Total Cholesterol, Alanine Aminotransferase And the Risk of Primary Liver Cancer: A Population-based prospective study

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Research

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Abstract

Background: Previous studies have shown that serum total cholesterol(TC) and serum alanine aminotransferase(ALT) were associated with liver cancer risk, respectively. However, the common contribution of TC and normal-high ALT to primary liver cancer(PLC) has not been reported to date. We aim to assess the separate and joint effect of low TC level and normal-high ALT level on the risk of PLC, a large prospective cohort was conducted in our study.

Methods: The participants were divided into 4 groups via cross-matching method according to TC[low level(-)/ non-low level(+)] and ALT[normal level(-)/ normal-high level(+)] status, and using the lower quartile(P_{25}) value(4.24 mmol/L) of TC and the upper quartile(P_{75}) value(22 U/L) of ALT as a threshold, respectively. Incident PLC was confirmed by review of medical records. Cox proportional hazards regression models and interactive additive models were used to evaluate whether the joint effect of low TC level and normal-high ALT level is associated with the risk of PLC.

Results: During 1,248,895 person-years follow-up, 298 participants were diagnosed with PLC among 114,972 subjects. TC≤4.24 mmol/L for the “TC(-)” group; TC≥4.24 mmol/L for the “TC(+)” group; ALT≤22U/L for the “ALT(-)” group; ALT≥22U/L for the “ALT(+)”. Compared with the “TC(+)” group, “ALT(-)” group, respectively, the adjusted hazard ratio(HR) and 95% confidence interval(95%CI) for PLC risk was 1.71 (1.34-2.19) in “TC(-)” group and for PLC risk was 1.52 (1.18-1.95) in “ALT(+)” group. In combinatorial analysis, compared with “TC(+) and ALT(-)” group, the significant increased risk of PLC were observed in “TC(+) and ALT(+)” group (HR=1.45; 95%CI: 1.07-1.97), “TC(-) and ALT(-)” group(HR=1.64; 95%CI: 1.21-2.22) and “TC(-) and ALT(+)” group(HR=2.70; 95%CI: 1.84-3.96), respectively. The interaction between “TC(-)” and “ALT (+)” on the risk of PLC was not significant($P_{interaction}$=0.26).

Conclusions: Both low TC level and normal-high ALT level were risk factors of PLC. There is the separate and joint effect of low TC level and normal-high ALT level on the risk of PLC.

Trial registration: ChiCTR-TNRC-11001489. Registered August 24, 2011 (retrospectively registered)

Background

Liver cancer, a heavy disease burden worldwide, is one of malignant tumors which causes serious harm to human life and health. According to the cancer data produced by the International Agency for Research on Cancer, liver cancer was predicted to be the sixth most commonly diagnosed cancer and the fourth leading cause of cancer death around the world [1]. Particularly, the study estimated that annually half of new cases occur in China and the incidence of liver cancer has been on the rise [2]. In the past decade, the age-standardized incidence of liver cancer has increased from 8.1 per 100,000 person-years[3] to 13.9 per 100,000 person-years[1] worldwide. Although some new progress has been made in the treatment of liver cancer in the past 30 years, it still remains the poor outcome for advanced liver cancer[4]. Positively seeking and avoiding the risk factors for liver cancer are the most effective approaches to decrease the liver cancer risk.

Currently, the aging[5], male[6], obesity[7], elevated fasting plasma glucose[8], and especially infection of HBV[9] and HCV[9] are well-established risk factors for development of liver cancer, respectively. Additionally, the dyslipidemia and inflammation related to chronic liver damage are also associated with the incidence of liver cancer[10]. Study has suggested that the deregulation of cholesterol homeostasis could affect the cancer development[11]. The observational studies from Japan[12] and Korea[13] found that low total cholesterol(TC) level was associated with an increased risk of liver cancer. Wen *et al*[14]. by using risk prediction model indicated that transaminase was best able to predict liver cancer risk. As we know, elevated alanine aminotransferase is one of most markers for hepatocyte injury and necrosis[15]. Although some researches have confirmed the close association between obviously elevated alanine aminotransferase(ALT) level and the increased liver cancer risk [16, 17], the normal-high ALT level remains unclear for liver cancer risk to date.

Previous studies only assessed the role of low TC alone or elevated ALT alone in the risk of liver cancer, and none of them had investigated the combined effect of both with the risk of primary liver cancer(PLC). Based on Kailuan study, the aim of current research is to examine the separate and joint effects of low TC level and normal-high ALT level on the risk of PLC.

Methods

Research Design and Participants

The Kailuan study is a prospective cohort study based on community population in Tangshan city, northern China (Trial Registration Number: ChiCTR-TNRC-11001489) [18,19]. Since 2006, the employees(≥18 years, including the retired) of the Kailuan Group, Tangshan City, were invited to participate in biennial health check-up at 11 affiliated hospitals. The Kailuan Study was conducted to estimate the prevalence chronic disease, nutritional disorders and major risk factors for these diseases. The details of the study design and procedures are available elsewhere[18,19]. From 2006 to 2007, 101,510 participants completed the survey, which constituted Kailuan Study I. From 2008 to 2009, 2010 to 2011, both 25,337 adults and 10,519 adults formed the Kailuan Study II and Kailuan Study III, respectively. And all participants(137,366) underwent questionnaire survey, clinical and laboratory examinations.

In our current study, we excluded 463 subjects who had PLC and a history of malignant tumors at the baseline, excluded 1,830 and 490 subjects with missing information of TC and ALT, respectively. According to the adult standard of American College of Gastroenterology(ACG)[20], 19,611 subjects with abnormal data of ALT were excluded (male serum ALT >33U/L or female serum ALT >25U/L). A total of 114,972 individuals were finally included in the current analyses(Fig 1).This study was approved by Ethics Committee of Kailuan General Hospital and in compliance with the Declaration of Helsinki. Informed consent was obtained from the participants.

Assessment of Exposure Factor and Other Related Laboratory

At 7:00-9:00 a.m., the fasting (8h-12h) elbow venous blood of all participants was collected about 5 ml and placed in a vacuum tube that containing EDTA. The upper serum was taken after centrifugating for 10 minutes at 3000 rotations per minute at 24℃. The serum samples were assured to complete the detection within 4 hours. Serum TC and serum ALT were determined by professional laboratory physicians using an autoanalyzer(Hitachi 747; Hitachi, Tokyo, Japan) and strictly following the instructions of reagents. TC was measured enzymatically(CHOD-PAP) with an upper limit of detection of 20.68 mmol/L. ALT(ALT, in U/L) was measured by enzymatic rate methed. The upper limit of detection of ALT was 1000 U/L. Other biochemical parameters, including serum high-density lipoprotein cholesterol(HDL-C), triglyceride(TG), hemoglobin(HGB), fasting blood glucose(FBG), hypersensitive C-reactive protein(hs-CRP) were determined by automatic biochemical analyzer(Hitachi 747; Hitachi, Tokyo, Japan). All plasma samples were analyzed at the central laboratory of Kailuan General Hospital.

Assessment of Other Relevant Variables

On the day of physical examination, the trained medical and nursing personnel would assist the participants to fill in the questionnaires via face-to-face interviews. The information of the questionnaire included: age, gender, smoking habits, drinking status, physical activity, past medical history and so on(eg, Hypertension, diabetes mellitus, malignant tumors, etc.)[21,22]. Height and weight were measured by professionally trained staff. BMI was calculated as body weight(kg) divided by the square of height(m^2). Hypertension was defined as systolic blood pressure ≥140mmHg, and/or diastolic blood pressure ≥90mmHg, or using antihypertensive medication. Diabetes was defined as FBG ≥7.0mmol/L or use of oral hypoglycemic agent. Smoking was defined as having smoked at least 1 cigarette per day on average for at least 1 year. Alcohol consumption was defined as having taken alcohol of 100mL/day(alcohol contents>50%) of alcohol for more than 1 year. Physical activity was defined as taking exercises more than four times a week, each time lasting at least 30 minutes[23]. Ultrasound diagnosis standard of fatty liver: Comparing the liver echogenicity with the kidney, the diffuse echo enhancement in liver, image of intrahepatic blood vessels and the diaphragm was blurry or invisible[24]. The diagnostic criterias of cirrhosis in ultrasound: the coarse tissue and nodularity in liver surface or parenchyma, with or without ascites and splenomegaly; or subjects with medical history of cirrhosis[24, 25].

Definition and Ascertainment of Outcome Events

During the period from participants' first physical examination to December 31, 2018, subjects which were first diagnosed with hepatocellular carcinoma, intrahepatic cholangiocarcinoma and other liver cancers with unclear types(excluding liver metastasis), we defined as PLC. Follow-up began at the first physical examination, and ended at occurrence of cancer, death, or December 31, 2018, whichever event came first. In our cohort, cancer events were confirmed via biennially health screening with face-to-face questionnaires and medical examinations. Additionally, medical records from Municipal Medical Service System(including medical insurance system and social security system) were checked yearly in detail to obtain outcome information of participants that may have been missed[8]. The outcome information was

collected by professionally trained staff, and the CanReg 4.0 software that provided by the International Agency for Research on Cancer of the World Health Organization (IARC/WHO) was used to input and logically verify about new cases of LC. According to the International Classification of Diseases, Tenth Revision(ICD-10), and PLC was defined as C22.

Statistical analysis

Participants were divided into 4 groups according to TC(low level/ non-low level) and ALT(normal level/ normal-high level) status and using the lower quartile(P_{25}) value(4.24 mmol/L) of TC and the upper quartile(P_{75}) value(22 U/L) of ALT as a threshold, respectively. Low TC level was defined as TC less than its P_{25} value as “TC(-)” group; non-low TC level was defined as TC greater than or equal to its P_{25} value as “TC(+)” group. Normal ALT level was defined as ALT less than its P_{75} value as “ALT(-)” group; normal-high ALT level was defined as ALT greater than or equal to its P_{75} value as “ALT(+)” group[26,27]. Four groups were obtained as follows via cross-matching method: “TC (-) +ALT (+)”, “TC (-) +ALT (-)”, “TC (+) +ALT (+)” and “TC (+) +ALT (-)”. Quantitative data with normal distribution was expressed as mean±standard deviation, one-way analysis of variance was used for multiple comparison between groups. The measurement data with skewed distribution were described as M(P_{25}-P_{75}), the nonparametric Kruskal-Wallis test of variance was used for multiple comparison between groups. Categorical variables were described by percentage and compared using the Chi-square test. Incidence rates were calculated by dividing the number of events by person years of follow up in each group. To investigate the joint effect of TC and ALT for PLC, three dummy variables were included in the models, and “TC(+) + ALT(-)” with minimum incidence in all groups was used as reference group. The Cox proportional hazards model was used to estimate the hazard ratios(HRs) and 95% confidence intervals(CIs) for the separate and joint effect of TC and ALT on PLC. Furthermore, interactive additive model was constructed to further test the joint effect of TC and ALT for PLC risk. We calculated the relative excess risk due to interaction(RERI), proportion of disease attributable to interaction(AP), synergy index(SI) and P value for interaction [28,29].

As sensitivity analyses, we further excluded 2,488 HBsAg positive participants, 231 participants in cirrhosis, 31,567 fatty liver participants, 38 participants who took statins, 11,127 ALT ≥40U/L participants during follow-up, and 13 participants who occurred PLC within 1 year after entry to the cohort, respectively. And the Cox proportional hazards model was repeated again. The data management and all analyses were conducted using SAS statistical software, version 9.4(SAS Institute, Cary, NC). $P < 0.05$ was considered statistically significant for 2-sided tests.

Results

Total of 114,972 participants were included in this study with the mean age of 49.65 ± 13.68 years (males: n = 92522, 84.66%; females: n = 22450, 15.34%). The P_{25} value of TC is 4.24 mmol/L, TC < 4.24 mmol/L for “TC(-)” group; TC ≥ 4.24 mmol/L for “TC(+)” group. The P_{75} value of ALT is 22 U/L, ALT < 22 U/L for “ALT(-)” group; ALT ≥ 22 U/L for “ALT(+)” group. The general baseline characteristics of the participants according to mismarch combinations of TC and ALT status are presented in Table 1.

Table 1
Baseline Characteristics by TC and ALT Status.

Variable	TC(-) + ALT(+)	TC(-) + ALT(-)	TC(+) + ALT(+)	TC(+) + ALT(-)	F/X²	P Value
N	6,669	22,070	22,962	63,271		
Male, %	6263 (94.15%)	16584 (75.22%)	21169 (92.56%)	48506 (76.72%)	X²=3890.93	< 0.0001
Age, y	47.91 ± 14.14	48.25 ± 15.58	50.16 ± 11.96	52.29 ± 13.03	F = 642.38	< 0.0001
BMI, kg/m²	25.22 ± 3.48	23.98 ± 3.47	25.62 ± 3.30	24.63 ± 3.39	F = 932.07	< 0.0001
HDL-C, mmol/L	1.38 ± 0.61	1.39 ± 0.36	1.53 ± 0.40	1.58 ± 0.45	F = 1192.65	< 0.0001
HGB, g/L	152 (143–161)	146 (133–156)	153 (144–162)	148 (136–158)	X²=3256.49	< 0.0001
FBG, mmol/L	5.36 ± 1.49	5.19 ± 1.35	5.61 ± 1.72	5.51 ± 1.73	F = 283.36	< 0.0001
Hs-CRP, mg/L	0.85 (0.34–2.20)	0.80 (0.30–2.30)	0.96 (0.40–2.20)	0.90 (0.33–2.34)	X²=102.05	< 0.0001
TG, mmol/L	1.22 (0.85–1.96)	0.99 (0.70–1.46)	1.43 (1.05–2.15)	1.23 (0.88–1.79)	X²=4854.86	< 0.0001
TC, mmol/L	3.58 ± 0.77	3.64 ± 0.67	5.37 ± 0.97	5.31 ± 0.89	F = 28092.10	< 0.0001
ALT, U/L	25.00 (23.00–28.00)	14.00 (10.00–18.00)	25.00 (23.00–28.00)	14.00 (11.00–18.00)	X²=66124.85	< 0.0001
Fatty liver, %	2257 (34.43%)	4130 (19.38%)	9254 (41.38%)	15926 (25.97%)	X²=3006.89	< 0.0001
Hypertension, %	2734 (41.00%)	7471 (33.85%)	10935 (47.62%)	27064 (42.77%)	X²=918.07	< 0.0001
Diabetes mellitus, %	505 (7.57%)	1275 (5.78%)	2275 (9.91%)	5504 (8.70%)	X²=279.96	< 0.0001
Alcohol consumption, %	1008 (15.11%)	2488 (11.27%)	4389 (19.11%)	10447 (16.51%)	X²=550.06	< 0.0001

TC(+): TC ≥ 4.24 mmol/L, TC(-): TC < 4.24 mmol/L; ALT(+): ALT ≥ 22U/L, ALT(-): ALT < 22U/L; Hs-CRP: hypersensitive C-reactive protein; HDL-C: high-density lipoprotein cholesterol; TG: triglyceride; TC: total cholesterol; HGB: hemoglobin; FBG: fasting blood glucose; BMI: body mass index; ALT: alanine aminotransferase.

Variable	TC(-) + ALT(+)	TC(-) + ALT(-)	TC(+) + ALT(+)	TC(+) + ALT(-)	F/X^2	P Value
Smoking, %	2018 (30.26%)	5154 (23.35%)	7348 (32.00%)	17647 (27.89%)	X^2=436.15	< 0.0001
Physical activity, %	975 (14.62%)	3078 (13.95%)	3396 (14.79%)	10246 (16.19%)	X^2=76.10	< 0.0001

TC(+): TC ≥ 4.24 mmol/L, TC(-): TC < 4.24 mmol/L; ALT(+): ALT ≥ 22U/L, ALT(-): ALT < 22U/L; Hs-CRP: hypersensitive C-reactive protein; HDL-C: high-density lipoprotein cholesterol; TG: triglyceride; TC: total cholesterol; HGB: hemoglobin; FBG: fasting blood glucose; BMI: body mass index; ALT: alanine aminotransferase.

Incidence and Risk of PLC in Groups by TC and ALT Status

During total 1,248,895 person-years (average 10.86 ± 2.11 years) follow-up, 298 PLC (278 male and 20 female) occurred, and the incidence of PLC was 24 per 100,000 person-years (28 per 100,000 person-years for male and 8 per 100,000 person-years for female). “TC(+) + ALT(-)”, “TC(+) + ALT(+)”, “TC(-) + ALT(-)” and “TC(-) + ALT(+)” groups were 19 per 100,000 person-years, 28 per 100,000 person-years, 27 per 100,000 person-years and 50 per 100,000 person-years, respectively. The “TC(-) + ALT(+)” group had the highest incidence of PLC(Table 2).

Table 2
Hazard ratios and 95% confidence interval for Risk of PLC in Groups by TC and ALT Status.

	Cases	Follow-up time, person-years	Incidence rate, per 100,000 person-years	Model 1	Model 2	Model 3
TC [a] alone						
TC(+)	197	939,206	21	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
TC(-)	101	309,689	33	1.56 (1.23–1.98)	1.67 (1.32–2.13)	1.71 (1.34–2.19)
ALT [b] alone						
ALT(-)	193	927,859	21	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
ALT(+)	105	321,036	33	1.58 (1.24-2.00)	1.56 (1.23–1.99)	1.52 (1.18–1.95)
Combinations of TC and ALT [c]						
TC(+) + ALT(-)	128	690,346	19	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
TC(+) + ALT(+)	69	248,861	28	1.50 (1.12–2.01)	1.51 (1.13–2.04)	1.45 (1.07–1.97)
TC(-) + ALT(-)	65	237,513	27	1.48 (1.10-2.00)	1.62 (1.20–2.18)	1.64 (1.21–2.22)
TC (-) + ALT(+)	36	72,176	50	2.70 (1.86–3.90)	2.81 (1.93–4.07)	2.70 (1.84–3.96)
TC(+): TC ≥ 4.24 mmol/L, TC(-): TC < 4.24 mmol/L; ALT(+): ALT ≥ 22U/L, ALT(-): ALT < 22U/L. HR: Hazard ratios; CI: confidence interval; Ref: reference.						
Model 1: Univariate analysis.						
Model 2: Adjusted for age, gender.						
Model 3: [c] Adjusted for age, gender, BMI, HDL-C, hs-CRP, TG, hypertension, diabetes, alcohol consumption, smoking and physical activity; [a] Further adjusted for ALT based on [c]; [b] Further adjusted for TC based on [c].						

In the multivariable adjusted analysis, the adjusted hazard ratio(HR) and 95% confidence interval(95%CI) for the risk of PLC in “TC (-)” alone group and “ALT (+)” alone group were 1.71(1.34–2.19) and 1.52 (1.18–1.95), respectively, after adjustment for gender, age, BMI, TC, ALT, HDL-C, hs-CRP, TG, hypertension,

diabetes, alcohol consumption, smoking and physical activity. And the adjusted HR(95%CI) for PLC risk increased from 1.45 (1.07–1.97) to 1.64 (1.21–2.22) and 2.70 (1.84–3.96) in each combination group of “TC(+) + ALT(+)”, “TC(-) + ALT(-)”, “TC(-) + ALT(+)”, respectively, after adjustment for gender, age, BMI, HDL-C, hs-CRP, TG, hypertension, diabetes, alcohol consumption, smoking and physical activity(Table 2).

A strong association was observed for PLC by different TC and ALT status in male. The adjusted HR (95%CI) for the risk of PLC in “TC (-)” alone group and “ALT (+)” alone group were 1.74 (1.36–2.25) and 1.49 (1.15–1.94), respectively, after adjustment for age, BMI, TC, ALT, HDL-C, hs-CRP, TG, hypertension, diabetes, alcohol consumption, smoking and physical activity. The adjusted HR(95%CI) for PLC risk increased from 1.41 (1.02–1.95) to 1.67 (1.24–2.27) and 2.72 (1.81–4.09) in each combination group of “TC(+) + ALT(+)”, “TC(-) + ALT(-)”, “TC(-) + ALT(+)”, respectively, after adjustment for age, BMI, HDL-C, hs-CRP, TG, hypertension, diabetes, alcohol consumption, smoking and physical activity [see Additional file 1 (Table S1)]. However, no statistical significance was found among female [see Additional file 2 (Table S2)].

Interaction Between Tc And Alt For Plc

Figure 2 shows the adjusted HR(95%CI) and interaction terms for PLC in different status of TC and ALT. The results showed that there was no evidence of interaction effect between “TC(-)” and “ALT(+)”. RERI(95%CI), AP(95%CI) and SI(95%CI) were 0.61 (-0.45-1.67), 0.23 (-0.11-0.56) and 1.56 (0.73–3.33), respectively, indicating that the parameters of interaction effect between “TC(-)” and “ALT(+)” were not statistically significant($P_{interaction} > 0.05$).

Sensitivity Analysis

To further determine the stability of the results, we excluded HBsAg positive participants, participants in cirrhosis, fatty liver participants, participants who took statins, ALT ≥ 40U/L participants during follow-up and participants who occurred PLC within 1 year after entry to the cohort, respectively. We found that “TC(-) + ALT(+)” group still had a highest risk of PLC events in all models(Table 3). The results of sensitivity analyses concerning the major potential confounders cannot alter the main findings.

Table 3
Sensitivity analysis of hazard ratios and 95% confidence interval for the risk of LC in groups by TC and ALT status.

	Sensitivity Analysis I	Sensitivity Analysis II	Sensitivity Analysis III	Sensitivity Analysis IV	Sensitivity Analysis V	Sensitivity Analysis VI
TC [a] alone						
TC (+)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
TC (-)	1.45 (1.06–1.98)	1.64 (1.27–2.13)	1.82 (1.37–2.43)	1.69 (1.32–2.16)	1.72 (1.32–2.23)	1.56 (1.19–2.05)
ALT [b] alone						
ALT (-)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
ALT (+)	1.19 (0.87–1.64)	1.48 (1.14–1.92)	2.12 (1.61–2.79)	1.52 (1.18–1.95)	1.65 (1.27–2.13)	1.45 (1.11–1.90)
Combinations of TC and ALT [c]						
TC (+) + ALT (-)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
TC (+) + ALT (+)	1.18 (0.81–1.72)	1.43 (1.04–1.97)	2.03 (1.45–2.84)	1.43 (1.06–1.94)	1.59 (1.17–2.17)	1.41 (1.03–1.91)
TC (-) + ALT (-)	1.44 (1.00-2.08)	1.59 (1.16–2.19)	1.72 (1.18–2.51)	1.59 (1.17–2.16)	1.64 (1.17–2.31)	1.54 (1.12–2.10)
TC (-) + ALT (+)	1.76 (1.03-3.00)	2.50 (1.66–3.78)	4.02 (2.66–6.09)	2.71 (1.85–3.96)	2.93 (1.99–4.33)	2.50 (1.68–3.72)

TC(+): TC ≥ 4.24 mmol/L, TC(-): TC < 4.24 mmol/L; ALT(+): ALT ≥ 22U/L, ALT(-): ALT < 22U/L. HR: Hazard ratios; CI: confidence interval; Ref: reference.

Sensitivity Analysis I: Excluding HBsAg positive participants, [c] Adjusted for age, gender, BMI, HDL-C, hs-CRP, TG, hypertension, diabetes, alcohol consumption, smoking and physical activity; [a] Further adjusted for ALT based on [c]; [b] Further adjusted for TC based on [c].

Sensitivity Analysis II: Excluding participants in cirrhosis, the adjusted factors are the same as [a], [b], [c].

Sensitivity Analysis III: Excluding fatty liver participants, the adjusted factors are the same as [a], [b], [c].

Sensitivity Analysis IV: Excluding participants who took statins, the adjusted factors are the same as [a], [b], [c].

Sensitivity Analysis V: Excluding ALT ≥ 40U/L participants during follow-up, the adjusted factors are the same as [a], [b], [c].

	Sensitivity Analysis I	Sensitivity Analysis II	Sensitivity Analysis III	Sensitivity Analysis IV	Sensitivity Analysis V	Sensitivity Analysis VI
Sensitivity Analysis VI: Excluding participants who occurred liver cancer within 1 year after entry to the cohort, the adjusted factors are the same as [a], [b], [c].						

Discussion

In this study, we have confirmed previous studies that low TC level is associated with an increase risk of liver cancer. And we also found that normal-high ALT level could increase the risk of PLC. Furthermore, with significantly higher relative risk for PLC would be seen in subject who both keep low TC level and normal-high ALT level.

While the association between low TC level and the risk of liver cancer are rarely reported, our results are basically consistent with the previous studies. A large prospective study including 1,189,719 adults based on NHIC(National Health Insurance Corporation) cohort in Korea had clarified that the inverse association between the concentration of TC and incident liver cancer[13]. Additionally, Tanaka *et al*. [16] also found that low TC level(TC < 3.59 mmol/L) was significantly inversely associated with the risk of liver cancer(RR = 6.16; 95% CI: 1.39–27.35) based on data of voluntary blood donors in Japan. Our findings agree with this. In our study, we observed that low TC level(TC < 4.24 mmol/L) increased 1.71-fold risk of PLC(HR = 1.71; 95%CI: 1.34–2.19) compared with non-low TC level group. Even after excluding statins in our studies, there were no significant changes in our results (HR = 1.69; 95%CI: 1.32–2.16). Decreased TC concentration is significantly associated with the risk of PLC.

Transaminase has a strong power to predict the risk of liver cancer[14]. Although the JPHC Study(The Japan Public Health Center-based Prospective Study) has confirmed that elevated ALT would increase the risk of liver cancer(HR = 13.5; 95% CI: 8.0–22.0)[30], this slightly different from our research. Indeed, the association between normal-high ALT level and PLC risk is our focus. We found that normal-high ALT level alone increased 1.52-fold risk of PLC(HR = 1.52; 95%CI: 1.18–1.95) compared with normal ALT level after adjustment of potential confounders. This means that ALT in normal range is adverse for development of PLC. And we should paid enough attention to this phenomenon.

More importantly, by using cross-classification method, our study indicated that low TC level and normal-high ALT level have a combined effect on the risk of PLC. After adjusting confounders, we observed that combination of low TC level and normal-high ALT level showed 2.70-fold increased risk of PLC(HR = 2.70; 95%CI: 1.84–3.96) compared with combination of non-low TC level and normal ALT level. Futhermore, the joint effect of the these two factors was greater than their separate risks. To the best of our knowledge, this is the first time to prospectively evaluate the association of joint effect of low TC level and normal-high ALT level with PLC risk to date. These observations actually indicated that low TC level and normal-high ALT level had conjoint impact on PLC risk. This will remind us that in screening for early PLC, besides focusing on chronic liver diseases such as hepatitis, cirrhosis and fatty liver, dyslipidemia and slight increase of transaminase also play roles in PLC risk.

According to prior clinical and epidemiological studies, the development of liver cancer is usually accompanied with liver cirrhosis, and have probably lowered serum cholesterol level before hepatocarcinogenesis[31]. However, in sensitivity analysis, after excluding HBsAg positive participants, participants in cirrhosis, fatty liver participants, participants who used statins, ALT ≥ 40U/L participants during follow-up, respectively. We found that the results of sensitivity analysis are consistent with the overall results. This could speculate that the roles of low TC level and normal-high ALT level on the risk of PLC may be independent of chronic liver disease. In consideration of the prediagnostic PLC might influence the level of TC or ALT, thus, this result for PLC was slightly attenuated after excluding participants who occurred PLC within 1 year after entry to the cohort.

The mechanisms that low TC level and normal-high ALT level increased the risk of PLC remain uncertain. Omer F et al.[32] have reported that cancer development is associated with modulation of cholesterol homeostasis. Several carcinogenic signals, such as PI3K/AKT/mTOR, RTK/RAS and TP53, play important roles in modulating cholesterol synthesis in tumor cells[32]. Especially TP53, a key tumor suppressor, could affect the development of cancers via modulating cholesterol homeostasis[33]. Furthermore, serum ALT levels were easily available marker of chronic liver inflammation. The OhdG, a parameter of genetic risk for hepatocarcinogenesis, acts as a pro-mutagenic DNA lesion produced by oxygen(hydroxy) radicals [34–36]. Shimoda et al.[37] found that the OhdG is positively related to serum ALT levels in patients without liver cancer, and speculated that oxidative DNA damage is produced by chronic liver tissue inflammation, which would increase the risk of genomic alterations causing liver cancer.

In fact, the interventions for the development of liver cancer can be achieved. Such as effective therapy or lifestyle changes are available to reduce the incidence and mortality for high-risk individuals. Meanwhile, it should be noted that the association between correct use of cholesterol-lowering drugs and the potential health risks.

Our study has several limitations. Firstly, HCV is a known independent risk factor for PLC[38]. Around 170 million people worldwide were infected with HCV[39]. Our research lacked this information, and the risk of HCV infection affecting the risk of PLC cannot be verified. Secondly, in this study, there were still a part of potential unmeasured factors which we did not consider, such as Aflatoxin, dietary habit et al. Thirdly, serum TC and ALT levels fluctuate daily, our study needs to be measured several times to ensure the accuracy of the results. Finally, our data did not differentiate between hepatocellular carcinoma and intrahepatic cholangiocarcinoma, the risk factors of both might be different.

In conclusion, based on Kailuan Study, we have confirmed that low TC level is associated with an increase risk of liver cancer. In addition, the novel evidence was provided that normal-high ALT level is associated with the risk of PLC. And individuals with coexistence of low TC level and normal-high ALT level would have an higher risk of PLC.

Declarations

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Ethics approval and consent to participate

This research protocol was approved by Ethics Committee of Kailuan General Hospital, and it was in compliance with the Declaration of Helsinki. Informed consent was obtained from the participants.

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Conflict of interest: The authors declare that they have no conflicts of interest.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions: SMM and LSQ conceived and designed the work; WWC, LXN and WYM have performed data acquisition; SMM, CHZ and WYM have analyzed the data; SMM wrote the paper; CLY and LSQ reviewed the manuscript. All authors read and approved the final manuscript.

Consent for publication

This original research article has been submitted to this journal and is not under consideration by other publications.

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Abbreviations

TC, total cholesterol; ALT, alanine aminotransferase; PLC, primary liver cancer; ACG, American College of Gastroenterology; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; HGB, hemoglobin; FBG, fasting blood glucose; hs-CRP, hypersensitive C-reactive protein; US, Ultrasound scanning; BMI, body mass index; IARC, International Agency for Research on Cancer; WHO, World Health Organization; ICD, International Classification of Diseases; HR, hazard ratios; CI, confidence intervals; RERI, relative excess risk due to interaction; AP, proportion of disease attributable to interaction; SI, synergy index.

Figures

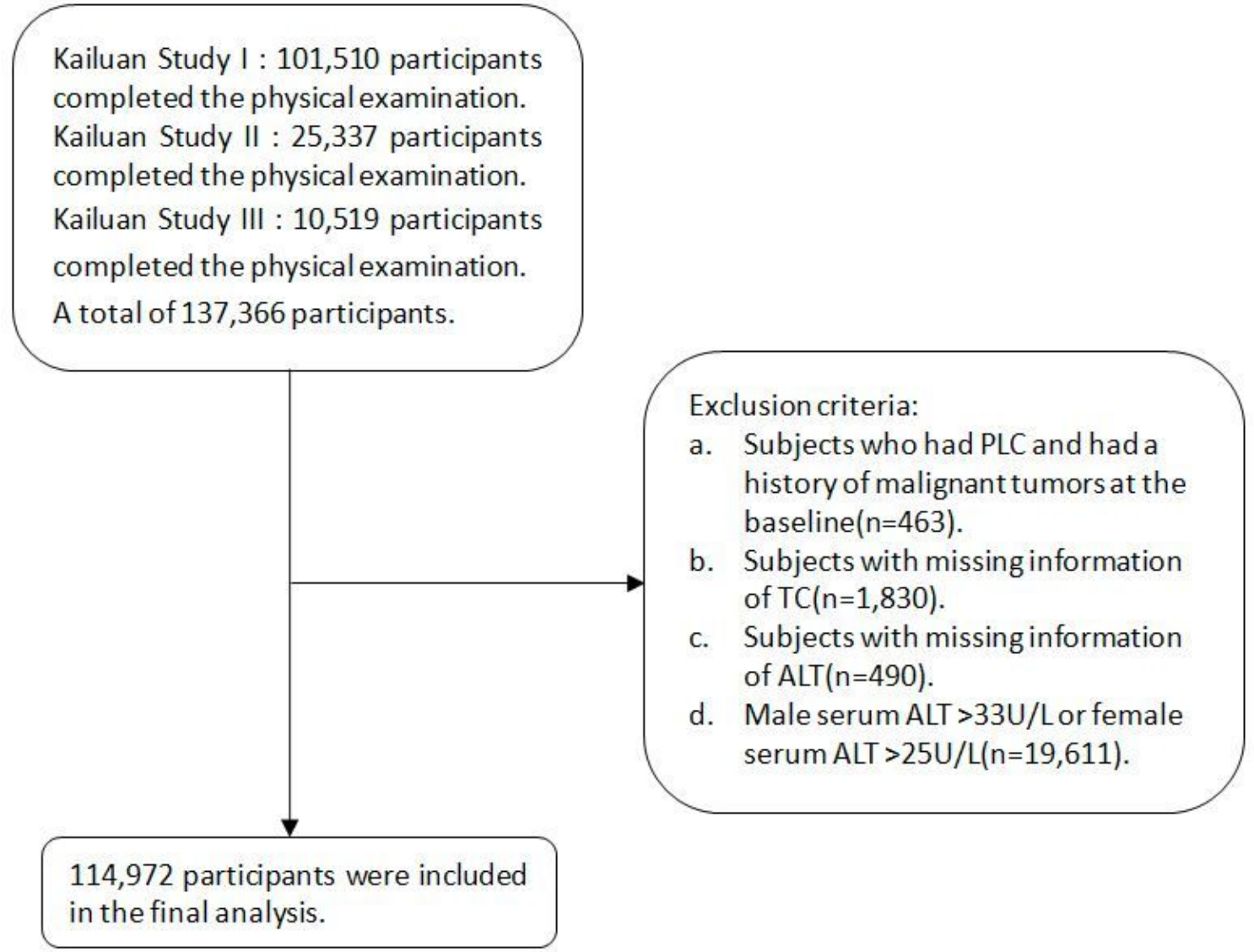

Figure 1

Flow chart of participants screening.

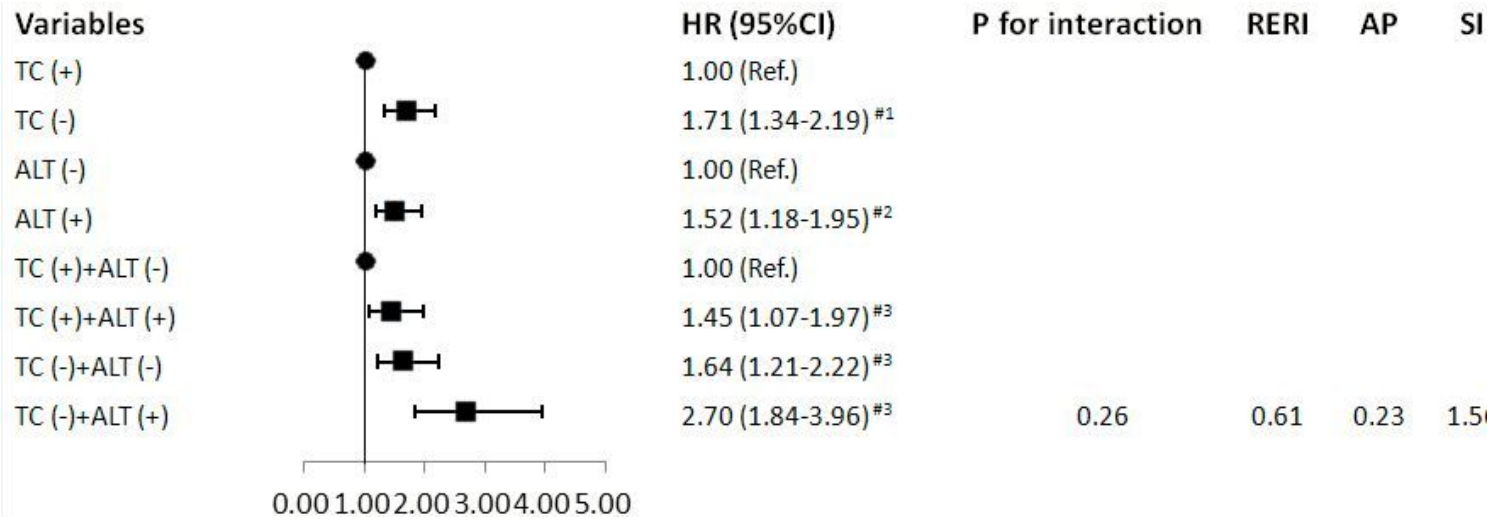

Figure 2

The adjusted HR(95%CI) and interaction terms for PLC in different status of TC and ALT. #3: Adjusted for age, gender, BMI, HDL-C, hs-CRP, TG, hypertension, diabetes, alcohol consumption, smoking and physical activity; #2: Further adjusted for TC based on #3; #1: Further adjusted for ALT based on #3. RERI: relative excess risk due to interaction; AP: proportion of disease attributable to interaction; SI: synergy index; HR: Hazard ratios; CI: confidence interval: Ref: reference.

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