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Serum Lipid Levels and the Risk of Biliary Tract Cancers and Biliary Stones: A Population-based Study in China

Gabriella Andreotti[1,*], **Jinbo Chen**[2], **Yu-Tang Gao**[3], **Asif Rashid**[4], **Shih-Chen Chang**[5], **Ming-Chang Shen**[6], **Bing-Sheng Wang**[7], **Tian-Quan Han**[8], **Bai-He Zhang**[9], **Kim N. Danforth**[1], **Michelle D. Althuis**[10], and **Ann W. Hsing**[1]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, DHHS, Bethesda, MD, USA

[2]Department of Epidemiology and Biostatistics, University of Pennsylvania, Philadelphia, PA, USA

[3]Department of Epidemiology, Shanghai Cancer Institute, Shanghai, China

[4]Department of Pathology, M.D. Anderson Cancer Center, Houston, TX, USA

[5]Department of Discovery Medicine and Epidemiology, AstraZeneca, Wilmington, DE, USA

[6]Shanghai Tumor Hospital, Fudan University, Shanghai, China

[7]Zhongshan Hospital, Fudan University, Shanghai, China

[8]Department of Surgery, Ruijin Hospital, Second Medical University, Shanghai, China

[9]Institute of Oriental Hepatobiliary Surgery, Second Military University, Shanghai, China

[10]Previously with Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, DHHS, Bethesda, MD, USA

Abstract

Biliary tract cancers, encompassing the gallbladder, extrahepatic bile ducts, and ampulla of Vater, are rare, but highly fatal malignancies. Gallstones, the predominant risk factor for biliary cancers, are linked with hyperlipidemia. As part of a population-based case-control study conducted in Shanghai, China, we examined the associations of serum lipid levels with biliary stones and cancers. We included 460 biliary cancer cases (264 gallbladder, 141 extrahepatic bile duct, and 55 ampulla of Vater), 981 biliary stone cases, and 858 healthy individuals randomly selected from the population. Participants completed an in-person interview and gave overnight fasting blood samples. Participants in the highest quintile of triglycerides (≥ 160 mg/dl) had a 1.4-fold risk of biliary stones (95% CI=1.1-1.9), a 1.9-fold risk of gallbladder cancer (95% CI=1.3-2.8), and a 4.8-fold risk of bile duct cancer (95% CI=2.8-8.1), compared to the reference group (third quintile: 90-124 mg/dl). Participants in the lowest quintile of high-density lipoprotein (HDL) (< 30 mg/dl) had a 4.2-fold risk of biliary stones (95% CI=3.0-6.0), an 11.6-fold risk of gallbladder cancer (95% CI=7.3-18.5), and a 16.8-fold risk of bile duct cancer (95% CI=9.1-30.9), relative to the reference group (third quintile: 40-49 mg/dl). In addition, total cholesterol, low-density lipoprotein (LDL) and apolipoprotein A (apo A) were inversely associated with biliary stones; whereas low levels as well as high levels of total cholesterol, LDL, apo A, and apolipoprotein B (apo B) were associated with excess risks of biliary tract cancers. Our findings support a role for serum lipids in gallstone development and biliary carcinogenesis.

[*]**Correspondence to:** Gabriella Andreotti, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd., EPS 8011, MSC 7240, Bethesda, MD 20892. Fax: 301-402-1819; andreotg@mail.nih.gov

Keywords

serum lipid levels; gallstones; biliary tract cancer

Introduction

Biliary tract cancers, consisting of tumors of the gallbladder, extrahepatic bile ducts, and ampulla of Vater, are rare, but highly fatal malignancies.[1,2] Gallstones are the most important known risk factor for all three biliary tract cancer subsites. Specifically, cholesterol gallstones (composed mainly of cholesterol) and mixed gallstones (composed of both cholesterol and bilirubin) are most strongly associated with gallbladder cancer.[2,3] Furthermore, cholesterol gallstones and gallbladder cancer share a number of putative risk factors, including obesity, high fat diet, and hyperlipidemia.[2,4]

Hyperlipidemia, is generally characterized by high serum levels of total cholesterol, triglycerides, low-density-lipoprotein (LDL), and low levels of high-density-lipoprotein (HDL). High triglycerides and low HDL have been most consistently associated with gallstones, whereas the associations of total cholesterol and LDL with gallstones are less consistent.[5,6,7] Cholesterol gallstone pathogenesis involves cholesterol saturation of the bile duct due to the hypersecretion of cholesterol from the liver;[8] however, the exact role of individual lipids, lipoproteins, and apolipoproteins is less clear.

Given the relationship between hyperlipidemia and gallstones and the strong link between gallstones and biliary tract cancers, it is plausible that serum lipids, particularly hyperlipidemia, may be associated with biliary tract cancers. To clarify further the role of serum lipids in biliary tract cancer etiology, we examined the effect of serum lipids (total cholesterol, triglycerides), lipoproteins (HDL, LDL), and apolipoproteins (apolipoprotein A (apo A), apolipoprotein B (apo B)) on the risks of biliary tract cancers and stones, and also assess whether lipids are related with biliary tract cancers through their association with gallstones, in a population-based case-control study in Shanghai, China.

Methods

Study Subjects

Details of the study methods have been previously reported elsewhere.[3,9,10] Briefly, cancer cases were identified by a rapid reporting system established by the Shanghai Cancer Institute (SCI) and 42 collaborating hospitals in Shanghai. Through this system we identified more than 95% of all incident biliary tract cancer cases (ICD9 code 156) diagnosed among Shanghai residents between June 1997 and May 2001. A total of 627 incident biliary tract cancer cases (368 gallbladder, 191 extrahepatic bile duct, and 68 ampulla of Vater cases), between 35-74 years of age, were included in this study. In order to evaluate the risk of biliary stones independently of biliary tract cancer, 1,037 hospital patients with biliary stones (774 gallstones and 263 extrahepatic bile duct stones), without a history of cancer, were included and were frequency-matched to cancer cases on age (5-year groups), gender, and diagnosing hospital. Population controls, without a history of biliary tract cancer, and with or without biliary stones, were randomly selected from the Shanghai Resident Registry that includes records of approximately 6 million Shanghai residents. We included a total of 959 population controls who were frequency-matched to cancer cases on age (5-year groups) and gender. Participation rates among eligible cancer patients and control subjects were 95% and 82%, respectively. All study subjects provided written informed consent, and the study protocol was approved by the Institutional Review Boards of the National Cancer Institute and SCI.

Case Confirmation

Biliary tract cancer and biliary stone diagnoses were confirmed by a panel of clinicians, ultrasonographers and pathologists. Seventy percent of biliary tract cancer cases were confirmed by histopathologic assesment, while the remaining 30% of cases, for whom we did not have histopathologic material, were confirmed using medical records, surgical reports, and imaging data, including magnetic resonance imaging (MRI), endoscopic retrograde cholangiopancreatography (ERCP), or computed tomography (CT). Biliary stone cases were confirmed by review of abdominal ultrasound, ERCP films, medical records, and surgical records, or by pathologic material for those who underwent a cholecystectomy.

Gallstone Assessment

Gallstone status was assessed for all biliary tract cancer cases and population controls. Specifically, gallstones were identified among cancer cases by self-reported history, surgical reports, imaging results (MRI, ERCP, CT), or ultrasound; and among population controls by self-reported history, and abdominal ultrasound (85% consented to the ultrasound).

Interview

Information on demographic characteristics, medical histories, and lifestyle factors was obtained through in-person interview conducted by trained interviewers, using a structured questionnaire. Cases were interviewed within 3 weeks of diagnosis. At interview, weight, height, and waist and hip circumferences were measured. All interviews were taped and verified in order to assure accuracy of interview protocol and coding. The response rate for interviews was over 95% among cases and 82% among controls. Five percent of the study subjects were randomly selected for re-interview three months after the initial interview, and the concordance of responses to key questions between the original and follow-up interviews was greater than 90%.

Blood Collection and Serum Lipid Assays

Overnight fasting blood samples were collected from over 80% of the participants who gave consent. Within 4 hours of collection, samples were transported to the central processing laboratory at the SCI for processing. Serum levels of total cholesterol, triglycerides, HDL, LDL, apo A and apo B were measured for all subjects who donated overnight fasting blood samples. Measurements were conducted at the Laboratory of Biochemistry, Institute of Cardiovascular Diseases, Zhongshan Hospital, Shanghai Medical University (Fudan University), using the following assays: 1) Total cholesterol: oxidation enzymatic assay using a cholesterol test kit prepared by Shanghai No.18 Pharmaceutical Company and spectrophotometer type 722;11 2) Triglycerides: glycerol phosphoricacid oxidase assay using a triglyceride kit prepared by the Shanghai Biochemical Company and spectrophotometer type 722;11 3) HDL: phosphotungstate-magnesium assay with the agent and instrument prepared and manufactured by the same companies as the total cholesterol assay;12 4) Apo A and apo B: immunoturbidimetric assay prepared by Shanghai Technology Company and ultraviolet spectrophotometer type 754;13 and 5) LDL was not directly measured but calculated by the Friedewald formula.[14, 15] Spectrophotometers were manufactured by the Shanghai Third Analysis Instrument Company. The sensitivity, specificity, and coefficient of variation of the lipid level assays were evaluated and were found to be satisfactory.

Statistical Analysis

The final analysis of this study included subjects for whom we had serum lipid level measurements (460 biliary tract cancer cases [264 gallbladder, 141 extrahepatic bile duct, 55 ampulla of Vater], 981 biliary tract stone cases [729 gallbladder, 252 bile duct], and 858

healthy control subjects). In order to make the appropriate case-control comparisons, gallbladder cancer cases were compared to controls without a history of cholecystectomy (n=803), bile duct cancer cases and ampulla of Vater cancer cases were compared to all controls, and biliary stone cases were compared to controls without biliary stones (n=653). Distributions of selected characteristics (gender, age group, education status, cigarette smoking status, alcohol drinking status, body mass index (BMI), waist-hip-ratio (WHR), hypertension, diabetes, and gallstone status (among cancer cases) were evaluated among cases and controls; characteristics with statistically significant different distributions between cases and controls (Fishers exact $p<0.05$) were considered potential confounding factors.

The risks of biliary tract stones and cancers in relation to each serum lipid (total cholesterol, triglycerides), lipoprotein (HDL, LDL), and apolipoprotein (apo A, apo B) (henceforth collectively referred to as "lipids") were assessed by computing the odds ratios (ORs) and 95% confidence intervals (95% CIs) using unconditional logistic regression. Lipid levels were categorized into quintiles based on distributions of the control participants. The middle category of every lipid was used as the reference category, since it most closely represents the normal range in healthy populations.[16,17] For all lipids, quintile distributions were similar for each control group (all controls, control without cholecystectomy, controls without stones), and therefore, the same lipid level categories were used for each case-control comparison. For the analysis of ampulla of Vater cancer, the two bottom and two highest quintiles were combined, resulting in three lipid level categories, due to the small number of cases. Initially, risk estimates were adjusted for age group and sex, but were further adjusted for BMI, WHR, cigarette smoking status, alcohol drinking, hypertension, diabetes and gallstone status (cancer risk only) in order to evaluate potential confounding by these factors. Although gallstones may be an intermediate step in the causal pathway between lipids and biliary tract cancer, it is possible that lipids may affect cancer risk independent of stones, especially for bile duct and ampulla of Vater cancers, which have a much lower prevalence of stones than gallbladder cancer, thus we examined the association of lipids and biliary tract cancer after adjustment for biliary stones. In addition, since the above mentioned factors may interact with serum lipids, we also assessed stratum-specific risks, and used the likelihood ratio test to formally test for multiplicative interactions between the factors and lipid levels on stone and cancer risk.

In order to assess the potential disease effects of biliary stones and cancers on serum lipid levels, we examined the impact of advanced biliary disease on the associations of serum lipid levels with stone and cancer risk. We calculated stratum-specific risk estimates by several indicators of advanced biliary disease. For biliary stone risk, we examined the impact of chronic cholestasis indicated by jaundice (absent, present), weight change (loss (≥ 5 lbs), no change (+/− 4 lbs), gain (≥ 5 lbs)), and diet change (no, yes). For biliary cancer risk, we also examined the effects of chronic cholestasis indicated by jaundice and weight change, as well as clinical stage (stage 1-3, stage 4-5), histologic grade (grade 1-2, grade 3), tumor size (< 6 cm, ≥ 6 cm), and metastasis (absent, present). We also compared the risks of biliary stones and cancers among all cases to the risks after excluding cases with possible advanced disease (indicated by jaundice, weight loss, diet change, high cancer stage and grade, large tumor size and metastasis).

RESULTS

Selected characteristics of study subjects are shown in Table 1. Compared to controls without stones, biliary stone cases were younger, less likely to drink alcohol and to have hypertension, but more likely to be overweight (BMI>=25 kg/m^2), have a high WHR (>0.92), have lost weight within the last 5 years (≥ 5 lbs), have a change in diet within the

last 5 years and have diabetes. Gallbladder cancer cases were also predominantly women (72.3%), while bile duct cancer cases were predominantly men (60%). All three biliary cancer subsites were more likely to have a higher WHR (>0.92), lost weight within the last 5 years, and have gallstones compared to controls. Bile duct and ampulla of Vater cancer cases were more likely to have ever smoked cigarettes, but less likely to have hypertension, while only gallbladder cancer cases were more likely to be overweight and have diabetes compared to controls.

Mean levels of serum lipids in cases and controls are presented in Table 2. Compared to controls, biliary stone cases had significantly lower mean levels of total cholesterol, HDL, LDL, and apo A, but a significantly higher mean level of triglycerides. Cases of all three cancer subsites had significantly higher mean levels of triglycerides and lower mean levels of HDL than controls. Mean levels of total cholesterol, LDL, apo A, and apo B varied by subsite. For example, mean levels of total cholesterol and LDL were significantly lower among gallbladder cancer cases, but significantly higher among bile duct cancer than controls, and not significantly differ between ampulla of Vater cancer cases and controls. These associations did not differ considerably between men and women.

The adjusted risks of biliary stones and cancers in relation to serum lipid levels are shown in Table 3. In order to account for potential confounding, biliary stone and cancer risk estimates were adjusted for age group and gender. Risk estimates were also calculated separately by several demographic and lifestyle factors, but none of the factors modified any of the associations, thus, risk estimates are presented for all subjects combined. As shown in Table 3, biliary stones were positively associated with triglycerides and inversely associated with HDL. For triglycerides, relative to subjects in the reference category (third quintile: 90-124 mg/dl), those in the lowest quintile (<70 mg/dl) had a 51% reduced risk of biliary stones (95% CI=0.35-0.68), while those in highest quintile (≥160 mg/dl) had a 43% increased risk (95% CI=1.08-1.90). For HDL, relative to subjects in the reference category (third quintile: 40-49 mg/dl), those in the lowest quintile (< 30 mg/dl) had a 4.2-fold risk of biliary stones (95% CI=2.98-6.01), while those in the highest quintile (≥ 58 mg/dl) had a 56% lower risk (95% CI=0.30-0.64). In addition, total cholesterol, LDL, and apo A were inversely associated with biliary stones. There was no association between apo B and biliary stones. The associations between serum lipids and biliary stones were independent of other lipids examined, and were not measurably changed after further adjustment for BMI, WHR, hypertension, diabetes, cigarette smoking, and alcohol drinking, nor were they considerably changed after excluding cases with advanced disease.

The risk of biliary tract cancers in relation to serum lipid levels did not differ greatly by cancer subsite. All three cancer subsites were positively associated with triglycerides and inversely associated with HDL level. For triglycerides, relative to subjects in the reference category (third quintile: 90-124 mg/dl), those in the highest quintile (≥ 160 mg/dl) had a 1.9-fold risk of gallbladder cancer (95% CI=1.33-2.84) and a 4.8-fold risk of bile duct cancer (95% CI=2.84-8.07). For HDL, relative to subjects in the reference category (third quintile: 40-49 mg/dl), those in the lowest quintile of HDL (< 30 mg/dl) had an 11.6-fold risk of gallbladder cancer (95% CI=7.31-18.53) and a 16.8-fold risk of bile duct cancer (95% CI=9.14-30.91). In addition, we observed excess risks of all three cancer subsites for subjects in the lowest as well as the highest categories of total cholesterol, LDL, apo A, and apo B. The associations between serum lipids and biliary tract cancers were independent of each lipid examined, and were not substantially changed after further adjustment for BMI, WHR, hypertension, diabetes, gallstones, cigarette smoking, and alcohol drinking, nor were they considerably changed after excluding cases with advanced disease. Thus, it is particularly noteworthy that serum lipids were associated with biliary tract cancers independent of their association with biliary stones.

DISCUSSION

In this population-based case-control study, high serum levels of triglycerides and low levels of HDL were significantly associated with an increased risk of biliary stones as well as excess risks of biliary tract cancers. In addition, total cholesterol, LDL and apo A were inversely associated with biliary stones. There was a U-shaped relationship between biliary tract cancers and serum levels of total cholesterol, LDL, apo A, and apo B, with excess risks for both the lowest and highest quintiles. These findings support a role for serum lipids in gallstone development and biliary carcinogenesis.

Consistent with the established relationship between hyperlipidemia and gallstones, we found that high levels of serum triglycerides and low levels of HDL and apo A were associated with excess risks of biliary stones, independently of the other lipids and risk factors we examined. Our findings are in agreement with previous cross-sectional and prospective studies of serum lipids and gallstones, which have consistently reported high triglycerides and low HDL in association with gallstone risk.[5-7,18,19]

Paradoxical to the generally accepted association between hyperlipidemia and gallstones, we observed that lower levels of total cholesterol, LDL, and apo B, characteristic of hypolipidemia, were also associated with biliary stones, independently of the other lipids and risk factors we examined. High levels of total cholesterol, LDL, and apo B were not associated with biliary stones. Results from previous studies of total cholesterol and LDL with gallstones are conflicting, with several cross-sectional studies reporting inverse,[20,21] positive,[22,23] and null[24,25] associations; while several prospective studies of gallstones reported no associations with total cholesterol and/or LDL.[6,26] Reasons for the inconsistencies across studies are unclear but may be due to differences in study design, study populations, lipid measurement methods, or inadequate control for confounding.

Since blood samples were collected shortly after diagnosis but before treatment, it is possible that lipid levels were affected by the presence of biliary stones. In order to assess this, we considered the possible effects of chronic cholestasis (bile duct obstruction due to biliary stones), which manifests as jaundice (increased serum levels of bilirubin), and is associated with increased serum levels of total cholesterol, triglycerides, and LDL, as well as decreased HDL.27 We found that the association of high triglycerides, low HDL, and low apo A with biliary stones persisted after biliary stone cases with jaundice were excluded from the analysis. We also assessed the effects of weight loss and dietary changes, which are possible consequences of biliary stones that are usually related to decreased levels of total cholesterol, triglycerides, and LDL and increased levels of HDL.28,29 However, we found that the associations of low total cholesterol and LDL remained significant after the exclusion of biliary stone cases who had a weight loss (≥ 5 lb) or dietary change within the last 5 years. In addition, we examined several symptoms that may be related to severity of gallstone disease, including stone size, number of stones, and stomach pain, but did not find that these factors modified the associations between serum lipid levels and stone risk. Together, these findings suggest that there was no substantial disease effect on serum lipids due to biliary stones in this study.

Since we found no considerable evidence for disease effects due to biliary stones, it is possible that high levels of triglycerides, and low levels of HDL and apo A, may affect the risk of biliary stones through their involvement in several key processes of gallstone pathogenesis. For example, high serum triglyceride levels are associated with increased cholesterol saturation of the bile[7,30,31] and rapid nucleation of cholesterol crystals,[32] important precursors to gallstones. Similarly, low serum levels of apo A have been linked to decreased cholesterol crystallization in the bile,[33] and low serum levels of HDL have been

associated with increased biliary cholesterol saturation and bile acid hyposecretion,[8, 30-32] which reduces cholesterol solubility in the bile and leads to gallstone formation.[34]

Although the exact role of low levels of total cholesterol and/or LDL in gallstone formation is unclear, a number of indirect observations suggest a possible inverse association between total cholesterol and gallstone pathogenesis. A higher prevalence of gallstones has been suggested among men on cholesterol-lowering diets and among subjects who experienced rapid weight loss, a behavior often correlated with lower cholesterol levels.[35,36] Therefore, our results, in conjunction with these observations, indicate the need to clarify the roles of total cholesterol, LDL and apo B in gallstone formation.

We observed that high levels of total cholesterol, triglycerides, LDL and apo B, and low levels of HDL and apo A, which are characteristic of hyperlipidemia, were associated with excess risks of biliary tract cancers, at all three subsites. These associations were independent of other lipids examined in the study and were not confounded or modified by other demographic or risk factors, including gallstones. Hyperlipidemia, especially higher levels of triglycerides and lower levels of HDL, has been reported in several other malignancies, including tumors of the colon, breast and prostate,[37-39] and has been implicated in gallbladder cancer etiology, due to its close relationships with gallstones, obesity, high-fat diet, and diabetes[40,41] which are also linked to gallbladder cancer.[2,4]

In contrast to the hyperlipidemic effects of total cholesterol, LDL, apo A, and apo B on biliary tract cancer risk, we also observed excess risks of all three subsites of biliary tract cancers at low levels of total cholesterol, LDL, and apo B, and high levels of apo A, independent of the other lipids and risk factors, including gallstones. Previous studies, both cross-sectional,[42,43] and prospective[44,45] have reported inverse associations of total cholesterol and/or LDL with several types of cancers; however, they were unable to rule out the possibility that the lower lipid levels were due to the effects of cancer. Decreases in total cholesterol and/or LDL have been associated with late-stage cancer, but have also been suggested to manifest as much as 5 years prior to cancer diagnosis.[46]

As with biliary stones, reverse causation due to the potential effects of biliary tumors had to be considered. Biliary tumors, like biliary stones, can cause chronic cholestasis due to bile duct obstruction, which presents with jaundice and is associated with increased serum levels of total cholesterol, triglycerides and LDL, and decreased HDL.[27] In our study, the excess risks of gallbladder cancer in relation with high triglycerides and low HDL persisted after exclusion of gallbladder cancer cases with jaundice. We were unable to assess whether the risks of bile duct and ampulla of Vater cancers were affected by jaundice, since over 85% of these cancer cases had jaundice at the time of diagnosis. We also considered the possible effects of cancer cachexia, which is characterized by severe weight loss at late stages of cancer, and is generally linked to low levels of total cholesterol and or LDL.[46] We found no evidence that the effects of low total cholesterol, LDL and apo B on biliary tract cancers were due to the late-stage cancer, as there was no measurable difference in risk after subjects with weight loss, advanced tumor stage, high histologic grade, large tumor size, and metastasis were excluded from the analysis. However, we cannot completely rule out the possibility that the associations of low total cholesterol, LDL, and apo B were due to the effects of cancer, since minor decreases in these lipids may have occurred prior to the cancer diagnosis, as has been suggested by previous prospective studies.

The mechanism by which hyperlipidemia may be involved in biliary tract cancer is unclear. Since, the associations between serum lipids and biliary tract cancers were statistically independent of gallstones, pathways other than those related to gallstones may also be involved in biliary carcinogenesis. Inflammation, independent of or in conjunction with

gallstones, is a likely mechanism, since proinflammatory cytokines, such as tumor necrosis factor (TNF-α) and interleukins (IL-1, IL-6) have been expressed in gallbladder[47] and/or bile duct cancer tissue,[48,49] and since most of the identified risk factors for biliary tract cancers, including hyperlipidemia and diabetes,[40,41] are linked to inflammation.[2] Also, increased levels of total cholesterol, triglycerides, and LDL and decreased levels of HDL and apo A have been associated with increased circulating levels of proinflammatory cytokines, including TNF-α, IL-1, and IL-6.[50,51] In addition, high LDL levels are associated with increased oxidized LDL, which is linked to an increase in reactive oxygen species.[52,53] Reactive oxygen species cause DNA damage, activate oncogenes, and inactivate tumor suppressor genes,[54] all of which have been found to play a role in carcinogenesis, including biliary cancer.[55] Together, these data suggest that high serum levels of total cholesterol, triglycerides, and LDL and low levels of HDL may play a role in biliary tract carcinogenesis through their lithogenic and inflammatory properties.

It is of interest that high levels of triglycerides and low levels of HDL as well as low levels of total cholesterol, LDL, apo A, and apo B had similar effects on biliary stones and biliary tract cancer risk, while high levels of total cholesterol, LDL, apo A and apo B were only associated with biliary tract cancers but not with biliary stones. Although high levels of triglycerides and low levels of HDL were associated with both biliary stones and biliary tract cancers, and despite the strong association between biliary stones and biliary tract cancers, biliary stones did not appear to be an intermediate factor between serum lipids and biliary tract cancers, since the association between these two lipids and biliary tract cancers persisted among subjects without stones. This finding suggests that serum lipids may play a role in biliary tract cancer risk independent of biliary stones. Further supporting this observation, we also found that high levels of total cholesterol, LDL, apo A and apo B were only associated with biliary tract cancers and not with biliary stones.

Several strengths of this population-based study should be noted: 1) selection bias was minimal due to the population-based design and very high case ascertainment and high response rates in cases and controls; 2) misclassification of cancer and stone cases was also minimal due to the detailed review of pathology and clinical data; 3) the nearly complete assessment of gallstone status among cancer cases and controls allowed for the assessment of cancer risk while controlling for gallstone status; and 4) measurement error of serum lipids was minimal given the high sensitivity and specificity, and small coefficient of variation of the lipid level assays.

Our study has several limitations: 1) despite our rigorous effects, about 30% of the cases did not have histological confirmation largely due to the nature of that biliary tract cancer are usually diagnosed at late-stage; however, a clinical panel carefully reviewed medical records, surgical reports and imaging data to improve the confirmation of cases without pathological material; 2) since blood was collected shortly after enrollment, we were unable to assess the temporal relationship between serum lipid levels and stone and cancer development, and were unable to completely rule out reverse causation due to potential disease effects; 3) we were unable to assess biliary lipid levels due to the invasiveness of the procedure for obtaining bile, especially for control subjects; 4) we had no information on cholesterol-lowering medication use, which could have caused a decrease in serum lipid levels in subjects taking these medications. It is possible that more biliary stone and cancer cases than controls could have been taking lipid-lowering medications; however, after excluding subjects with a history of high cholesterol, heart disease, and hypertension, subjects who would most likely be taking lipid-lowering medications, we found no substantial change in risk for biliary stones or cancers, suggesting minimal impact of lipid-lowering drugs on the association between serum lipid levels and biliary stones and cancers in our study; and 5) although this is the largest study of biliary tract cancers to date, we had

limited statistical power to evaluate ampulla of Vater cancer risk, and to test for interactions between serum lipids and other factors.

In conclusion, this population-based study showed that high serum levels of triglycerides and low levels of HDL and apo A were associated with excess risks of biliary stones as well as biliary tract cancer at all three subsites. In addition high levels, as well as low levels, of total cholesterol, LDL, and apo B were associated with excess risks of biliary tract cancer. Our findings suggest that hyperlipidemia may be associated with biliary tract cancers independently of gallstones. Future prospective studies are needed to confirm these results and investigate the individual and combined effects of serum lipids, gallstones, and other risk factors on the development of biliary tract cancers.

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Table 1

Selected characteristics of subjects by case-control status

Selected Characteristics	Controls		Biliary Stones[1]		Biliary Tract Cancers					
					Gallbladder[2]		Bile Duct[3]		Ampulla of Vater[3]	
	n	%	n	%	n	%	n	%	n	%
All subjects	858	(100.0)	981	(100.0)	264	(100.0)	141	(100.0)	55	(100.0)
Gender										
Male	333	(38.8)	369	(37.6)	72	(27.3)	83	(58.9)	30	(54.5)
Female	525	(61.2)	612	(62.4)	192	(72.7*)	58	(41.1*)	25	(45.5*)
Age at Interview (years)										
34-54	114	(13.3)	285	(29.0)	37	(14.0)	22	(15.6)	5	(9.1)
55-64	247	(28.8)	284	(29.0)	64	(24.2)	37	(26.2)	13	(23.6)
65-75	497	(57.9)	412	(42.0*)	163	(61.8)	82	(58.2)	37	(67.3)
Education										
Elementary	352	(41.0)	302	(30.8)	141	(53.4)	55	(39.0)	24	(43.6)
High school	381	(44.4)	509	(51.9)	98	(37.1)	65	(46.1)	26	(47.3)
Above high school	125	(14.6)	170	(17.3*)	25	(9.5*)	21	(14.9)	5	(9.1)
Ever Smoked Cigarettes [4]										
No	600	(69.9)	713	(72.7)	195	(74.1)	79	(56.0)	30	(54.5)
Yes	258	(30.1)	268	(27.3)	68	(25.9)	62	(44.0*)	25	(45.5*)
Ever Drank Alcohol										
No	681	(79.4)	823	(84.0)	223	(84.5)	97	(68.8)	41	(74.5)
Yes	177	(20.6)	157	(16.0*)	41	(15.5)	44	(31.2*)	14	(25.5)
Body Mass Index [5] **(kg/m^2)**										
<18.5	73	(8.5)	45	(4.5)	13	(4.9)	6	(4.3)	1	(1.8)
18.5-22.9	354	(41.3)	320	(32.6)	89	(33.7)	61	(43.3)	23	(41.8)
23.0-24.9	171	(19.9)	241	(24.6)	53	(20.0)	36	(25.5)	12	(21.8)
25.0-29.9	224	(26.1)	341	(34.8)	98	(37.1)	35	(24.8)	19	(34.6)
≥30	36	(4.2)	34	(3.5*)	11	(4.2*)	3	(2.1)	0	(0.0)

Selected Characteristics	Controls		Biliary Stones[1]		Biliary Tract Cancers					
					Gallbladder[2]		Bile Duct[3]		Ampulla of Vater[3]	
	n	%	n	%	n	%	n	%	n	%
Waist Circumference [6] (cm)										
<74.0	166	(19.3)	149	(15.2)	34	(12.9)	36	(25.5)	17	(30.9)
74.0-79.9	156	(18.2)	205	(20.9)	48	(18.2)	28	(19.9)	11	(20.0)
80.0-84.9	175	(20.4)	229	(23.3)	48	(18.2)	27	(19.1)	10	(18.2)
85.0-89.9	172	(20.1)	191	(19.5)	48	(18.2)	12	(8.5)	6	(10.9)
≥90.0	189	(22.0)	207	(21.1*)	86	(32.6*)	38	(27.0*)	11	(20.0)
Hip Circumerence [6] (cm)										
<90.0	165	(19.2)	278	(28.3)	84	(31.8)	62	(44.0)	25	(45.4)
90.0-93.9	167	(19.5)	194	(19.9)	41	(15.5)	26	(18.4)	12	(21.8)
94.0-98.9	169	(19.7)	214	(21.8)	38	(14.4)	17	(12.1)	9	(16.4)
99.0-103.9	181	(21.1)	175	(17.8)	34	(12.9)	4	(2.8)	4	(7.3)
≥104.0	176	(20.5)	120	(12.2*)	67	(25.4*)	32	(22.7*)	5	(9.1)
Waist-to-Hip Ratio [6]										
<0.80	165	(19.2)	118	(12.0)	21	(8.0)	11	(7.8)	7	(12.7)
0.80-0.82	154	(17.9)	102	(10.4)	16	(6.0*)	10	(7.1)	4	(7.3)
0.83-0.86	187	(21.9)	217	(22.1)	53	(20.1)	22	(15.6)	10	(18.2)
0.87-0.90	196	(22.8)	241	(24.6)	63	(23.9)	32	(22.7)	12	(21.8)
≥0.91	156	(18.2)	303	(30.9*)	111	(42.0*)	66	(46.8*)	22	(40.0*)
Weight change [7]										
No Change (+/− 4 lbs)	330	(38.8)	305	(31.3)	83	(32.4)	36	(25.9)	10	(18.9)
Gained weight (≥ 5lbs)	395	(46.4)	332	(34.0)	31	(12.1)	15	(10.8)	8	(15.1)
Lost (≤ 5lbs)	126	(14.8)	339	(34.7*)	142	(55.5*)	88	(63.3*)	35	(66.0*)
Lost < 5% of weight	11	(8.7)	36	(10.6)	13	(9.2)	2	(2.3)	2	(5.7)
Lost ≥ 5% of weight	115	(91.3)	303	(89.4)	129	(90.8)	86	(97.7)	33	(94.3)
Diet Change [8]										
No	594	(69.2)	540	(55.0)	178	(67.4)	104	(73.8)	40	(72.7)

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Selected Characteristics	Controls		Biliary Stones[1]		Biliary Tract Cancers					
					Gallbladder[2]		Bile Duct[3]		Ampulla of Vater[3]	
	n	%	n	%	n	%	n	%	n	%
Yes	264	(30.8)	441	(45.0*)	86	(32.6)	37	(26.2)	15	(27.3)
Hypertension										
No	498	(58.0)	660	(67.3)	169	(64.0)	98	(69.5)	41	(74.5)
Yes	360	(42.0)	321	(32.7*)	95	(36.0)	43	(30.5*)	14	(25.5*)
Diabetes										
No	788	(91.8)	872	(89.0)	229	(87.1)	129	(91.5)	52	(94.5)
Yes	70	(8.2)	108	(11.0*)	34	(12.9*)	12	(8.5)	3	(5.5)
Gallstone Status										
No	653	(76.1)	0	(0.0)	40	(15.2)	47	(33.3)	26	(47.3)
Yes	205	(23.9)	981	(100.0)	224	(84.8*)	94	(66.7*)	29	(52.7*)

Note: Total number of subjects may vary because of missing values

[1] Biliary stone cases include gallstone and bile duct stones. Biliary stone cases compared with controls without biliary stones (n=653)

[2] Gallbadder cancer cases compared with population controls who had a gallbladder (n=803)

[3] Bile duct and ampulla of Vater cancer cases compared to all population controls (n=858)

[4] Ever smoked cigarettes for at least 6 consecutive months

[5] Body mass index = weight/(height^2); distribution based on WHO classification for obesity among Asians

[6] Cutoffs for waist and hip circumference, and waist-to-hip-ratio based on quintiles among population controls

[7] Weight change = weight 5 years prior interview - weight at interview; percent weight change=((weight change)/weight 5 years prior interview)*100

[8] Self-reported diet change during 5 years prior to interview

* $p<0.05$ for Fishcer's exact test for difference between cases and controls

Table 2

Adjusted[1] mean levels of serum lipid levels by case-control status

						Biliary Tract Cancers							
	Controls (n=858)		Biliary Stones[2] (n=981)			Gallbladder[3] (n=264)			Bile Duct[3] (n=141)			Ampulla of Vater[4] (n=55)		
Lipids (mg/dl)	Mean	(SE)	Mean	(SE)	p^5	Mean	(SE)	p^5	Mean	(SE)	p^5	Mean	(SE)	p^5
Total Cholesterol	182.3	(1.9)	170.9	(1.4)	*<.0001*	165.4	(3.7)	*<.0001*	193.1	(4.5)	*0.03*	187.4	(8.1)	*0.54*
Triglycerides	107.3	(2.3)	127.4	(2.2)	*<.0001*	130.8	(5.4)	*<.0001*	178.8	(9.3)	*<.0001*	156.9	(15.7)	*0.0002*
HDL	43.9	(0.5)	37.4	(0.4)	*<.0001*	32.8	(1.0)	*<.0001*	28.8	(1.3)	*<.0001*	32.2	(2.5)	*<.0001*
LDL	108.2	(1.8)	101.5	(1.4)	*0.001*	100.5	(3.5)	*0.05*	121.4	(4.3)	*0.01*	114.6	(7.8)	*0.42*
Apo A	113.9	(0.9)	109.5	(0.7)	*<.0001*	106.1	(1.7)	*<.0001*	102.6	(2.3)	*<.0001*	110.0	(4.0)	*0.34*
Apo B	85.8	(0.9)	85.1	(0.7)	*0.63*	88.6	(1.8)	*0.19*	106.1	(2.2)	*<.0001*	96.2	(4.0)	*0.01*

[1] Adjusted for age group and gender

[2] Biliary stone cases include gallstone and bile duct stones. Biliary stone cases compared with controls without biliary stones (n=653)

[3] Gallbadder cancer cases compared with population controls who had a gallbladder (n=803)

[4] Bile duct and ampulla of Vater cancer cases compared to all population controls (n=858)

[5] Omnibus null hypothesis that mean lipid levels between cases and controls are equal

Table 3

Odds ratios (ORs) and 95% confidence intervals (CIs) for biliary tract stones and cancers in relation to serum lipid levels

Lipid Level[1] (mg/dl)	Controls	Biliary Stones[2]			Gallbladder Cancer[3]			Bile Duct Cancer[4]		
	n	n	OR[5]	(95% CI)[5]	n	OR[5]	(95% CI)[5]	n	OR[5]	(95% CI)[5]
TOTAL	**858**	**981**	-	-	**264**	-	-	**141**	-	-
Total Cholesterol										
<160	228	425	1.92	(1.41-2.61)	153	4.03	(2.52-6.43)	61	2.84	(1.50-5.38)
160-179	189	182	0.89	(0.64-1.24)	25	0.76	(0.42-1.37)	24	1.43	(0.70-2.92)
180-199	155	154	**1.00**	**reference**	27	**1.00**	**reference**	13	**1.00**	**reference**
200-239	212	157	0.70	(0.50-0.97)	25	0.62	(0.34-1.11)	17	1.04	(0.49-2.22)
≥240	74	63	0.84	(0.54-1.31)	34	2.38	(1.32-4.29)	26	4.92	(2.36-10.25)
P omnibus[6]				*<.0001*			*<.0001*			*<.0001*
Triglyceride										
<70	160	106	0.49	(0.35-0.68)	29	0.72	(0.44-1.12)	10	0.61	(0.28-1.34)
70-89	147	118	0.65	(0.47-0.89)	30	0.77	(0.47-1.25)	9	0.64	(0.29-1.45)
90-124	228	275	**1.00**	**reference**	62	**1.00**	**reference**	21	**1.00**	**reference**
125-159	137	185	1.26	(0.92-1.72)	47	1.34	(0.86-2.09)	18	1.52	(0.78-2.98)
≥160	186	297	1.43	(1.08-1.90)	96	1.94	(1.33-2.84)	83	4.79	(2.84-8.07)
P omnibus[6]				*<.0001*			*<.0001*			*<.0001*
HDL										
<35	82	425	4.23	(2.98-6.01)	162	11.63	(7.31-18.53)	102	16.81	(9.14-30.91)
35-41	214	237	1.93	(1.46-2.54)	50	3.11	(2.02-4.78)	14	2.59	(1.37-4.89)
42-49	256	165	**1.00**	**reference**	32	**1.00**	**reference**	14	**1.00**	**reference**
50-57	173	77	0.57	(0.41-0.80)	12	0.52	(0.27-1.00)	7	0.40	(0.34-1.99)
≥58	133	77	0.44	(0.30-0.64)	8	0.27	(0.11-0.65)	3	0.30	(0.11-1.40)
P omnibus[6]				*<.0001*			*<.0001*			*<.0001*
LDL										
<80	166	294	1.77	(1.33-2.37)	100	4.17	(2.68-6.47)	50	2.37	(1.43-3.95)
80-109	275	305	1.06	(0.81-1.38)	81	2.00	(1.29-3.10)	29	0.86	(0.49-1.49)
110-139	236	245	**1.00**	**reference**	35	**1.00**	**reference**	28	**1.00**	**reference**

Lipid Level[1] (mg/dl)	Controls	Ampulla of Vater Cancer[4]		
	n	n	OR[5]	(95% CI)[5]
	858	**55**	-	-
Total Cholesterol				
<180	417	34	2.34	(0.89-6.11)
180-199	155	5	**1.00**	**reference**
≥200	286	16	1.82	(0.65-5.08)
P omnibus[6]				*0.02*
Triglyceride				
<90	307	7	0.38	(0.15-0.98)
90-124	228	13	**1.00**	**reference**
≥125	323	35	1.91	(0.98-3.71)
P omnibus[6]				*<.0001*
HDL				
<42	333	47	9.74	(2.99-31.73)
42-49	215	3	**1.00**	**reference**
≥50	310	5	1.20	(0.28-5.08)
P omnibus[6]				*<.0001*
LDL				
<80	441	32	2.10	(0.95-4.64)
80-109	236	8	**1.00**	**reference**

Lipid Level[1] (mg/dl)	Controls	Biliary Stones[2]			Gallbladder Cancer[3]			Bile Duct Cancer[4]			Lipid Level[1] (mg/dl)	Controls	Ampulla of Vater Cancer[4]		
	n	n	OR[5]	(95% CI)[5]	n	OR[5]	(95% CI)[5]	n	OR[5]	(95% CI)[5]		n	n	OR[5]	(95% CI)[5]
140-169	123	76	0.55	(0.38-0.78)	16	0.81	(0.43-1.53)	13	1.01	(0.50-2.04)	≥110	180	15	2.77	(1.14-6.75)
≥170	57	55	0.91	(0.59-1.42)	32	3.48	(1.97-6.15)	21	3.64	(1.90-6.97)					
P omnibus [6]				*<.0001*			*<.0001*			*<.0001*	*P omnibus* [6]				*0.01*
Apo A											**Apo A**				
<100	126	251	2.32	(1.73-3.12)	98	5.12	(3.44-7.62)	65	8.87	(5.09-15.45)					
100-114	281	350	1.45	(1.14-1.85)	86	1.87	(1.29-2.73)	32	1.92	(1.06-3.46)	<115	407	41	3.61	(1.73-7.55)
115-129	337	296	**1.00**	**reference**	56	**1.00**	**reference**	19	**1.00**	**reference**	115-129	337	9	**1.00**	**reference**
130-144	73	52	0.87	(0.57-1.32)	10	0.81	(0.39-1.68)	9	2.50	(1.08-5.79)	≥130	114	5	1.75	(0.57-5.37)
≥145	41	32	0.96	(0.56-1.63)	14	2.13	(1.08-4.23)	16	7.69	(3.63-16.32)					
P omnibus [6]				*<.0001*			*<.0001*			*<.0001*	*P omnibus* [6]				*0.001*
Apo B											**Apo B**				
<60	42	70	1.42	(0.90-2.25)	26	2.74	(1.55-4.85)	7	1.72	(0.70-4.24)					
60-74	197	223	0.95	(0.72-1.25)	64	1.25	(0.85-1.84)	21	1.13	(0.62-2.06)	<75	239	17	1.72	(0.80-3.68)
75-89	295	336	**1.00**	**reference**	75	**1.00**	**reference**	27	**1.00**	**reference**	75-89	295	12	**1.00**	**reference**
90-109	240	258	0.88	(0.68-1.14)	58	0.93	(0.63-1.37)	31	1.48	(0.85-2.56)	≥90	324	26	2.07	(1.02-4.19)
≥110	84	94	1.09	(0.75-1.60)	41	1.87	(1.18-2.96)	55	7.43	(4.38-12.63)					
P omnibus [6]				*<.0001*			*0.001*			*<.0001*	*P omnibus* [6]				*0.0002*

[1] Categories based on quintiles among population controls; lowest and highest categories combined for ampulla of Vater cancer

[2] Biliary stones include gallstones and bile duct stones. Biliary stone cases compared with controls who did not have biliary stones (n=653)

[3] Gallbadder cancer cases compared with population controls who had a gallbladder (n=803)

[4] Bile duct and ampulla of Vater cancer cases compared with all population controls (n=858)

[5] Adjusted for age and gender

[6] Omnibus null hypothesis that odds ratios are all equal to 1