Published in final edited form as:
Hepatology. 2013 February ; 57(2): 648–655. doi:10.1002/hep.26092.

Risk Factors for Intrahepatic Cholangiocarcinoma: Association between Metformin use and Reduced Cancer Risk

Roongruedee Chaiteerakij[1,2], **Ju Dong Yang**[1], **William S. Harmsen**[3], **Seth W. Slettedahl**[3], **Teresa A. Mettler**[1], **Zachary S. Fredericksen**[4], **W. Ray Kim**[1], **Gregory J. Gores**[1], **Rosebud O. Roberts**[5], **Janet E. Olson**[5], **Terry M. Therneau**[3], and **Lewis R. Roberts**[1]

[1]Division of Gastroenterology and Hepatology, College of Medicine, Mayo Clinic and Mayo Clinic Cancer Center, Rochester, MN, US [2]Department of Medicine, Faculty of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand [3]Department of Biomedical Statistics and Informatics, Mayo Clinic College of Medicine, and Mayo Clinic Cancer Center, Rochester, MN, US [4]Division of Biomedical Statistics and Informatics, Department of Health Sciences Research, Mayo Clinic College of Medicine, Rochester, MN, US [5]Division of Epidemiology, Department of Health Sciences Research, Mayo Clinic, Rochester, MN, US

Abstract

The associations between diabetes, smoking, obesity and intrahepatic cholangiocarcinoma (ICC) risk remain inconclusive. Metformin is purportedly associated with a reduced risk for various cancers. This case-control study evaluated risk factors for ICC and explored the effects of metformin on ICC risk in a clinic/hospital-based cohort. ICC patients seen at Mayo Clinic, Rochester, MN between January 2000 and May 2010 were identified. Age, sex, ethnicity, and residential area-matched controls were selected from among Mayo Clinic Biobank participants. The associations between potential factors and ICC risk were determined. Six hundred and twelve cases and 594 controls were identified. Factors associated with increased ICC risk included biliary tract diseases (Adjusted Odds Ratio [AOR] 81.8, 95% confidence interval [CI]: 11.2–598.8, $P<0.001$), cirrhosis (AOR 8.0, 95%CI: 1.8–36.5, $P=0.007$), diabetes (AOR 3.6, 95%CI: 2.3–5.5, $P<0.001$), and smoking (AOR 1.6, 95%CI: 1.3–2.1, $P<0.001$). Compared to diabetic patients not treated with metformin, odds ratio (OR) for ICC for diabetic patients treated with metformin was significantly decreased (OR 0.4, 95%CI: 0.2–0.9, $P=0.04$). Obesity and metabolic syndrome were not associated with ICC.

Conclusion—This study confirmed diabetes and smoking as independent risk factors for ICC. A novel finding was that treatment with metformin was significantly associated with a 60% reduction in ICC risk in diabetic patients.

Keywords

bile duct cancer; epidemiology; oral hypoglycemic agent; diabetes

Correspondence: Lewis R. Roberts, M.B. Ch.B., Ph.D. (Corresponding Author), Division of Gastroenterology and Hepatology, College of Medicine, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, US, Roberts.lewis@mayo.edu; Phone: 507-538-4877; Fax: 507-284-0762.

Financial disclosure: This work was supported by Grants CA100882, CA128633, and CA165076 from the National Institutes of Health; the Mayo Clinic Center for Cell Signaling in Gastroenterology (NIDDK P30DK084567); the Mayo Clinic Cancer Center (CA15083), and the Mayo Foundation (to LRR)

Introduction

Cholangiocarcinoma is categorized based on anatomic location as intrahepatic or extrahepatic cholangiocarcinoma, which are considered as separate diseases with different genetic alterations and clinical characteristics. Intrahepatic cholangiocarcinoma (ICC) is increasing in importance because its incidence has been rising around the world, including in the U.S.(1–3) We recently showed that the incidence of ICC in Olmsted County, Minnesota increased 7-fold between the 1976–1990 time period and the 2000–2008 time period.(3) The cause of this increasing trend in ICC incidence is unknown.

A number of case-control studies have consistently identified several risk factors for ICC.(4–12) These include biliary tract diseases (i.e., primary sclerosing cholangitis (PSC), choledochal cyst, or hepatolithiasis), parasitic infestation of the biliary tract by *Clonorchis sinensis* or *Opisthorchis viverrini,* and chronic liver diseases such as chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection or cirrhosis from other causes. (4–7,9–12) Although diabetes mellitus (DM), smoking and obesity have been shown to be risk factors for many cancers, the associations remain inconclusive for ICC.(4–10)

The metabolic syndrome is an increasingly important health problem in the U.S., with a prevalence as high as 25%.(13) A recent report using combined SEER and Medicare data suggests that the metabolic syndrome is associated with an increased risk of ICC in the population aged over 65.(14) As the rising incidence of the metabolic syndrome is a possible cause of the rising incidence of ICC, validation of this result in other age groups, particularly in ICC patients aged under 65, is important.

Recent epidemiologic studies have shown that metformin use by patients with type 2 DM, but not use of other glucose lowering agents, is associated with a decreased risk for a number of cancers, including hepatocellular carcinoma (HCC).(15–20) Statin use has also been shown to decrease the risk for HCC in 2 large case-control studies in the U.S. and Taiwan.(21) However, it is unknown whether metformin or statin use are associated with a decreased risk for ICC.

The aims of our study were: 1) to investigate the associations of controversial risk factors including DM, smoking, and obesity with risk of ICC, 2) to validate the association between metabolic syndrome and ICC risk, and 3) to explore the effects of metformin or statin use on ICC risk.

Methods

Study population

All patients with ICC seen at Mayo Clinic, Rochester, MN between January 2000 and May 2010 were included in the study. We searched for ICC cases in the Mayo Clinic Life Sciences System (MCLSS) using the ICD-9-CM code of “155.1” and/or the keywords “cholangiocarcinoma” and “bile duct cancer” to identify all potential ICC patients (n=1828).

The diagnosis of cholangiocarcinoma was confirmed by histopathology and the anatomic location of the tumor determined by review of histopathology and radiology (computerized tomography, magnetic resonance imaging or endoscopic retrograde cholangiopancreatography). Cholangiocarcinomas were categorized as “intrahepatic” if the lesion arose within the hepatic parenchyma and did not extend beyond the secondary hilar branches of the biliary tree. After review, 1216 of the 1828 potential ICC patients were excluded (965 had extrahepatic cholangiocarcinoma, 60 HCC, 92 other malignancy or liver

metastasis, 23 benign liver lesions and 76 had no pathological or radiologic information). The remaining 612 patients with confirmed ICC were included in the analysis.

Control subjects were selected from the Mayo Clinic Biobank, which comprises patients receiving care at the Mayo Clinic who have agreed to participate in this clinic-based database. This database includes a large group of patients seen at Mayo Clinic and is designed to provide control groups for studies performed at Mayo Clinic, allowing selection of controls that are matched to cases by age, gender, ethnicity and residence. Mayo Clinic Biobank participants include local patients seeking their routine medical care in the Department of Family Medicine or the Division of Community Internal Medicine and non-local referral patients seeking care for both routine and serious medical conditions in the Division of General Internal Medicine. Biobank participants provide a blood sample, complete a health questionnaire, and give authorization for use of their medical records in research. Recruitment of Biobank participants began in April 2009.

Cases were matched by age (±5 years), sex, ethnicity, and residence (Olmsted County Minnesota, Southeast Minnesota, Other Minnesota, Iowa, Wisconsin, North and South Dakota, and other regions of the U.S. (Northeast, Southeast, Southwest, Northwest and Midwest)) to subjects who enrolled in the Mayo Clinic Biobank between April 2009 and May 2010. Controls did not have a history of any cancers.

Clinical information

Demographic data, clinical information, medications and laboratory results were abstracted from the electronic medical record. Data on risk factors were abstracted from a general health and family information form. This self-administered questionnaire is routinely completed by patients and included in the medical record.

Risk factors abstracted included body mass index, history of liver disease (HBV or HCV infection, cirrhosis, nonalcoholic steatohepatitis (NASH), PSC, choledochal cyst or hepatolithiasis), DM, hyperlipidemia, family history of liver cancer, and smoking status. We excluded alcohol from the analysis because data on the amount and duration of alcohol use was missing in over 10% of both the case and control groups.

We abstracted the results of tests for HBV and HCV infection for all cases and controls. HBV infection was defined as a positive hepatitis B surface antigen and HCV infection was defined as a positive HCV RNA. A diagnosis of HBV or HCV in the physician's note was accepted as proof of viral infection.

Obesity was defined by a body mass index ≥ 30 kg/m^2. Metabolic syndrome was defined according to the American Heart Association/National Cholesterol Education Program Adult Treatment Panel III (AHA/NCEP ATP III) criteria (at least three of the following 5 criteria: triglyceride level ≥ 150 mg/dL, high-density lipoprotein cholesterol <40 mg/dL in men or <50 mg/dL in women, systolic blood pressure ≥ 130 mmHg or diastolic pressure ≥ 85 mmHg, fasting plasma glucose ≥ 110 mg/dL and waist circumference >102 cm in men or >88 cm in women).(22) Since data on waist circumference was not available, we used obesity as a proxy variable for elevated waist circumference. NASH was diagnosed by histopathology or evidence of fatty infiltration on radiologic imaging with elevation of serum aminotransferase enzymes and exclusion of other chronic liver diseases and excessive alcohol drinking (>140 and >70 grams/week in men and women, respectively). Cirrhosis was diagnosed by radiologic evidence of a nodular liver, caudate lobe hypertrophy, or portal hypertension (collateral vessels, varices, and splenomegaly).

Current or previous use of metformin or a statin was ascertained from the medication list and physician's notes. For cases, we reviewed the medication list from 1 year before ICC diagnosis until the date of ICC diagnosis to ensure that metformin or the statin was not withdrawn due to the diagnosis of malignancy. Similarly, for controls, we reviewed the medication list from 1 year before until study enrollment. Smoking status was classified as never-smoker or ever-smoker. The amount and duration of cigarette smoking were abstracted for subjects who ever smoked.

The durations of risk factors prior to diagnosis of ICC in cases or prior to study enrollment in controls were abstracted. Fifteen percent of cases and controls were randomly selected to assess the agreement of self-reported patient questionnaire data with physicians' notes, laboratory results and/or radiologic imaging (as the gold standard).

Statistical analysis

Kappa statistics were calculated for the agreement of self-reported data from the patient questionnaire with data directly abstracted from physicians' notes in the medical record, with a mean Kappa value of 0.91 (substantial to almost perfect observer agreement).(23) Since cases and controls were not enrolled within the same period of time (cases were from January 2000 – May 2010; controls were from April 2009 – May 2010), changes in the frequencies of variables over time might influence the results. To account for this, ICC cases were categorized into 4 groups based on the year of diagnosis (group 1: 2000–2002, group 2: 2003–2005, group 3: 2006–2008 and group 4: 2009–2010). The change in frequency of each risk factor variable in ICC patients by year group was assessed by trend analysis.

Logistic regression was used to estimate the univariate association of each variable with ICC. Since the frequency of statin use increased over time during the study period, propensity scores for statin use variable were calculated and included in the analysis model. Similarly, to correct for possible imbalances in the frequency of metformin use, we balanced the data using sampling weights. This method of adjusting for imbalance is a standard approach in survey sampling, particularly complex surveys.(24) Variables with $P<0.05$ in the univariate models were included in the multivariate model. Age, gender and ethnicity, which were considered to be potential confounders, were also included in the multivariate model. The duration of existing conditions significantly associated with ICC was compared using the Wilcoxon Rank Sum test. Data analysis was performed using SAS 9.1 (SAS Institute, Gary, NC).

Sensitivity analysis—Given the disparity between date of diagnosis of cases and date of enrollment of controls into the Mayo Biobank database, we repeated the analyses restricted to cases who were enrolled from 2006 to 2010 (groups 3 and 4, n=279 cases) and controls enrolled from 2009–2010, thus limiting the cases to those diagnosed within 3 years of enrollment of controls.

Results

Patient characteristics

Six hundred and twelve ICC cases and 594 controls were included in the analysis. Table 1 summarizes the baseline characteristics and the frequency of risk factors for the case and control groups. Demographics were comparable between the groups. There were 149 cases seen from 2000–2002 (group 1), 184 seen from 2003–2005 (group 2), 186 seen from 2006–2008 (group 3), and 93 seen from 2009–May 2010 (group 4). The trend analysis showed that the frequencies of risk factor variables did not change significantly over time except for the frequencies of metformin use among diabetic patients and of statin use among

hyperlipidemic patients (Supplemental table 1 online). The frequency of metformin use among diabetic patients showed a significantly increasing trend during the time period over which cases were seen, i.e. 7.7%, 27.3%, 34.6% and 30.0% in groups 1, 2, 3 and 4, respectively ($P_{for\ trend}$ =0.04), particularly increasing between the first and second time periods. Between the second and fourth time periods (groups 2, 3 and 4), there was no statistically significant difference in the frequency of metformin use ($P_{for\ trend}$ =0.77). Unlike the early rise in use of metformin, the frequency of statin use increased continuously over the time of the study, from 23.8% in group 1 to 43.5%, 46.9% and 67.9% in groups 2, 3 and 4, respectively ($P_{for\ trend}$ <0.01).

Diabetes and smoking are associated with an increased risk for ICC

As expected, the relative odds of ICC were markedly increased in patients with a history of biliary tract diseases (PSC, choledochal cysts, or hepatolithiasis) (OR 81.6, 95%CI: 11.3–589.0, P<0.001). Univariate analysis of other risk factor variables with ICC showed that cirrhosis (OR 21.8, 95%CI: 5.3–90.5, P<0.001), HCV infection (OR 6.4, 95%CI: 1.4–28.5, P=0.001), DM (OR 3.3, 95%CI: 2.2–4.9, P<0.001), and smoking (OR 1.5, 95%CI: 1.2–1.8, P=0.02) were significantly associated with an increased OR of ICC. In contrast, the OR for ICC was significantly reduced (OR 0.5, 95%CI: 0.4–0.6, P<0.001) in patients with hyperlipidemia compared to individuals without hyperlipidemia. HBV infection (OR 1.0, 95%CI: 0.2–4.8), obesity (OR 0.9, 95%CI: 0.7–1.1), metabolic syndrome (OR 0.9, 95%CI: 0.7–1.2), NASH (OR 0.6, 95%CI: 0.2–1.4), and family history of liver cancer (OR 1.3, 95%CI: 0.3–5.8) were not associated with ICC (P>0.05 for all 5 variables). The univariate analyses results remained unchanged in the sensitivity analyses restricting to groups 3 and 4 ICC cases (data not shown).

Table 2 shows the multivariate adjusted OR (AOR) for biliary tract diseases, cirrhosis, HCV infection, DM, smoking and hyperlipidemia of all ICC cases and of case groups 3 and 4. Biliary tract diseases (AOR 81.8, 95%CI: 11.2–598.8, P<0.001) and cirrhosis (AOR 8.0, 95%CI: 1.8–36.5, P=0.007) were associated with ICC. HCV infection was not associated with ICC in the multivariate model (AOR 2.6, 95%CI: 0.5–13.5, P=0.25). Since the very high OR for biliary tract diseases could potentially conceal the effect of other risk variables, we performed a sensitivity analysis by excluding the biliary tract diseases variable in the univariate and multivariate model. The overall results did not change (Supplemental Table 2 online).

Diabetes was associated with ICC (AOR 3.6, 95%CI: 2.3–5.5, P<0.001). The median (interquartile range) duration of DM was 10.1 (4.2–16.5) years before ICC diagnosis in the 60 ICC cases with DM for whom data was available (out of a total of 105 ICC cases with DM). The duration of DM was 8.4 (5.1–12.0) years before the date of Biobank enrollment in the 31 controls with DM for whom data was available (of a total of 35 controls with DM). There was no significant difference in duration of DM before ICC diagnosis or Biobank enrollment (P=0.44).

Smoking conferred a significantly increased risk for ICC (AOR 1.6, 95%CI: 1.3–2.1, P<0.001), however, no dose-response relationship between smoking and ICC was demonstrated (data not shown).

Hyperlipidemia was associated with a decreased risk for ICC (AOR 0.4, 95%CI: 0.3–0.6, P<0.001). This association might be due to a protective effect of treatment with statins. Compared to hyperlipidemic patients who were not treated with statins, the OR for ICC for hyperlipidemic patients treated with statins was significantly decreased to 0.6 (95%CI: 0.4–0.9, P=0.03). However, the rate of statin use increased significantly over the course of the study. To test for spurious associations, we performed sensitivity analyses restricting the

comparison to ICC cases with hyperlipidemia in year groups 3 and 4 versus controls with hyperlipidemia and to ICC cases with hyperlipidemia in group 4 who were diagnosed within the same time period of controls versus controls with hyperlipidemia. For these comparisons, the OR (95%CI) for ICC was 0.9 (0.5–1.6, P=0.81) and 1.2 (0.5–2.7, P=0.73), respectively (Table 3A), suggesting that the purported association between statin use and ICC risk among hyperlipidemic patients was due to changes in statin use over time.

There is a strong inverse relationship between metformin use and ICC risk

Twenty six of 105 (24.8%) ICC cases with DM and 22 of 34 (64.7%) controls with DM were treated with metformin (P<0.001). Diabetic patients on metformin had a significantly smaller risk of ICC as compared to those not on metformin (OR 0.2, 95%CI: 0.1–0.4, P<0.001). Since the use of metformin increased during the study period, we repeated the analysis excluding patients in group 1 and including patients in groups 2, 3 and 4, for whom the frequency of metformin use was not statistically different ($P_{for\ trend}$ =0.77). The repeat analysis showed that diabetic patients treated with metformin had an OR of 0.4 (95%CI: 0.2–0.9, P=0.04) for developing ICC. This result also remained unchanged in the sensitivity analyses restricted to ICC cases with DM in groups 3 and 4 (OR 0.4, 95%CI: 0.1–0.9, P=0.04) and to ICC cases with DM in group 4 who were diagnosed within the same time period of controls (OR 0.3, 95%CI: 0.1–0.96, P=0.047) (Table 3B).

Discussion

We found significant associations of biliary tract diseases, cirrhosis, DM, smoking, and metformin use with ICC risk in this large hospital/clinic-based case-control study at a major referral center in the U.S.

Our findings confirm that DM and smoking are independent risk factors for ICC. This is important because both DM and smoking are modifiable risk factors. Diabetes prevention and smoking cessation may therefore reduce the risk of ICC, which is usually diagnosed at an advanced stage and has an extremely poor prognosis. Diabetes conferred a 3.6-fold increased risk for ICC in this study, a higher magnitude than was found in previous U.S. case-control studies (AOR 1.8–2).(5,7) The carcinogenic effect of DM in humans is well established.(25) An *in vitro* study has suggested that the insulin-like growth factor signaling pathway is involved in the pathogenesis of cholangiocarcinoma.(26) There is therefore a biologically plausible hypothesis for our observation that DM increases the risk of ICC. This hypothesis is also supported by our finding of a long duration (10.1 years) of DM before ICC diagnosis.

The carcinogenic effect of smoking is also well established, including for HCC.(27) Studies from Asia found no association between smoking and ICC whereas most studies from Western countries show modest associations of smoking and ICC with ORs of 1.4–1.8.(5–8) In the present study, smoking was associated with a 1.9-fold increased risk for ICC, a magnitude consistent with those of other Western studies.

In contrast to the results of the few case-control studies performed thus far in the U.S., HCV was not found to be an independent risk factor for ICC.(5–7) HCV was significantly associated with ICC risk in the univariate model and became a trend towards significance in the sensitivity analysis restricted to ICC case groups 3 and 4 (P=0.08), thus the lack of significance in the multivariate model may be due to a lack of statistical power. Given the high AOR of 8.0 for cirrhosis, and the fact that HCV infection is a major risk factor for cirrhosis in the U.S., it is possible that there is an interaction/confounding between these two variables in attribution of risk. Similar to previous U.S. case-control studies, the frequency

of HBV infection was very low and there was therefore no association of HBV with ICC in this population.(5,6)

Our data showed that metformin use is associated with a 60% reduction in ICC risk in diabetic patients, a magnitude comparable to that of shown in other cancers (50–85% risk reduction) including HCC, pancreatic, colorectal, breast and lung cancer.(15–20) This is biologically plausible as shown by *in vivo* and *in vitro* experiments demonstrating anti-tumor effects of metformin in breast and prostate cancer cells due to activation of adenosine monophosphate-activated protein kinase (AMPK) which suppresses the activity of the mammalian Target Of Rapamycin/Ribosomal protein S6 kinase beta-1 (mTOR/S6K1).(28,29) Whether metformin also has an effect on malignant cholangiocytes is currently unknown.

It can be argued that the protective effects of metformin against ICC shown in this study may be due to differences in the baseline characteristics of diabetic patients treated with metformin compared to those not treated with metformin, i.e., metformin is a marker of lesser duration or less severe stage of DM, leading to a lower prevalence of ICC among patients with shorter duration or less severe DM. Since metformin is usually the initial therapy given when DM is diagnosed and is typically an effective treatment only for those who do not have very high serum glucose levels, it is possible that patients treated with metformin in our cohort had less severe DM than those who were not treated with metformin.(30) Nevertheless, metformin can be used, regardless of the duration of DM, in combination with other oral hypoglycemic agents or insulin in severe diabetic patients or in those whose target serum glucose level cannot be achieved with metformin treatment alone.(30) In our ICC cohort patients with DM, the median duration of DM was not different between those treated with metformin and not treated with metformin. We recognize that the duration of DM does not directly correlate with the severity of disease and that HbA1C or the presence of complications from DM (e.g. diabetic nephropathy or retinopathy) better reflect the severity of disease. These variables were not abstracted from the medical record as this was not in the scope of the present study. It will be interesting to explore the effect of severity of DM on ICC risk in future studies and investigate whether patients with mild DM have a lower risk for ICC than those with more severe DM.

Somewhat surprisingly, we found that hyperlipidemia was associated with a decreased risk for ICC. Since the association between hyperlipemia and ICC risk has never been reported, additional validation studies are needed before we can conclude that this association is real. If this association is true, it may either be a causal relationship, or simply reflect that the absence of hyperlipidemia is a marker of an occult cancer, i.e. the lower frequency of hyperlipidemia in ICC patients could possibly be due to cancer related fat malabsorption, a decrease in caloric intake and weight loss during cancer growth, or a decrease in lipid synthesis on account of impairment in liver function. To prove causality, further studies on the mechanistic effect of hyperlipidemia on ICC pathogenesis are also needed.

The mechanistic functions of statins on cancer have not been completely elucidated. Statins appear to have pleiotrophic effects which can either increase or decrease cancer risk.(31) In this study, we did not find an association between statin use and ICC risk among patients with hyperlipidemia. Additional studies are warranted to validate this finding.

In contrast to recently published results, we did not find an association between the metabolic syndrome and ICC.(14) This discrepancy may be related to the short duration of the diagnosis of metabolic syndrome and the small numbers of subjects with NASH in our cohorts. The link between metabolic syndrome and ICC risk may be related to the progression from steatohepatitis to fibrosis during the natural history of NASH. In our

cohorts, the mean durations of metabolic syndrome were only 6.5 and 8.3 years in the case and control groups, respectively. This duration may not have been long enough for the development of liver fibrosis.

The major strength of our study was the diagnosis of ICC in all patients was confirmed by histological and radiological results. We used prospective collection of data from a patient questionnaire and the method of data collection was comparable between the case and control groups. Our study had a large sample size and confirmed the associations between DM and smoking and ICC. Importantly, our study revealed the novel observation of inverse association between metformin use and the risk of ICC. There were two main limitations of our study. First, the time periods during which cases and controls were assessed were not matched, in order to allow us to include the largest number of ICC cases as possible, as this is a relatively uncommon cancer. However, this limitation was mitigated by examining whether there were any changing trends in the prevalence of each variable over time and accounting for these trends in our analysis and by the sensitivity analyses. Second, as most patients in the case group were referred to our institution after the diagnosis of ICC, detailed information on baseline BMI prior to ICC diagnosis, the duration of underlying diseases, and medication use were not always available. Therefore, our findings should be further validated in independent cohorts. In particular, the protective effect of metformin should ideally be studied in patients known to be taking metformin in the period at least 2–5 years prior to the development of ICC. In addition, the prevalence of NASH in the case group might be underestimated since we did not have data on serum transaminase enzyme levels prior to ICC diagnosis in most cases. However, the prevalence of NASH in the control group (2.0%) was consistent with that in the general population (3–5%).(32)

In conclusion, our findings are consistent with previous reports of associations between diabetes, smoking, and ICC. The novel observation of inverse association between metformin use and ICC risk found in our study warrants further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

ICC	Intrahepatic cholangiocarcinoma
AOR	Adjusted odds ratio
OR	Odds ratio
CI	Confidence interval
PSC	Primary sclerosing cholangitis
HBV	Hepatitis B virus
HCV	Hepatitis C virus
DM	Diabetes mellitus
HCC	Hepatocellular carcinoma
NASH	Nonalcoholic steatohepatitis

References

1. Patel T. Increasing incidence and mortality of primary intrahepatic cholangiocarcinoma in the United States. Hepatology. 2001; 33:1353–1357. [PubMed: 11391522]
2. Shaib YH, Davila JA, McGlynn K, El-Serag HB. Rising incidence of intrahepatic cholangiocarcinoma in the United States: a true increase? J Hepatol. 2004; 40:472–477. [PubMed: 15123362]
3. Yang JD, Kim B, Sanderson SO, Sauver JS, Yawn BP, Larson JJ, et al. Biliary tract cancers in Olmsted County, Minnesota, 1976–2008. Am J Gastroenterology. 2012; 107:1256–62.
4. Lee TY, Lee SS, Jung SW, Jeon SH, Yun SC, Oh HC, et al. Hepatitis B virus infection and intrahepatic cholangiocarcinoma in Korea: a case-control study. Am J Gastroenterol. 2008; 103:1716–1720. [PubMed: 18557716]
5. 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; 128:620–626. [PubMed: 15765398]
6. Shaib YH, El-Serag HB, Nooka AK, Thomas M, Brown TD, Patt YZ, et al. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma: a hospital-based case-control study. Am J Gastroenterol. 2007; 102:1016–1021. [PubMed: 17324130]
7. Welzel TM, Graubard BI, El-Serag HB, Shaib YH, Hsing AW, Davila JA, et al. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma in the United States: a population-based case-control study. Clin Gastroenterol Hepatol. 2007; 5:1221–1228. [PubMed: 17689296]
8. Grainge MJ, West J, Solaymani-Dodaran M, Aithal GP, Card TR. The antecedents of biliary cancer: a primary care case-control study in the United Kingdom. Br J Cancer. 2009; 100:178–180. [PubMed: 19018260]
9. Zhou YM, Yin ZF, Yang JM, Li B, Shao WY, Xu F, et al. Risk factors for intrahepatic cholangiocarcinoma: a case-control study in China. World J Gastroenterol. 2008; 14:632–635. [PubMed: 18203300]
10. Yamamoto S, Kubo S, Hai S, Uenishi T, Yamamoto T, Shuto T, et al. Hepatitis C virus infection as a likely etiology of intrahepatic cholangiocarcinoma. Cancer Sci. 2004; 95:592–595. [PubMed: 15245596]
11. Parkin DM, Srivatanakul P, Khlat M, Chenvidhya D, Chotiwan P, Insiripong S, et al. Liver cancer in Thailand. I. A case-control study of cholangiocarcinoma. Int J Cancer. 1991; 48:323–328. [PubMed: 1645697]
12. Donato F, Gelatti U, Tagger A, Favret M, Ribero ML, Callea F, et al. Intrahepatic cholangiocarcinoma and hepatitis C and B virus infection, alcohol intake, and hepatolithiasis: a case-control study in Italy. Cancer Causes Control. 2001; 12:959–964. [PubMed: 11808716]
13. McCullough AJ. Epidemiology of the metabolic syndrome in the USA. J Dig Dis. 2011; 12:333–340. [PubMed: 21091931]
14. Welzel TM, Graubard BI, Zeuzem S, El-Serag HB, Davila JA, McGlynn KA. Metabolic syndrome increases the risk of primary liver cancer in the United States: a study in the SEER-Medicare database. Hepatology. 2011; 54:463–471. [PubMed: 21538440]
15. Donadon V, Balbi M, Mas MD, Casarin P, Zanette G. Metformin and reduced risk of hepatocellular carcinoma in diabetic patients with chronic liver disease. Liver Int. 2010; 30:750–758. [PubMed: 20331505]
16. Lee MS, Hsu CC, Wahlqvist ML, Tsai HN, Chang YH, Huang YC. Type 2 diabetes increases and metformin reduces total, colorectal, liver and pancreatic cancer incidences in Taiwanese: a representative population prospective cohort study of 800,000 individuals. BMC cancer. 2011; 11:20. [PubMed: 21241523]
17. Li D, Yeung SC, Hassan MM, Konopleva M, Abbruzzese JL. Antidiabetic therapies affect risk of pancreatic cancer. Gastroenterology. 2009; 137:482–488. [PubMed: 19375425]
18. Hassan MM, Curley SA, Li D, Kaseb A, Davila M, Abdalla EK, et al. Association of diabetes duration and diabetes treatment with the risk of hepatocellular carcinoma. Cancer. 2010; 116:1938–1946. [PubMed: 20166205]

19. Lai SW, Chen PC, Liao KF, Muo CH, Lin CC, Sung FC. Risk of hepatocellular carcinoma in diabetic patients and risk reduction associated with anti-diabetic therapy: a population-based cohort study. Am J Gastroenterol. 2012; 107:46–52. [PubMed: 22085817]
20. Lee JH, Kim TI, Jeon SM, Hong SP, Cheon JH, Kim WH. The effects of metformin on the survival of colorectal cancer patients with diabetes mellitus. Int J Cancer. 2012; 131:752–759. [PubMed: 21913184]
21. El-Serag HB, Johnson ML, Hachem C, Morgana RO. Statins are associated with a reduced risk of hepatocellular carcinoma in a large cohort of patients with diabetes. Gastroenterology. 2009; 136:1601–1608. [PubMed: 19208359]
22. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation. 2005; 112:2735–2752. [PubMed: 16157765]
23. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977; 33:159–174. [PubMed: 843571]
24. The National Health and Nutrition Examination Survey (NHANES). Analytic and Reporting Guidelines. 2006. (www.cdc.gov/.../nhanes/nhanes.../nhanes_analytic_guidelines_dec_2005.pdf)
25. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, et al. Diabetes and cancer: a consensus report. Diabetes Care. 2010; 33:1674–1685. [PubMed: 20587728]
26. Alvaro D, Barbaro B, Franchitto A, Onori P, Glaser SS, Alpini G, et al. Estrogens and insulin-like growth factor 1 modulate neoplastic cell growth in human cholangiocarcinoma. Am J Pathol. 2006; 169:877–888. [PubMed: 16936263]
27. Yang JD, Roberts LR. Hepatocellular carcinoma: a global view. Nat Rev Gastroenterol Hepatol. 2010; 7:448–458. [PubMed: 20628345]
28. Zakikhani M, Dowling R, Fantus IG, Sonenberg N, Pollak M. Metformin is an AMP kinase-dependent growth inhibitor for breast cancer cells. Cancer Res. 2006; 66:10269–10273. [PubMed: 17062558]
29. Ben Sahra I, Laurent K, Loubat A, Giorgetti-Peraldi S, Colosetti P, Auberger P, et al. The antidiabetic drug metformin exerts an antitumoral effect in vitro and in vivo through a decrease of cyclin D1 level. Oncogene. 2008; 27:3576–3586. [PubMed: 18212742]
30. Standards of medical care in diabetes--2011. Diabetes Care. 2011; 34 (Suppl 1):S11–61. [PubMed: 21193625]
31. Gonyeau MJ, Yuen DW. A clinical review of statins and cancer: helpful or harmful? Pharmacotherapy. 2010; 30:177–194. [PubMed: 20099992]
32. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther. 2011; 34:274–285. [PubMed: 21623852]

Table 1

Baseline characteristics of ICC cases and controls *

	ICC cases (n=612)	Controls (n=594)	*P* value
Age, year (mean ± SD, range)	61.2 ± 13.1 (20–92)	61.6 ± 12.9 (21–92)	0.60
Male	308 (50.3%)	291 (49.0%)	0.64
White	448 (94.3%)	565 (96.4%)	0.1
Biliary tract diseases †	74 (12.1%)	1 (0.2%)	< 0.001
PSC	60 (9.8%)	1 (0.2%)	< 0.001
Choledochal cyst	11 (1.8%)	0 (0.0%)	< 0.001
Hepatolithiasis	4 (0.7%)	0 (0.0%)	0.02
Cirrhosis	42 (6.9%)	2 (0.3%)	< 0.001
HCV infection	13 (2.1%)	2 (0.3%)	0.02
HBV infection	3 (0.5%)	3 (0.5%)	0.97
Other comorbidities			
Obesity	191 (31.2%)	205 (34.6%)	0.21
Hyperlipidemia	165 (27.0%)	256 (43.1%)	< 0.001
Metabolic syndrome	140 (22.9%)	142 (23.9%)	0.67
DM	105 (17.2%)	34(5.7%)	< 0.001
NASH	7 (1.1%)	12 (2.0%)	0.22
Smoking status			0.001
Ever smoker	308 (53.0%)	255 (43.7%)	
Never smoker	273 (47.0%)	329 (56.3%)	
Family history of liver cancer	4 (0.7%)	3 (0.5%)	0.73
Statin use among hyperlipidemic patients	72 of 165 (43.6%)	165 of 256 (64.5%)	< 0.001
Metformin use among diabetic patients	26 of 105 (24.8%)	22 of 34 (64.7%)	< 0.001

* All data except for age are shown in number (%).

† One ICC case had both choledochal cyst and hepatolithiasis.

ICC: Intrahepatic cholangiocarcinoma, PSC: Primary sclerosing cholangitis, HCV: Hepatitis C virus, HBV: Hepatitis B virus, DM: Diabetes mellitus, NASH: Nonalcoholic steatohepatitis

Table 2

Multivariate logistic regression analysis of potential risk factors for ICC: Sensitivity analysis of All ICC cases (n=612) versus controls (n=594) (A)* and case groups 3 and 4 ICC (n=279) versus controls (n=594) (B)*

	(A) All ICC cases (n=612)			(B) Case groups 3 and 4 (n=279)		
Risk factor	**AOR**	**95% CI**	***P* value**	**AOR**	**95% CI**	***P* value**
Biliary tract diseases	81.8	11.2–598.8	<0.001	96.0	12.7–724.4	<0.001
Cirrhosis	8.0	1.8–36.5	0.007	11.3	2.4–53.4	0.002
DM	3.6	2.3–5.5	<0.001	3.1	1.8–5.3	<0.001
HCV infection	2.6	0.5–13.5	0.25	4.6	0.8–16.4	0.08
Ever smoker	1.6	1.3–2.1	<0.001	1.5	1.1–2.1	0.01
Hyperlipidemia	0.4	0.3–0.6	<0.001	0.4	0.3–0.6	<0.001

* Model included age, gender and ethnicity

ICC: Intrahepatic cholangiocarcinoma, DM: Diabetes mellitus, HCV: Hepatitis C virus, AOR: Adjusted odds ratio, 95%CI: 95% Confidence interval

Hepatology. Author manuscript; available in PMC 2014 February 01.

Table 3

Univariate analyses of the association between statin use and ICC risk among hyperlipidemic ICC cases (number indicated in table) versus hyperlipidemic controls (n=256) (A)* and between metformin use and ICC risk among diabetic ICC cases (number indicated in table) versus diabetic controls (n=34) (B)†

(A)

Hyperlipidemia	**All ICC cases (n=165)**			**Groups 3 and 4 ICC cases (n=77)**			**Group 4 ICC cases (n=28)**		
Statin use	OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value
Yes	0.6	0.4–0.9	0.03	0.9	0.5–1.6	0.81	1.2	0.5–2.7	0.73
No	1.0	reference		1.0	reference		1.0	reference	

(B)

DM	**All ICC cases (n=105)**			**Groups 2, 3 and 4 ICC cases (n=79)**			**Groups 3 and 4 ICC cases (n=46)**			**Group 4 ICC cases (n=20)**		
Metformin use	**OR**	**95% CI**	***P* value**	**OR**	**95% CI**	***P* value**	**OR**	**95% CI**	***P* value**	**OR**	**95% CI**	***P* value**
Yes	0.2	0.1–0.4	<0.001	0.4	0.2–0.9	0.04	0.4	0.1–0.9	0.04	0.3	0.1–0.96	0.047
No	1.0	reference		1.0	reference		1.0	reference		1.0	reference	

*Model included propensity scores for statin use to account for change in the frequency of use of statins over the study period.

†The frequency of metformin use was balanced using the sampling weights method. The weights assigned to each patient are specific to metformin and are not appropriate for the other variables in the multivariate analyses.

ICC: Intrahepatic cholangiocarcinoma, DM: Diabetes mellitus, OR: Odds ratio, 95%CI: 95% confidence interval