U-Shaped Association Between Serum Bilirubin Levels and Peripheral Arterial Disease in Chinese Males with Hypertension

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Abstract

Background: Previous studies indicated that serum total bilirubin might play an important role in peripheral artery disease (PAD). However, the effects of different levels of serum total bilirubin (TbIL) on PAD development remain an uncertainty and had limited data in male participants. We aimed to examine the prevalence of PAD and TbIL among Chinese male adults with hypertension.

Methods: A total of 5129 hypertension male subjects aged 27-93 years was included in the current study. The outcome was a peripheral arterial disease (PAD), defined as present when ABI of either sides was ≤ 0.90 .

Results: Of 5,129 hypertension male participants, 194 (3.78%) had PAD, and the median serum concentrations of TbIL were 14.10 $\mu\text{mol/L}$ (interquartile range 11.00 to 18.60 $\mu\text{mol/L}$). Compared to participants in Q2-Q3 of LgTbIL, there was a significantly increased risk of PAD for participants in both Q1 (OR, 1.49; 95%CI: 1.04-2.14) and Q4 (OR, 1.70; 95%CI: 1.16-2.48). After adjusting for potential confounders, a nonlinear U-shaped relationship was detected between TbIL and PAD, and calculated the inflection point was 1.08 (LgTbIL = 1.08, TbIL = 12.02 $\mu\text{mol/L}$). The ORs (95% CIs) were 0.11 (0.02, 0.83) on the left side of an inflection point and 5.26 (1.59, 17.38) on the right side of the inflection point, respectively. Similar results were found in various subgroups.

Conclusions: This cross-sectional study had shown that there was a U-shaped curve for the risk of PAD with TbIL concentration in Chinese males with hypertension, with a turning point at 12.02 $\mu\text{mol/L}$.

Introduction

As a prevalent cardiovascular disease (CVD), peripheral arterial disease (PAD) had a high fatality rate(1), and in recent years, the incidence rate of PAD has increased year by year(2). Hypertension is one of the significant risk factors for PAD; the number of patients with hypertension is estimated at 245 million in China(3). Several studies have shown that increases in blood pressure are strictly related to increased PAD risk(4–6). Therefore, there is an urgent need to identify novel modifiable risk factors to inform PAD prevention in the hypertensive population. The ankle-brachial index (ABI) is a noninvasive method for diagnosis and detection of PAD and is widely used for risk assessment of atherosclerosis and cardiovascular diseases(7), and PAD was defined as an ABI of ≤ 0.90 in either leg(8).

The effects of serum total bilirubin (TbIL) on the risk of CVD have received considerable attention(9–12). Bilirubin is a potent antioxidant under physiological conditions; a higher TbIL level could be a protector factor for atherosclerosis(13). At the same time, some studies have shown that elevated TbIL levels were negatively correlated with coronary artery disease (CAD) (14–16), arterial stiffness(12), and PAD(17–19). Excessive TbIL is probably an indicator of the potential liver cell damage associated with an increased risk of CVD(20). Nevertheless, the possible effect of excessive TbIL on the risk of increased PAD has not been examined in previous studies. Moreover, androgens in men inhibit bilirubin excretion, and estrogen

in women increases their excretion(21). Compared with females, males have more risk factors for PAD and higher bilirubin levels.

Therefore, this study aimed to assess the relationship between the TBiL and PAD in Chinese hypertensive male subjects to address this gap, as mentioned earlier in knowledge.

Methods

Study Design and population

All patients gave written informed consent. The Ethics Committees of the Institute of Biomedicine, Anhui Medical University, approved study protocols. In the current study, we included rural subjects from ongoing China H-type hypertension Registry Study (Registration number: ChiCTR1800017274). China H-type hypertension Registry Study is a real-world, multicenter, observational study, conducted from Wuyuan, Jiangxi province of China, beginning in July 2018. The aim of this study was to confirm that establish a national registry of patients with hypertension, investigate the prevalence and treatment of hypertension in China, and assess the related factors affecting its prognosis. Details regarding the inclusion and exclusion criteria of the study have been described elsewhere(22).

A total of 14,268 participants completed the investigation. Subject were excluded if they: 1.were not hypertension (n = 34); 2. had missed ABI (n = 3328) and TBiL value (n = 6); 3. were females (n = 5771). The final analysis included 5,129 participants (Figure S1).

Data collection

Baseline information on sociodemographic characteristics, lifestyle habits, comorbidities, and medication use was obtained in-person interviews by well-trained research staff according to a standard protocol. Anthropometric parameters indicators included weight, height, waist circumference (WC), hip circumference (HC), systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse, and ABI measurements were collected. BMI was calculated as the body weight in kilograms divided by the square of the height in meters (kg/m^2).

After an overnight fast of 12–15 h, blood samples were collected utilizing venipuncture and were immediately frozen and stored -80°C until analysis. The measured variables included serum total bilirubin (TBiL), serum homocysteine (Hcy), serum creatinine, lipids, and fasting blood glucose (FBG). The formula for estimated glomerular filtration rate (eGFR) used the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Eq. (23). All of these parameters were measured using automatic clinical analyzers (Beckman Coulter) at the core laboratory of the National Clinical Research Center for Kidney Disease, Guangzhou, China. All laboratory measurements met a standardization and certification program.

Ankle-Brachial Index Measurement

The Ankle-Brachial Index (ABI) was automatically measured with the subject in the supine position after having rested for more than 10 minutes, using Omron Colin BP-203RPE III device (Omron Health Care, Kyoto, Japan) and calculated for each leg by dividing the SBP obtained at the ankle level in the respective leg by the SBP of the brachial artery(24). The lowest value of the ABI was used in the analysis. PAD was defined as an ABI of ≤ 0.90 in either leg(8).

Other Definitions

Hypertension was defined as SBP ≥ 140 mm Hg and/or DBP ≥ 90 mm Hg, and also if the individual was on antihypertensive medication within 2 weeks(25). Diabetes mellitus was defined as a self-reported physician diagnosis of diabetes or FBG concentration of ≥ 7.0 mmol/L or the use of glucose-lowering drugs. Chronic kidney disease (CKD) was defined as eGFR < 60 mL/min/1.73 m² or a self-reported physician diagnosis of CKD.

Covariates

These selected covariates are closely related to PAD. Continuous variables included age, systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse, BMI, HC, WC, laboratory results [FBG, Hcy, alanine aminotransferase(ALT), aspartate aminotransferase(AST), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), estimated glomerular filtration rate (eGFR)] Categorical variables consisted of smoking status (never, former or current), drinking status (never, former or current), coronary heart disease (no or yes), and medication use (antihypertensive drugs, glucose-lowering drugs, lipid-lowering drugs).

Statistical Analysis

We divided the study population into four groups based on participants by quartiles of LgTbIL levels. Baseline characteristics presented as mean, median (SD or interquartile range) for continuous variables and count (percentage) for categorical variables. Differences in population characteristics were compared using Student's t-test, chi-square test, or Wilcoxon signed-rank test. The distribution of serum concentrations of TbIL was strongly skewed toward the left. Thus, we performed the Log10 transformation (LgTbIL) before analysis. We constructed three models to examine the association between LgTbIL and the prevalence of PAD. The crude model was adjusted for none; model 1 was adjusted for age, BMI, WC, HC, SBP, DBP, pulse, smoking status, alcohol consumption, and model 2 was adjusted for all variables in Model 1 plus adjusted for FBG, TC, TG, Hcy, HDL-C, AST, ALT, eGFR, CHD, antihypertensive drugs, glucose-lowering drugs, lipid-lowering drugs. Multivariate logistic regression was used to assess the odds ratios (ORs) and 95% confidence intervals (CI) for PAD in each LgTbIL tertile. To characterize the shape of the relationship between LgTbIL and PAD prevalence, a generalized additive model, and a smooth curve fitting (penalized spline method) was performed. If nonlinearity was detected, we first use a recursive algorithm to calculate the inflection points and then construct a two-segment binary logistic model on both sides of the inflection points. As additional exploratory analyses, possible modifications of the LgTbIL effects on the prevalence of PAD in participants separated by the turning point of LgTbIL were also assessed for variables including age (< 65 vs. ≥ 65 years), BMI (< 24 vs.

$\geq 24 \text{ kg/m}^2$), smoking status (never vs. Former vs. current), AST (≤ 40 vs. ≥ 40 U/L), diabetes mellitus (no vs. yes), CKD (no vs. yes). A 2-tailed $P < 0.05$ was considered to be statistically significant in all analyses. The Empower (R; www.empowerstats.com; X&Y Solutions, Inc, Boston, MA, USA) and the statistical package R (<http://www.R-project.org>, The R Foundation) were used for all data analyses.

Results

Study participants and baseline characteristics

As shown in the flow chart (Figure S1), a total of 5,129 hypertension male subjects were included in the current study. The mean age of our study participants was 63.92 ± 9.62 years, the median serum concentrations of TBiL was $14.10 \text{ } \mu\text{mol/L}$ (interquartile range 11.00 to $18.60 \text{ } \mu\text{mol/L}$); 194 (3.78%) had PAD, and 828 (16.14%) had diabetes.

Baseline characteristics of study participants stratified by quartiles of LgTBiL levels are summarized in Table 1. Participants with higher levels of LgTBiL tended to be a current alcohol drinker, and had higher rates of diabetes mellitus; and higher value in DBP, pulse, FBG, BMI, WC, HC, TC, HDL, AST, ALT, eGFR, albumin; had lower rates of PAD, CKD and smokers; and lower value in age (all $P < 0.01$). There were no statistically significant between the four groups in terms of SBP, Hcy, TG, stroke, CHD, antihypertensive drugs, glucose-lowering drugs, or lipid-lowering drugs (all $P > 0.05$).

Table 1
Baseline characteristics of study participants by quartiles of baseline LgTbIL levels

Characteristics	Total	LgTbIL, $\mu\text{mol/L}$				<i>P</i> value
		Q1 (< 1.04)	Q2 (1.04–1.15)	Q3 (1.15–1.27)	Q4 (≥ 1.27)	
N	5129	1281	1272	1293	1283	
Age, year	63.92 $\pm$ 9.62	64.37 $\pm$ 9.23	64.23 $\pm$ 9.44	63.60 $\pm$ 9.86	63.50 $\pm$ 9.91	0.043
BMI, kg/m ²	23.36 $\pm$ 4.01	22.98 $\pm$ 3.44	23.43 $\pm$ 3.60	23.44 $\pm$ 3.46	23.58 $\pm$ 5.23	0.001
WC	83.85 $\pm$ 9.69	82.79 $\pm$ 9.88	84.14 $\pm$ 9.72	84.08 $\pm$ 9.36	84.40 $\pm$ 9.71	< 0.001
HC	91.83 $\pm$ 6.82	91.31 $\pm$ 6.79	92.02 $\pm$ 7.20	92.03 $\pm$ 6.71	91.98 $\pm$ 6.53	0.016
Smoking status, N (%)						< 0.001
Never	1070 (20.86%)	213 (16.63%)	241 (18.95%)	292 (22.58%)	324 (25.25%)	
Former	1516 (29.56%)	358 (27.95%)	358 (28.14%)	395 (30.55%)	405 (31.57%)	
Current	2543 (49.58%)	710 (55.43%)	673 (52.91%)	606 (46.87%)	554 (43.18%)	
Drinking status, N (%)						< 0.001
Never	1906 (37.17%)	546 (42.62%)	470 (36.98%)	461 (35.65%)	429 (33.44%)	
Former	1090 (21.26%)	289 (22.56%)	294 (23.13%)	290 (22.43%)	217 (16.91%)	
Current	2132 (41.58%)	446 (34.82%)	507 (39.89%)	542 (41.92%)	637 (49.65%)	

Abbreviation: PAD, peripheral arterial disease; BMI, body mass index; WC, waist circumference; HC, hip circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; CHD, coronary heart disease; CKD, chronic kidney disease; FBG: fasting blood glucose; Hcy, homocysteine; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; TG, Triglycerides; TbIL, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate.

[§]diabetes mellitus was defined as self-reported physician diagnosis of diabetes or FBG concentration ≥ 7.0 mmol/L or use of glucose-lowering drugs.

[#]CKD was defined as eGFR < 60 mL/min/1.73 m² or a self-reported physician diagnosis of CKD.

Characteristics	Total	LgTbIL, $\mu\text{mol/L}$				<i>P</i> value
		Q1 (< 1.04)	Q2 (1.04–1.15)	Q3 (1.15–1.27)	Q4 (≥ 1.27)	
SBP, mmHg	146.63 $\pm$ 17.96	147.16 $\pm$ 18.60	146.68 $\pm$ 17.37	146.04 $\pm$ 17.32	146.65 $\pm$ 18.52	0.475
DBP, mmHg	90.35 $\pm$ 11.06	89.30 $\pm$ 11.22	90.06 $\pm$ 10.93	90.48 $\pm$ 11.12	91.55 $\pm$ 10.85	< 0.001
pulse, bpm	74.41 $\pm$ 14.06	73.28 $\pm$ 13.84	73.73 $\pm$ 13.11	74.57 $\pm$ 13.53	76.06 $\pm$ 15.51	< 0.001
Laboratory data						
Hcy, $\mu\text{mol/L}$	20.50 $\pm$ 13.64	20.17 $\pm$ 12.99	19.94 $\pm$ 12.67	21.13 $\pm$ 15.31	20.77 $\pm$ 13.42	0.103
FBG, mmol/L	6.07 $\pm$ 1.50	5.94 $\pm$ 1.40	6.01 $\pm$ 1.47	6.12 $\pm$ 1.52	6.19 $\pm$ 1.59	< 0.001
TC, mmol/L	4.93 $\pm$ 1.06	4.83 $\pm$ 1.09	4.93 $\pm$ 1.06	4.97 $\pm$ 1.05	4.99 $\pm$ 1.05	< 0.001
TG, mmol/L	1.65 $\pm$ 1.26	1.67 $\pm$ 1.54	1.61 $\pm$ 1.09	1.65 $\pm$ 1.15	1.68 $\pm$ 1.20	0.570
HDL-C, mmol/L	1.55 $\pm$ 0.44	1.47 $\pm$ 0.41	1.53 $\pm$ 0.43	1.57 $\pm$ 0.43	1.63 $\pm$ 0.49	< 0.001
AST, U/L	27.98 $\pm$ 21.81	26.26 $\pm$ 16.53	26.73 $\pm$ 11.58	27.44 $\pm$ 10.84	31.48 $\pm$ 36.91	< 0.001
ALT, U/L	22.25 $\pm$ 20.96	20.79 $\pm$ 15.60	21.27 $\pm$ 13.95	22.36 $\pm$ 15.21	24.55 $\pm$ 32.85	< 0.001
Albumin, g/L	46.49 $\pm$ 4.16	45.26 $\pm$ 4.13	46.29 $\pm$ 3.90	47.06 $\pm$ 4.09	47.35 $\pm$ 4.20	< 0.001
TbIL, median (IQR), $\mu\text{mol/L}$	14.10 (11.00–18.60)	9.20 (7.90–10.10)	12.50 (11.70–13.30)	16.00 (15.00–17.10)	22.90 (20.30–27.60)	< 0.001

Abbreviation: PAD, peripheral arterial disease; BMI, body mass index; WC, waist circumference; HC, hip circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; CHD, coronary heart disease; CKD, chronic kidney disease; FBG: fasting blood glucose; Hcy, homocysteine; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; TG, Triglycerides; TbIL, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate.

$\S$ diabetes mellitus was defined as self-reported physician diagnosis of diabetes or FBG concentration ≥ 7.0 mmol/L or use of glucose-lowering drugs.

$\#$ CKD was defined as eGFR < 60 mL/min/1.73 m² or a self-reported physician diagnosis of CKD.

Characteristics	Total	LgTbIL, $\mu\text{mol/L}$				<i>P</i> value
		Q1 (< 1.04)	Q2 (1.04–1.15)	Q3 (1.15–1.27)	Q4 (≥ 1.27)	
eGFR, mL/min/1.73 m ²	86.18 $\pm$ 20.46	82.96 $\pm$ 23.79	86.68 $\pm$ 19.40	86.48 $\pm$ 19.98	88.61 $\pm$ 17.83	< 0.001
Comorbidities, N (%)						
Stroke	403 (7.86%)	107 (8.35%)	102 (8.02%)	106 (8.20%)	88 (6.86%)	0.484
CHD [#]	273 (5.32%)	55 (4.29%)	72 (5.66%)	68 (5.26%)	78 (6.08%)	0.217
Diabetes mellitus ^{\$}	828 (16.14%)	188 (14.68%)	184 (14.47%)	219 (16.94%)	237 (18.47%)	0.015
CKD	781 (15.23%)	263 (20.53%)	191 (15.02%)	182 (14.08%)	145 (11.30%)	< 0.001
PAD	194 (3.78%)	64 (5.00%)	33 (2.59%)	45 (3.48%)	52 (4.05%)	0.013
Medication use, N (%)						
Antihypertensive drugs	3362 (65.55%)	844 (65.89%)	829 (65.17%)	849 (65.66%)	840 (65.47%)	0.985
Glucose-lowering drugs	232 (4.52%)	58 (4.53%)	55 (4.32%)	55 (4.25%)	64 (4.99%)	0.807
Lipid-lowering drugs	177 (3.45%)	40 (3.12%)	45 (3.54%)	48 (3.71%)	44 (3.43%)	0.871
Abbreviation: PAD, peripheral arterial disease; BMI, body mass index; WC, waist circumference; HC, hip circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; CHD, coronary heart disease; CKD, chronic kidney disease; FBG: fasting blood glucose; Hcy, homocysteine; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; TG, Triglycerides; TbIL, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate.						
^{\$} diabetes mellitus was defined as self-reported physician diagnosis of diabetes or FBG concentration ≥ 7.0 mmol/L or use of glucose-lowering drugs.						
[#] CKD was defined as eGFR < 60 mL/min/1.73 m ² or a self-reported physician diagnosis of CKD.						

Associations between LgTbIL and PAD

The multivariate logistic regression model was performed to evaluate the associations between LgTbIL and PAD. The effect values (OR) and 95% confidence interval (CI) for full-adjustment were listed in Table 2, per 1 unit of LgTbIL higher were associated with 56% increased risk of PAD, but the results did not

reach statistical significance. For sensitivity analysis, we also handled LgTbIL as quartiles and a categorical variable, respectively. Compared with participants in Q2, a higher risk of PAD was found in participants in Q1 (OR,1.89; 95%CI: 1.20–2.96), Q3 (OR,1.58; 95%CI: 0.98–2.53) and Q4 (OR,2.16; 95%CI: 1.35–3.45), respectively. It can be seen from Table 2 that among participants in Q3, the risk of increased PAD was not significant; therefore, Q3 and Q2 populations are combined as a control group. The result showed that in comparison with participants in Q2-Q3 of LgTbIL, there was a significantly increased risk of PAD for participants in both Q1 (OR,1.49; 95%CI: 1.04–2.14) and Q4 (OR,1.70; 95%CI: 1.16–2.48). The result suggested that the relations between LgTbIL and PAD were not merely linear.

Table 2
ORs and 95% CI of PAD incidence according to TbIL levels (μmol/L)

LgTbIL	N	Events (%)	PAD OR (95%CI), <i>P</i> value		
			Crude model	Model 1	Model 2
Continuous	5129	350 (3.21%)	0.74 (0.33, 1.68) 0.473	1.12 (0.48, 2.63) 0.789	1.56 (0.64, 3.81) 0.332
Quartiles					
Q1 (< 1.04)	1281	64 (5.00%)	1.97 (1.29, 3.03) 0.002	1.94 (1.25, 3.02) 0.003	1.89 (1.20, 2.96) 0.006
Q2 (1.04–1.15)	1272	33 (2.59%)	1.00	1.00	1.00
Q3 (1.15–1.27)	1293	45 (3.48%)	1.35 (0.86, 2.14) 0.193	1.54 (0.96, 2.46) 0.071	1.58 (0.98, 2.53) 0.059
Q4 (≥ 1.27)	1283	52 (4.05%)	1.59 (1.02, 2.47) 0.041	1.93 (1.22, 3.06) 0.005	2.16 (1.35, 3.45) 0.001
Categories					
Q1 (< 1.04)	1281	64 (5.00%)	1.68 (1.20, 2.35) 0.003	1.55 (1.09, 2.20) 0.015	1.49 (1.04, 2.14) 0.031
Q2-Q3 (1.04–1.27)	2565	78 (3.04%)	1.00	1.00	1.00
Q4 (≥ 1.27)	1283	52 (4.05%)	1.35 (0.94, 1.93) 0.103	1.53 (1.06, 2.23) 0.024	1.70 (1.16, 2.48) 0.007
Crude model was adjusted for none.					
Model 1 was adjusted for age, BMI, WC, HC, SBP, DBP, pulse, smoking status, drinking status.					
Model 2 was adjusted for all variables in Model 1 plus adjusted for FBG, TC, TG, Hcy, pulse, HDL-C, AST, ALT, antihypertensive drugs, glucose-lowering drugs, lipid-lowering drugs.					

Threshold effect analysis of LgTbIL on PAD

To find the nonlinear relationship between LgTbIL and PAD, we used a generalized additive model and penalized spline method (Fig. 1). The smoothing curve showed that there existed a U-shaped curve association of LgTbIL with PAD in Chinese male adults with hypertension (after adjusting age, BMI, WC, HC, SBP, DBP, pulse, smoking status, alcohol consumption, FBG, TC, TG, Hcy, HDL-C, AST, ALT, eGFR, CHD, antihypertensive drugs, glucose-lowering drugs, lipid-lowering drugs). Furthermore, we further fitted the relationship between LgTbIL and PAD using the two-piecewise logistic regression model (Table 3), and calculated the inflection point was 1.08 (LgTbIL = 1.08, TbIL = 12.02 $\mu\text{mol/L}$). Among the participants whose LgTbIL < 1.08 $\mu\text{mol/L}$ (TbIL < 12.02 $\mu\text{mol/L}$), there was a significant trend towards decreasing risk of PAD development with increment of LgTbIL (OR, 0.11; 95%CI: 0.02–0.83). However, the risk of PAD development significantly increased with the increasing level of LgTbIL (OR, 5.26; 95%CI: 59-17.38) in participants with LgTbIL $\geq$ 1.08 (TbIL $\geq$ 12.02 $\mu\text{mol/L}$).

Table 3					
Threshold effect analysis of TbIL on PAD using two piecewise logistic regression model					
LgTbIL	N	Events (%)	PAD OR (95%CI)†, P value		
			Crude model	Model 1	Model 2
Continuous	5129	194 (3.78%)	0.74 (0.33, 1.68) 0.473	1.12 (0.48, 2.63) 0.789	1.56 (0.64, 3.81) 0.332
Inflection point					
$\leq$ 1.08	1739	72 (4.14%)	0.07 (0.01, 0.44) 0.004	0.10 (0.01, 0.73) 0.023	0.11 (0.02, 0.83) 0.032
> 1.08	3390	122 (3.59%)	2.40 (0.77, 7.51) 0.131	3.45 (1.08, 11.05) 0.037	5.26 (1.59, 17.38) 0.007
P for log likelihood ratio test			0.009	0.012	0.007
Crude model was adjusted for none.					
Model 1 was adjusted for age, BMI, WC, HC, SBP, DBP, pulse, smoking status, drinking status.					
Model 2 was adjusted for all variables in Model 1 plus adjusted for FBG, TC, TG, Hcy, HDL-C, AST, ALT, eGFR, CHD, antihypertensive drugs, glucose-lowering drugs, lipid-lowering drugs.					
Figure S1 Flow chart of study participants					

Subgroup analyses

We performed exploratory subgroup analyses to assess the association between LgTbIL and PAD in two groups of participants separated by the turning point of LgTbIL (1.08) (Fig. 2). The effect of LgTbIL on PAD showed no significant difference in the following subgroups: age (< 65 vs. $\geq$ 65 years), BMI (< 24 vs. $\geq$ 24 kg/m^2), smoking status (never vs. Former vs. current), AST ($\leq$ 40 vs. $\geq$ 40 U/L), diabetes mellitus (no vs. yes), CKD (no vs. yes) in both groups (all P for interactions > 0.05), after adjustment for age, BMI, WC, HC, SBP, DBP, pulse, smoking status, alcohol consumption, FBG, TC, TG, Hcy, HDL-C, AST, ALT, eGFR,

CHD, antihypertensive drugs, glucose-lowering drugs, lipid-lowering drugs, except for the stratifying variable.

Discussion

For the first time, we found a independently U-shaped association between TBIl and PAD among hypertensive male subjects, and then revealed a turn point ($LgTBIl = 1.08$, $TBIl = 12.02 \mu\text{mol/L}$) by threshold effect analysis.

Several previous studies had examined the relationships between TBIl levels and PAD(17–19). Ozeki et al.(17)reported that serum bilirubin concentration was significantly negatively associated with PAD prevalence in 935 cardiology patients. Lan et al.(18)conducted a cross-section study, which included 543 participants with hypertension (mean age: 62.7 ± 12.4 years). The result showed that per 1 unit increment of TBIl was associated with 8.6% (OR, 0.914; 95% CI: 0.845–0.990) lower risk of PAD; besides, an independently negatively relationship between TBIl and PAD (OR,0.884; 95% CI: 0.792–0.985) was merely found in males but not in females. A cross-sectional examination from the National Health and Nutrition Examination Survey (1999 to 2004) analyzed 7075 adults with data available on the ankle brachial index, serum total bilirubin level, and PAD risk factors. The result showed that a 0.1 mg/dL increase in bilirubin level was associated with a 6% reduction in the odds of PAD (OR 0.94 [95% CI 0.90 to 0.98]), and this association is more influential in men compared with women(19). However, these studies did not discuss a nonlinear relationship between TBIl and PAD.

In the current study, some new insights were demonstrated in hypertensive males patients. Our in-depth study showed that the association between TBIl and PAD risk was not a simple linear association but a U-shaped curve, suggesting that low and high TBIl levels were associated with increased PAD risk. The reasons for these contradictory findings might be different total serum bilirubin levels, and the distribution of total serum bilirubin levels may vary depending on the race(26, 27), age(28), health status(29, 30), and sample size of the subjects. We conducted a cross-section study included 5,129 Chinese hypertensive males subjects, the mean age of our study participants was 63.86 ± 9.25 years, the median serum concentrations of TBIl was $14.10 \mu\text{mol/L}$ (interquartile range 11.00 to $18.60 \mu\text{mol/L}$); However, Ozeki et al.'s study(17) enrolled 935 Japanese cardiology patients (median serum bilirubin: about $8.55 \mu\text{mol/L}$), and Lan et al.'s study(18) analyzed 543 Chinese participants with hypertension, the mean serum bilirubin was $12.2 \pm 5.6 \mu\text{mol/L}$, respectively. At the same time, Perlstein et al.(19)conducted a cross-sectional examination that included 7075 various racial groups adults; the median total bilirubin level was 11.97 (interquartile range 8.55 to 13.68) $\mu\text{mol/L}$. Due to the small sample size of the above study and the relatively lower bilirubin levels. Thence, we speculate that the negative relationship might be part of the U-shaped curve in this study. Secondly, previous studies were carried out in patients with cardiology and hypertension, and the general people, while the current study conducted in participants with hypertension accompanied by hyper-homocysteinemia (HHcy). About 75% of hypertension patients in China have hyper-homocysteinemia simultaneously(31), HHcy was defined as Hcy level $\geq$

10 $\mu\text{mol/L}$ (32), TBIl may be heterogeneous for different diseases; the threshold point of injury is different in different diseases.

The current study found that the OR value of Model 1 has changed direction compared with the crude model. The differences in outcomes between the crude model and Model 1 in our study may be explained by the widespread influence of covariates, such as smoking, blood pressure, age, and BMI. Lu et al.(33) conducted a meta-analysis of the association between cigarette smoking and PAD. The result demonstrated that smoking increased risk of PAD. According to the 2017 ESC Guidelines on the Diagnosis and Treatment of Peripheral Arterial Diseases, in collaboration with the European Society for Vascular Surgery, the major risk factors for PAD are smoking, hypertension, diabetes, abnormal lipid metabolism, obesity, etc.(8). Because the effect of these covariates on PAD is too significant to cover up the effect of serum bilirubin on PAD, the independent effect of serum bilirubin on PAD is only reflected after adjusting it.

To our knowledge, TBIl is a potent endogenous antioxidant protecting cells from a 10 000-fold higher concentration of oxidants(34–36); Enhance, lower bilirubin levels could induce the oxidative stress and inflammation which are related to the pathogenesis and development of arteriosclerosis(37). However, the exact mechanisms of excessive TBIl levels with PAD remain unknown. One possible reason could account for the association between excessive TBIl and increased risk of PAD, which was that excessive TBIl such as dominant jaundice might indicate potential liver cell damage, such as hepatocellular or obstructive jaundice, which in turn causes elevated levels of transaminases and alkaline phosphatase(38), the increased levels of transaminase and alkaline phosphatase are associated with an increased risk of CVD(20, 39).

The potential limitations of our study should also be noted. First, we cannot draw any causal relationship between serum bilirubin and PAD from the data because this is a cross-sectional study. Second, the serum bilirubin was only assessed at the baseline in the present study; multiple tests may make the results more accurate. Lastly, this study was conducted in Chinese hypertension male participants, the generalizability of the findings to other populations remains to be determined.

Conclusions

In summary, this cross-sectional study showed a U-shaped curve for the risk of PAD with TBIl concentration in hypertensive male patients, with a turning point at about 12.02 $\mu\text{mol/L}$. Further well-designed prospective cohort studies are needed to determine the association's causality and clarify the potential underlying mechanisms of TBIl in PAD.

Declarations

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Authors' contributions: YMS participated in the literature search, data analysis, and data interpretation. YMS wrote the manuscript. LHH extracted and collected data. LHH, MHL, XH, CCD, WZ, TW, LJZ conceived of the study and participated in its design and coordination. HHB and XSC participated in the study design and provided critical revision. All authors read and approved the final manuscript.

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Ethical approval: All procedures performed in studies involving human participants were following the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Figures

Figure 1

Association between LgTBil and the prevalence of PAD A nonlinear association between TBil and the prevalence of PAD was found ($P < 0.05$). The solid line and dashed line represent the estimated values and their corresponding 95% confidence interval. Adjustment factors included age, BMI, WC, HC, SBP, DBP, pulse, smoking status, drinking status, FBG, TC, TG, Hcy, HDL-C, AST, ALT, eGFR, CHD, antihypertensive drugs, glucose-lowering drugs, lipid-lowering drugs.

Figure 2

Stratified analysis for the PAD and LgTBil in various subgroups divided by 1.08. (A: LgTBil ≤1.08 μmol/IL, B: LgTBil >1.08 μmol/IL). *Each subgroup analysis adjusted for age, BMI, WC, HC, SBP, DBP, pulse, smoking status, drinking status, FBG, TC, TG, Hcy, HDL-C, AST, ALT, eGFR, CHD, antihypertensive drugs, glucose-lowering drugs, lipid-lowering drugs—except for the stratifying variable.

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