plus albuminuria on the overall cancer risk in T2D (Yang et al. 2009a), which was further enhanced by the presence of HDLC ≥ 1.30 mmol/l (Yang et al. 2012c). In a recent review article, based on a large body of clinical and experimental findings, we hypothesized that these epidemiological findings might reflect increased oxidative stress and upregulation of the RAS activity, secondary to hyperglycemia (Yang et al. 2012b) with inflammation (Yang et al. 2010c) and/or dysregulated cholesterol metabolism (Yang et al. 2009b) as mediating pathways. In this study, we did not detect significant interaction of co-presence of low LDLC plus albuminuria with HBsAg for HCC. On the other hand, we found markedly an enhancing effect of lack of insulin treatment and HbA1c $\geq 7\%$ on the risk association between HBV infection and HCC, suggesting that insulin insufficiency might be a major culprit in the development of HCC in T2D.

In a large matched case-control study from the USA, use of statins among patients with diabetes was associated with a 26% reduction in the HCC risk (El-Serag et al. 2009). In another large population-based cohort study from Taiwan, use of statins in HBV-infected patients was associated with a reduced risk for HCC in a dose-dependent manner (Tsan et al. 2012). Our study further shows that use of statins might have particular benefits in reducing HCC risk among patients with both T2D and HBV infection. Previously, we reported that low TG was associated with increased overall cancer risk in T2D, which was attenuated by use of statins (Yang et al. 2011a). Further exploration indicated that only co-presence of low TG plus low LDLC and neither of these two factors alone was associated with increased overall cancer risk in T2D (Yang et al. 2012a). Of note, insulin selectively activates SREBP1c, leading to upregulated synthesis of free fatty acids (Shimomura et al. 1999), while IGF1 activates SREBP1a, resulting in upregulated synthesis of both cholesterol and free fatty acids (Smith et al. 2008). As there is a feedback regulation loop from intracellular low cholesterol to IGF1 and SREBP1a to upregulate cholesterol synthesis (Brown & Goldstein 1997), we hypothesized that insulin insufficiency might lead to low

Figure 1

Adjusted cumulative incidence of hepatocellular carcinoma (HCC) stratified by combinations of HBsAg and enhancers or attenuators of its effect on the risk of HCC. (A) From top to bottom: i) HBsAg positive and HbA1c $\geq 7.0\%$ (top line); ii) HBsAg positive and HbA1c $< 7.0\%$; iii) HBsAg negative and HbA1c $\geq 7.0\%$; and iv) HBsAg negative and HbA1c $< 7.0\%$ (bottom line). (B) From top to bottom: i) HBsAg positive and LDLC < 2.8 mmol/l plus triglyceride < 1.7 mmol/l = yes (top line); ii) HBsAg positive and LDLC < 2.8 mmol/l plus triglyceride < 1.7 mmol/l = no; iii) HBsAg negative and LDLC < 2.8 mmol/l plus triglyceride < 1.7 mmol/l = yes; and iv) HBsAg negative and LDLC < 2.8 mmol/l plus triglyceride < 1.7 mmol/l = no (bottom line). (C) From top to bottom: i) HBsAg positive and nonuse of insulin (top line); ii) HBsAg positive and use of insulin; iii) HBsAg negative and nonuse of insulin; and iv) HBsAg negative and use of insulin (bottom line). (D) From top to bottom: i) HBsAg positive and nonuse of statins (top line); ii) HBsAg positive and use of statins; iii) HBsAg negative and nonuse of statins; and iv) HBsAg negative and use of statins (bottom line). The covariates adjusted and *P* values are the same as in Tables 3 and 5.

TG and low LDLC resulting in dysregulation of the IGF1, SREBP1a, and cholesterol synthesis. The activation of the cholesterol pathway can increase RAS signals and other oncogenes resulting in increased cancer risk (Yang *et al.* 2012b). In this connection, the enhanced effect of HBV infection on HCC risk by co-presence of low LDLC plus low TG and its attenuation by use of insulin or statins strongly supported our hypothesis. Of note, the residual effects of either HBV infection or co-presence of low LDLC plus low TG remained substantial after controlling for

their synergistic effect on the risk of HCC. Use of insulin or statins failed to attenuate the increased HRs for HCC to a level close to 1. These observations suggested while the pathway from HBV infection to HCC and that from insulin insufficiency to dysregulated cholesterol synthesis were distinct entities, there might be cross talks between them with dysregulated intermediary metabolisms enhancing the promoting effect of chronic HBV infection on HCC. In this regard, recent studies have shown that diabetes could accelerate formation and accumulation of

Table 5 Hazard ratios (HRs) of carrier status of HBsAg for the risk of HCC in relation to co-presence of low LDLC and low triglyceride, insulin, and statin usage.

Exposures	HCC (%)	HR	95% CI of HR	P value
Interactive models for HBsAg positive and nonuse of insulin (460 subjects who ever used insulin in 2.5 years before enrollment were excluded)				
Model 1				
HBsAg positive and use of insulin	2 (4.55%)	7.82	0.98–62.37	0.0522
HBsAg negative and nonuse of insulin	5 (1.11%)	2.74	0.52–14.32	0.2331
HBsAg positive and nonuse of insulin	6 (6.38%)	30.99	5.56–172.60	<0.0001
HBsAg negative and use of insulin	2 (0.74%)		Reference	
Model 2				
HBsAg positive and use of insulin	2 (4.55%)	5.87	0.52–66.11	0.1522
HBsAg negative and nonuse of insulin	5 (1.11%)	2.70	0.36–20.52	0.3375
HBsAg positive and nonuse of insulin	6 (6.38%)	37.51	4.11–342.31	0.0013
HBsAg negative and use of insulin	2 (0.74%)		Reference	
Interactive models for HBsAg positive and nonuse of statins (264 subjects who ever used statins in 2.5 years before enrollment were excluded)				
Model 1				
HBsAg positive and use of statins	1 (2.86%)	13.64	0.84–221.23	0.0660
HBsAg negative and nonuse of statins	7 (1.35%)	5.49	0.67–45.15	0.1134
HBsAg positive and nonuse of statins	13 (8.90%)	57.22	6.95–470.96	0.0002
HBsAg negative and use of statins	1 (0.28%)		Reference	
Model 2				
HBsAg positive and use of statins	1 (2.86%)	16.99	0.90–320.98	0.0588
HBsAg negative and nonuse of statins	7 (1.35%)	5.27	0.52–53.92	0.1612
HBsAg positive and nonuse of statins	13 (8.90%)	64.94	5.59–754.12	0.0009
HBsAg negative and use of statins	1 (0.28%)		Reference	

HR, hazard ratio; CI, confidence interval; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma. Model 1: adjusted for age sex, intake of tobacco (ex-smoker and current smoker) and alcohol (ex-drinker and current drinker), BMI (<24 and ≥ 27.6 kg/m²), and cirrhosis. Model 2: further adjusted for LDLC-related risk indicators (LDLC <2.80 mmol/l plus albuminuria or plus TG <1.70 mmol/l and LDLC ≥ 3.80 mmol/l where appropriate), HDLC (<1.0 and ≥ 1.3 mmol/l), systolic BP, as well as use of statins, RAS inhibitors (ACEIs or ARBs), insulin, metformin, glibenclamide/gliclazide, and rosiglitazone/pioglitazone during follow-up period as well as the propensity score for use of insulin (the c-statistic=0.78) or statins (the c-statistic=0.81) during follow-up period where appropriate.

advanced glycation end products (AGEs), which have been implicated in development of liver disease (Hyogo & Yamagishi 2008). Further experimental studies are needed to study the effects of AGEs on promoting HBV-associated HCC risk in diabetes.

Study limitations

There are several limitations of this study. First, this study had a small sample size with only 23 cases of HCC, which resulted in wide confidence intervals. However, due to the large effect sizes of variables of interest, we were able to detect highly significant enhancers and attenuators of HBV infection on the risk of HCC. Although the final models were overfitted, the relatively constant HRs derived in both the minimally and the fully adjusted models suggested that the impact of overfitting on the effect sizes was small, if any.

Second, there might be a selection bias as not every patient in the cohort had HBsAg measurements. Patients

with HBsAg measurement had worse glucose control and renal functions and were more likely to have HCC and to die. Therefore, findings from this analysis could not be extrapolated to low-risk populations with T2D.

Third, this was an observational study with inherent biases, which could not be completely removed despite statistical adjustments. Thus, these results only generated hypotheses for further testing and should not influence clinical practice. Besides, a small number of patients who had negative HBsAg but positive hepatitis B core antigen might have been misclassified.

Finally, liver function at enrollment was not available for analysis. We acknowledged that cirrhosis might contribute to the lipid pattern of low LDLC and low TG, for example, due to poor nutrition. However, we did not observe differences in BMI or distribution of co-presence of low LDLC and low TG between patients with or without cirrhosis. Up to 31.5% ($n=415$) of patients in the cohort had co-presence of low LDLC and low TG and 97.3% ($n=404$) of patients with this phenotype did not have

cirrhosis, although 50% ($n=11$) of patients with cirrhosis had this cancer risk phenotype and 27.3% ($n=6$) of them had low BMI defined as BMI <24 kg/m². Besides, adjustment for cirrhosis tended to increase the HR of co-presence of low LDLC and low TG for HCC from 7.20 (95% CI, 1.82–28.48) to 8.74 (95% CI, 2.36–32.37; Table 3, independent models, model 2). Taken together, the association between HCC and co-presence of low LDLC and low TG was unlikely due to cirrhosis or its metabolic consequences.

Conclusion

In conclusion, in Chinese T2D patients with known HBsAg status, suboptimal glycemic control (HbA1c $\geq 7\%$) and co-presence of low LDLC plus low TG greatly enhanced the promoting effect of chronic HBV infection on HCC, while use of insulin or statins attenuated the increased risk. These observations support the notion that upregulation of the IGF1, SREBP1a, and cholesterol synthesis pathway due to insulin insufficiency might link diabetes and HCC and that dysregulation of these interlinked pathways might markedly enhance the promoting effect of chronic HBV infection on HCC in T2D. Given the high prevalence of T2D and HCC as well as the poor prognosis of patients with HCC, there is an urgent need to replicate these novel findings in separate cohorts and to investigate the underlying molecular mechanisms. As insulin and statins are safe and widely used in T2D, we also call for randomized clinical trials to test whether use of insulin and statins in T2D patients who are carriers of HBV and risk-conferring phenotypes will reduce the risk of HCC.

Declaration of interest

J C N Chan has received research grant and/or honorarium for consultancy or giving lectures from Bayer, Daiichi-Sankyo, Lilly, GlaxoSmithKline, Merck Sharp & Dohme, Merck Serono, Pfizer, Astra Zeneca, Sanofi-Aventis, Novo-Nordisk, and/or Bristol-Myers Squibb. A P S Kong has received honorarium for consultancy or giving lectures from Merck Serono and Lilly. G Xu has received research grant and/or honorarium for consultancy or giving lectures from Merck Sharp & Dohme. R C W Ma has received honorarium for consultancy or giving lectures from Boehringer-Ingelheim, Eli Lilly, Astra-Zeneca, Danone, Nestle, and Pfizer. All proceeds have been donated to the Chinese University of Hong Kong to support the ongoing research. Other authors declared no conflict of interest with this manuscript.

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