Total cholesterol, alanine aminotransferase and the risk of primary liver cancer: A population-based prospective study in China

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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.

Method: The participants were divided into 4 groups via mismarch 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 cut point, 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 of 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 and “ALT(-)” group, respectively, the adjusted hazard ratio(HR) and 95% confidence interval(95%CI) of “TC(-)” group for PLC risk was 1.71 (1.34-2.19) and of “ALT(+)” group for PLC risk was 1.52 (1.18-1.95). In combinatorial analysis, compared with the group of “TC(+) with ALT(-)”, the significant increased risk of PLC were observed in “TC(+) with ALT(+) group”(HR=1.45; 95%CI: 1.07-1.97), “TC(-) with ALT(-)” group(HR=1.64; 95%CI: 1.21-2.22) and “TC(-) with ALT(+)” group(HR=2.70; 95%CI: 1.84-3.96), respectively. The interaction term 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 strong predictors of PLC. Individuals with low TC level and normal-high ALT level, would extremely increase 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 latest cancer data produced by the International Agency for Research on Cancer in 2018, liver cancer was predicted to be the sixth most commonly diagnosed cancer and the fourth leading cause of cancer death around the world, with about 841,000 new cases and 782,000 deaths for liver cancer annually [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 associated with the incidence of liver cancer[10]. Study has suggested that the deregulation of cholesterol homeostasis could lead to 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*[10], by using risk prediction model found that transaminase was best able to predict liver cancer risk. As we know, elevated alanine aminotransferase is one of most markers for hepatocyte injury, necrosis, inflammation-related cell death[14]. Although some researches have confirmed the close association between obviously elevated alanine aminotransferase(ALT) level and the increased liver cancer risk [15, 16], 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 an ongoing prospective cohort study based on functional community population in Tangshan city, northern China (Trial Registration Number: ChiCTR-TNRC-11001489) [17, 18]. Since 2006, the employees(≥ 18 years, including the retired) of the Kailuan Group, Tangshan City, were invited to participate in biennial health check-up. 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[17, 18]. 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. 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)[19], 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 (8 h-12 h) 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, and ALT(ALT, in U/L) was measured with an enzymatic rate methed with an upper limit of detection of 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). The interassay coefficient of variation for each measurement was less than 10%. All the plasma samples were analyzed at the central laboratory at Kailuan General Hospital.

Assessment of Other Relevant Variables

On the day of physical examination, the trained medical and nursing personnel would fill in the questionnaires with participants together via face-to-face interviews. The information of the questionnaire mainly included: age, gender, smoking habits, drinking status, physical activity, past medical history(eg, Hypertension, diabetes mellitus, malignant tumors, etc.)[20, 21]. 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 ≥ 140 mmHg, and/or diastolic blood pressure ≥ 90 mmHg, or using antihypertensive medication. Diabetes was defined as FBG ≥ 7.0 mmol/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 100 mL/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[22]. The diagnostic criterias of fatty liver in US(Ultrasound scanning) as follows: the hepatic echogenicity was diffuse enhancement compare with kidney; the vessels and the diaphragm of liver altered blurred; or the brilliance of posterior hepatic segments penetrated poorly and the intrahepatic vessels or the diaphragm was even invisible[23, 24]. The diagnostic criterias of cirrhosis as follows: the nodularity was detected in the surface of liver by US; the coarse tissue and nodularity were detected in the liver parenchyma with ascites or splenomegaly; or obvious collateral circulation was observed in US; or subjects with medical history of cirrhosis[23, 24].

Definition and Ascertainment of Outcome Events

During the period from participants' first physical examination to December 31, 2018, subjects which were diagnosed with hepatocellular carcinoma, intrahepatic cholangiocarcinoma and other PLC with unclear types, 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 addition, medical records from Tangshan medical insurance system and death certificates from Kailuan social security system were checked yearly to get outcome information that may have been missed[8]. This part of information is collected by professionally trained staff, and the CanReg 4.0 software 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 LC is 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 cut point, 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[25, 26]. Four groups were obtained as follows via using mismarch 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, to test the joint effect of TC and ALT for PLC risk, interactive additive model was constructed. We calculated the relative excess risk due to interaction(RERI), proportion of disease attributable to interaction(AP) and synergy index(SI). RERI or AP = 0 means no interaction; RERI or AP > 0 means positive interaction; and RERI or AP < 0 means negative interaction. SI = 1 means no interaction; SI > 1 means positive interaction; SI < 1 means negative interaction[27, 28].

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/X²	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 per participant) follow-up, 298 PLC occurred, and the incidence of PLC was 0.24 per 1000 person-years in all subjects. “TC(+) + ALT(-)”, “TC(+) + ALT(+)”, “TC(-) + ALT(-)” and “TC(-) + ALT(+)” groups were 0.19 per 1000 person-years, 0.28 per 1000 person-years, 0.27 per 1000 person-years and 0.50 per 1000 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 LC in Groups by TC and ALT Status.

	Cases	Follow-up time, person-years	Incidence rate, per 1000 person-years	Model 1	Model 2	Model 3
TC [#1] alone						
TC(+)	197	939,206	0.21	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
TC(-)	101	309,689	0.33	1.56 (1.23–1.98)	1.67 (1.32–2.13)	1.71 (1.34–2.19)
ALT [#2] alone						
ALT(-)	193	927,859	0.21	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
ALT(+)	105	321,036	0.33	1.58 (1.24-2.00)	1.56 (1.23–1.99)	1.52 (1.18–1.95)
Combinations of TC and ALT …						
TC(+) + ALT(-)	128	690,346	0.19	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
TC(+) + ALT(+)	69	248,861	0.28	1.50 (1.12–2.01)	1.51 (1.13–2.04)	1.45 (1.07–1.97)
TC(-) + ALT(-)	65	237,513	0.27	1.48 (1.10-2.00)	1.62 (1.20–2.18)	1.64 (1.21–2.22)
TC (-) + ALT(+)	36	72,176	0.50	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: …Adjusted for age, gender, BMI, HDL-C, hs-CRP, TG, hypertension, diabetes, alcohol consumption, smoking and physical activity; #1 Further adjusted for ALT based on …; #2 Further adjusted for TC based on ….						

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).

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[#1] 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[#2] 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…						
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, …Adjusted for age, gender, BMI, HDL-C, hs-CRP, TG, hypertension, diabetes, alcohol consumption, smoking and physical activity; #1 Further adjusted for ALT based on …; #2 Further adjusted for TC based on ….

Sensitivity Analysis II: Excluding participants in cirrhosis, the adjusted factors are the same as #1, #2, ….

Sensitivity Analysis III: Excluding fatty liver participants, the adjusted factors are the same as #1, #2, ….

Sensitivity Analysis IV: Excluding participants who took statins, the adjusted factors are the same as #1, #2, ….

Sensitivity Analysis V: Excluding ALT ≥ 40U/L participants during follow-up, the adjusted factors are the same as #1, #2, ….

	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 #1, #2, ….						

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*. [15] 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. And 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[10]. 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)[29], 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.

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 main results. This could speculate that the joint effect 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.[30] reported that cancer is associated with modulation of cholesterol homeostasis. Several carcinogenic signals, such as PI3K/AKT/mTOR, RTK/RAS and TP53, is associated with cholesterol synthesis in cells. TP53, a key tumor suppressor, could affect the development of cancers via modulating cholesterol homeostasis. As all we know, the major marker of chronic liver inflammation were serum ALT levels. The OhdG, a parameter of genetic risk for hepatocarcinogenesis, acts as a pro-mutagenic DNA lesion produced by oxygen(hydroxy) radicals [31–33]. Shimoda et al.[34] found that the OhdG is positively associated with 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 correct use of cholesterol-lowering drugs is also important for avoiding the potential health risks.

Our study has several limitations. Firstly, HCV is a known independent risk factor for PLC[35]. Around 170 million people worldwide were infected with HCV[36]. 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.

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.

Declarations

Consent for publication

During the hospitalization, written informed consents in view of prospective research and publication of the clinical data were obtained from every included patient or their guardians.

Availability of data and materials

Please contact corresponding author for further data requests.

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Competing interests

The authors declare that they have no competing interests.

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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Authors’ contributions

M.S. and S.L. conceived and designed the work; W.W., Y.W. and X.L. have performed data acquisition; M.S., H.C. and Y.W. have analyzed the data; M.S. wrote the paper; L.C. and S.L. reviewed the manuscript.

All authors read and approved the final manuscript.

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Figures

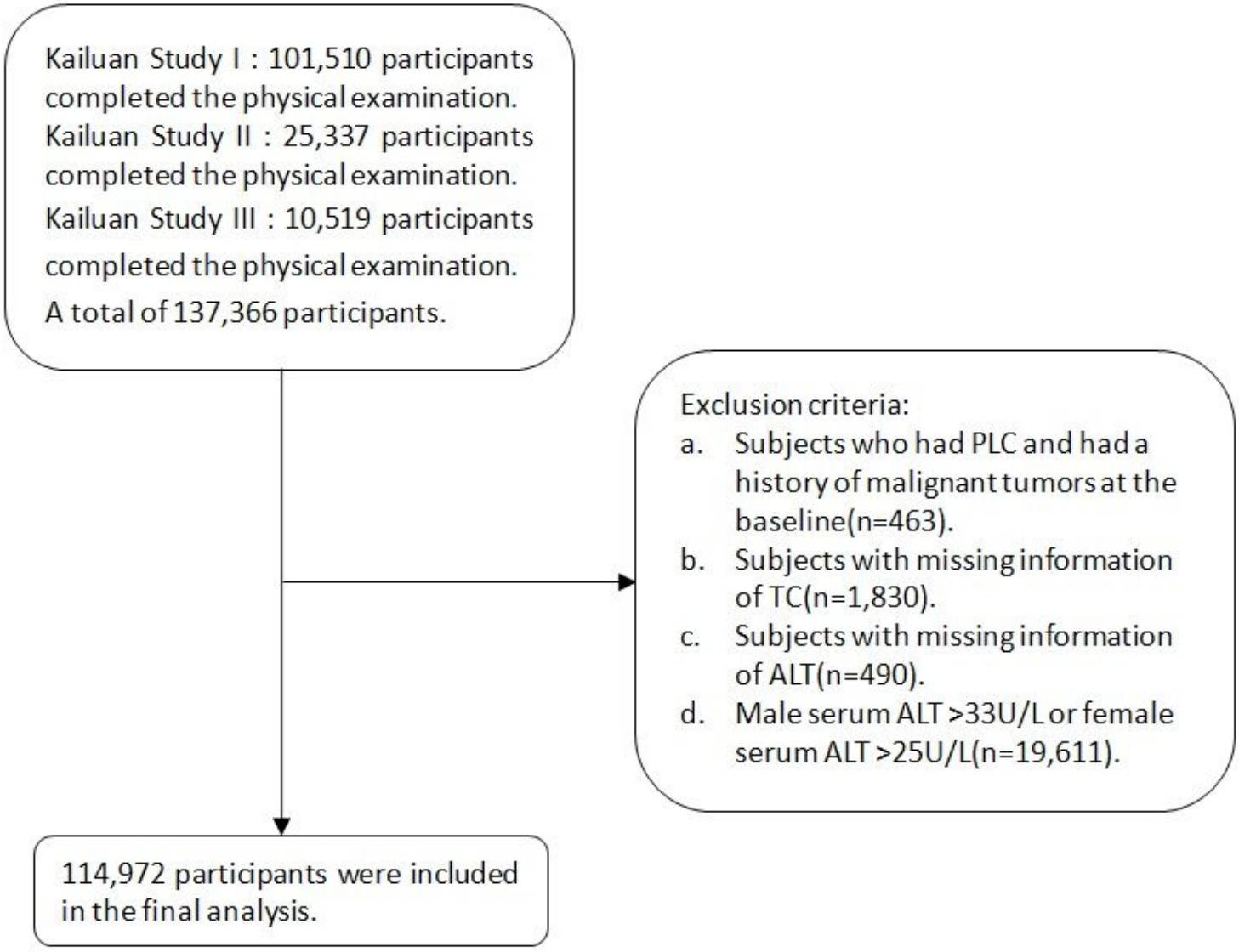

Figure 1

Flow chart of participants screening

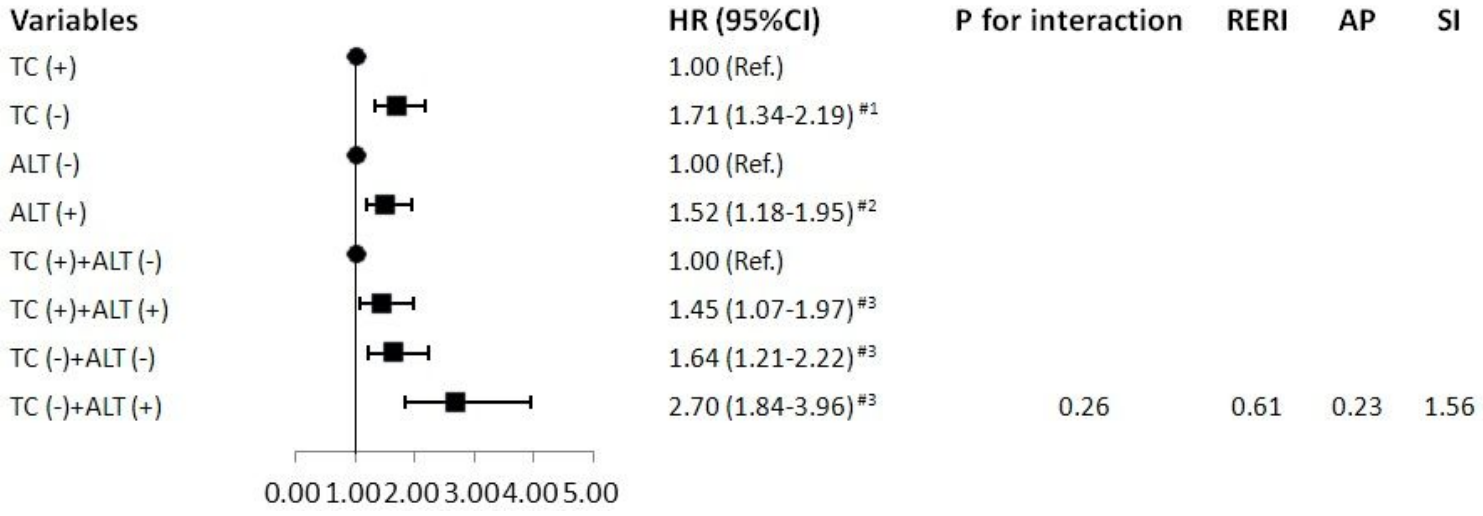

Figure 2

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