

U-shaped association between serum bilirubin levels and peripheral arterial disease in Chinese males with hypertension

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Research article

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Abstract

Background: Previous studies have 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 uncertain, and there are 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 hypertensive male subjects aged 27-93 years were included in the current study. The outcome was peripheral arterial disease (PAD), defined as present when the ABI of either side was \leq 0.90.

Results: Of 5129 hypertensive male participants, 194 (3.78%) had PAD, and the mean serum total bilirubin was 15.67 (7.76) $\mu\text{mol/L}$. Compared to participants in Q2-Q3 of LgTbIL, there was a significantly increased prevalence 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 the prevalence of PAD, and the inflection point was calculated to be 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 the inflection point and 5.26 (1.59, 17.38) on the right side of the inflection point. Similar results were found in various subgroups.

Conclusions: This cross-sectional study showed that there was a U-shaped curve for the prevalence 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) has 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 the 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 \leq 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 protective 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 increase its 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 TBI_L and the prevalence of PAD in Chinese hypertensive male subjects to address this gap in knowledge, as mentioned earlier.

Methods

Study Design and population

All patients gave written informed consent. The Ethics Committees of the Institute of Biomedicine, Anhui Medical University, approved the study protocols. In the current study, we included rural subjects from the ongoing China H-type hypertension Registry Study (Registration number: ChiCTR1800017274). The China H-type hypertension Registry Study is a real-world observational study conducted in Wuyuan, Jiangxi Province, China, beginning in July 2018. The aim of this study was to 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]. Eligible participants were men and women aged 18 years and older who were diagnosed with hypertension. The exclusion criteria of the study included (1) psychological or nervous system impairment resulting in an inability to demonstrate informed consent; (2) unable to be followed-up according to the study protocol or plans to relocate in the near future; and (3) patients who are not suitable for inclusion or for long-term follow-up as assessed by the study physicians.

A total of 14,268 participants completed the investigation. Subjects were excluded if they 1. were not hypertensive (n = 34); 2. had missing ABI (n=3328) and TBI_L values (n = 6); and 3. were females (n=5771). The final analysis included 5129 participants (Figure S1).

Data collection

Baseline information on sociodemographic characteristics, lifestyle habits, comorbidities, and medication use was obtained through in-person interviews conducted by trained researchers according to a standard operating procedure. Anthropometric parameter indicators, including weight, height, waist circumference (WC), hip circumference (HC), systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), and ABI measurements, were collected. BMI was calculated as the body weight in kilograms divided by the square of the height in meters (kg/m²).

All the study subjects were told one day in advance that fasting venous blood samples would be collected the next morning. After an overnight fast of 12-15 h, blood samples were collected utilizing venipuncture and were immediately frozen and stored at -80°C until analysis. The measured variables included serum total bilirubin (TBI_L), serum homocysteine (Hcy), serum creatinine, lipids, and fasting blood glucose (FBG). The formula used for the estimated glomerular filtration rate (eGFR) was the

Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation[23]. All of these parameters were measured using automatic clinical analyzers (Beckman Coulter) at the BiaoJia Biotechnology Laboratory, Shenzhen, China.

Ankle-Brachial Index Measurement

The ankle-brachial index (ABI) was automatically measured with the subject in the supine position after resting for more than 10 minutes using an 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 if the individual was on antihypertensive medication in the past 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.

Statistical Analysis

We divided the study population into four groups based on quartiles of LgTbIL levels. Baseline characteristics are presented as the mean \pm SD for continuous variables and the count (percentage) for categorical variables. Differences in population characteristics were compared using one-way Analysis of Variance (ANOVA) test or chi-square test. Serum concentrations of TbIL were expressed as $\mu\text{mol/L}$. The distribution of serum concentrations of TbIL was strongly skewed toward the left. Thus, we performed the Log₁₀ transformation (LgTbIL) before analysis. Multivariate logistic regression was used to investigate the association between LgTbIL and the prevalence of PAD. We constructed three models: the crude model was not adjusted; Model 1 was adjusted for age, BMI, WC, HC, SBP, DBP, pulse, smoking status, and alcohol consumption; and Model 2 was adjusted for all variables in Model 1 plus adjustment for FBG, TC, TG, Hcy, HDL-C, AST, ALT, eGFR, CHD, antihypertensive drugs, glucose-lowering drugs, and lipid-lowering drugs. In the regression analyses model, the variables were selected because of their clinical importance, statistical significance in the univariable analysis, and the potential confounders effect estimates individually changed by at least 10%. According to published guidelines and studies, The main risk factors of PAD are smoking, hypertension, diabetes, abnormal lipid metabolism, obesity and family history of cardiovascular disease[2, 26]. To characterize the shape of the relationship between LgTbIL and PAD prevalence, a generalized additive model and smooth curve fitting (penalized spline method) were performed. If nonlinearity was detected, we first used a recursive algorithm to calculate the inflection points and then constructed 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. ≥24 kg/m²), smoking status (never vs. former vs. current), AST/ALT (<1 vs. ≥1), diabetes mellitus (no vs. yes), and CKD (no vs. yes). A 2-tailed P<0.05 was considered to be statistically significant in all analyses. 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 5129 hypertensive male subjects were included in the current study. The mean age of our study participants was 63.92 ± 9.62 years, the mean serum total bilirubin was 15.67 (7.76) μmol/L, 194 subjects (3.78%) had PAD, and 828 (16.14%) had diabetes.

The baseline characteristics of the 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; higher values of DBP, pulse, FBG, BMI, WC, HC, TC, HDL, AST, ALT, eGFR, and albumin; lower rates of PAD, CKD and smoking; and lower values for age (all $P < 0.01$). There were no statistically significant differences among 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, $\mu\text{mol/L}$				P 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, cm	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, cm	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%)	
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
heart rate (HR), 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, $\mu\text{mol/L}$	15.67 \pm 7.76	8.87 \pm 1.51	12.51 \pm 0.90	16.08 \pm 1.27	25.17 \pm 9.47	<0.001
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	232 (4.52%)	58 (4.53%)	55 (4.32%)	55 (4.25%)	64 (4.99%)	0.807

drugs						
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; HR, heart rate; 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.

Associations between LgTBiL and PAD

A multivariate logistic regression model was performed to evaluate the associations between LgTBiL and the prevalence of PAD. The effect values (ORs) and 95% confidence intervals (CIs) for full adjustment are listed in Table 2. Every 1 unit increase in LgTBiL was associated with 56% increased odds of PAD (OR 1.56 [95% CI 0.64 to 3.81]), but the results did not reach statistical significance. For sensitivity analysis, we also handled LgTBiL as quartiles and as a categorical variable. Compared with participants in Q2, a higher odds 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). Table 2 shows that among participants in Q3, the odds of increased PAD were not significant; therefore, Q3 and Q2 populations were combined as a control group. The results showed that in comparison with participants in Q2-Q3 of LgTBiL, there was a significantly increased odds ratio 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 results suggested that the relationship between LgTBiL and the prevalence of PAD was not merely linear.

Table 2 ORs and 95% CI of PAD incidence according to TBiL levels (μ mol/L)

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
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, HDL-C, AST, ALT, eGFR, CHD, antihypertensive drugs, glucose-lowering drugs, lipid-lowering drugs.

Threshold effect analysis of LgTBiL on PAD

To find the nonlinear relationship between LgTBiL and the prevalence of PAD, we used a generalized additive model and penalized spline method (Figure 1). The smoothing curve showed that a U-shaped curve association existed between LgTBiL and the prevalence of PAD in Chinese male adults with hypertension (after adjusting 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, and lipid-lowering drugs). We further fitted the relationship between LgTBiL and the prevalence of PAD using the two-piecewise logistic regression model (Table 3) and calculated that the inflection point was 1.08 (LgTBiL = 1.08, TBiL = 12.02 μmol/L). Among the participants whose LgTBiL <1.08 μmol/L (TBiL <12.02 μmol/L), there was a significant trend toward decreasing odds of PAD development with increasing LgTBiL (OR, 0.11; 95% CI: 0.02- 0.83). However, the odds of PAD development significantly increased with increasing levels of LgTBiL (OR, 5.26; 95% CI: 59-17.38) in participants with LgTBiL ≥ 1.08 (TBiL ≥ 12.02 μmol/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					
≤ 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.

Subgroup analyses

We performed exploratory subgroup analyses to assess the association between LgTbIL and the prevalence of PAD in two groups of participants separated by the turning point of LgTbIL (1.08) (Figure 2). The effect of LgTbIL on PAD showed no significant difference in the following subgroups: age (<65 vs. ≥ 65 years), BMI (< 24 vs. ≥ 24 kg/m²), smoking status (never vs. former vs. current), AST/ALT (<1 vs. ≥ 1), diabetes mellitus (no vs. yes), and 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, and lipid-lowering drugs, except for the stratifying variable.

Discussion

For the first time, we found an independent U-shaped association between TbiL and the prevalence of PAD among hypertensive male subjects and then revealed a turning point (LgTbIL = 1.08, TbiL = 12.02 μ mol/L) by threshold effect analysis.

Several previous studies have examined the relationships between TbiL levels and PAD. 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-sectional study that included 543 participants with hypertension (mean age: 62.7 ± 12.4 years). The results showed that every 1 unit increment of TbiL was associated with an 8.6% (OR, 0.914; 95% CI: 0.845-0.990) lower risk of PAD; in addition, an independently negative relationship between TbiL and PAD (OR, 0.884; 95% CI: 0.792-0.985) was 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 results 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 than in 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 male patients. Our in-depth study showed that the association between TbiL and PAD prevalence was not a simple linear association but a U-shaped curve, suggesting that low and high TbiL levels were associated with increased PAD prevalence. The reasons for these contradictory findings might be the different total serum bilirubin levels, and the distribution of total serum bilirubin levels may vary depending on the race [27, 28], age [29], health status [30, 31], and sample size of the subjects. We conducted a cross-sectional study including

5129 Chinese hypertensive male subjects. The mean age of our study participants was 63.86 ± 9.25 years, and the mean serum total bilirubin was $15.67 (7.76) \mu\text{mol/L}$. However, Ozeki et al.'s study[17] enrolled 935 Japanese cardiology patients (median serum bilirubin: approximately $8.55 \mu\text{mol/L}$), and Lan et al.'s study[18] analyzed 543 Chinese participants with hypertension. The mean serum bilirubin was $12.2 \pm 5.6 \mu\text{mol/L}$. At the same time, Perlstein et al.[19] conducted a cross-sectional examination that included 7075 adults of various races; 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, we therefore speculate that the negative relationship might be part of the U-shaped curve in this study. Second, previous studies were carried out in patients with cardiovascular disease and hypertension and in the general population, while the current study was conducted in participants with hypertension accompanied by hyperhomocysteinemia (HHcy). Approximately 75% of patients with hypertension in China have hyperhomocysteinemia simultaneously[32], HHcy was defined as Hcy level $\geq 10 \mu\text{mol/L}$ [33], and TBIl may be heterogeneous for different diseases, so the threshold point of injury is different in different diseases.

The current study found that the OR value of Model 1 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.[26] conducted a meta-analysis of the association between cigarette smoking and PAD. The results demonstrated that smoking increased the risk of PAD. According to a national study of the prevalence and risk factors associated with peripheral arterial disease from China, the major risk factors for PAD are smoking, hypertension, diabetes, abnormal lipid metabolism, obesity, etc[34]. 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[35-37]; hence, lower bilirubin levels could induce oxidative stress and inflammation, which are related to the pathogenesis and development of arteriosclerosis[38]. However, the exact mechanisms of excessive TBIl levels with PAD remain unknown. One possible reason that could account for the association between excessive TBIl and increased risk of PAD is 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[39]. The increased levels of transaminase and alkaline phosphatase are associated with an increased risk of CVD[20, 40]. Diseases of the liver may interfere with the production of the active metabolites of vitamin D[41], reduced serum vitamin D levels were associated with an increased risk of PAD[42]. Therefore, we suspect that excessive TBIl level can not offset the influence of the decline of vitamin D level on the prevalence of PAD.

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. Third, in our questionnaire, we did not collect the information of symptoms in common vascular presentations, which was our limitation. PAD was only defined by ABI in our study. Many studies used ABI alone for PAD diagnosis in epidemiology investigation. Nevertheless, it would be better if our study use Edinburgh Claudication Questionnaire to estimate intermittent claudication and CT angiography (CTA) to exam lower extremity[43]. 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 prevalence of PAD with TBIl concentration in hypertensive male patients, with a turning point at approximately 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 the prevalence of PAD.

List Of Abbreviations

PAD, peripheral arterial disease; BMI, body mass index; WC, waist circumference; HC, hip circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; 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. OR, odds ratios; CI, confidence interval.

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the The Ethics Committees of the Institute of Biomedicine, Anhui Medical University. Written informed consent was obtained from each participant.

Consent to publish

Written informed consent for publication of clinical details and/or clinical images was obtained from the all of the participants.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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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 have read and substantially revised the manuscript for intellectual content, and approved the last version of the manuscript.

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Figures

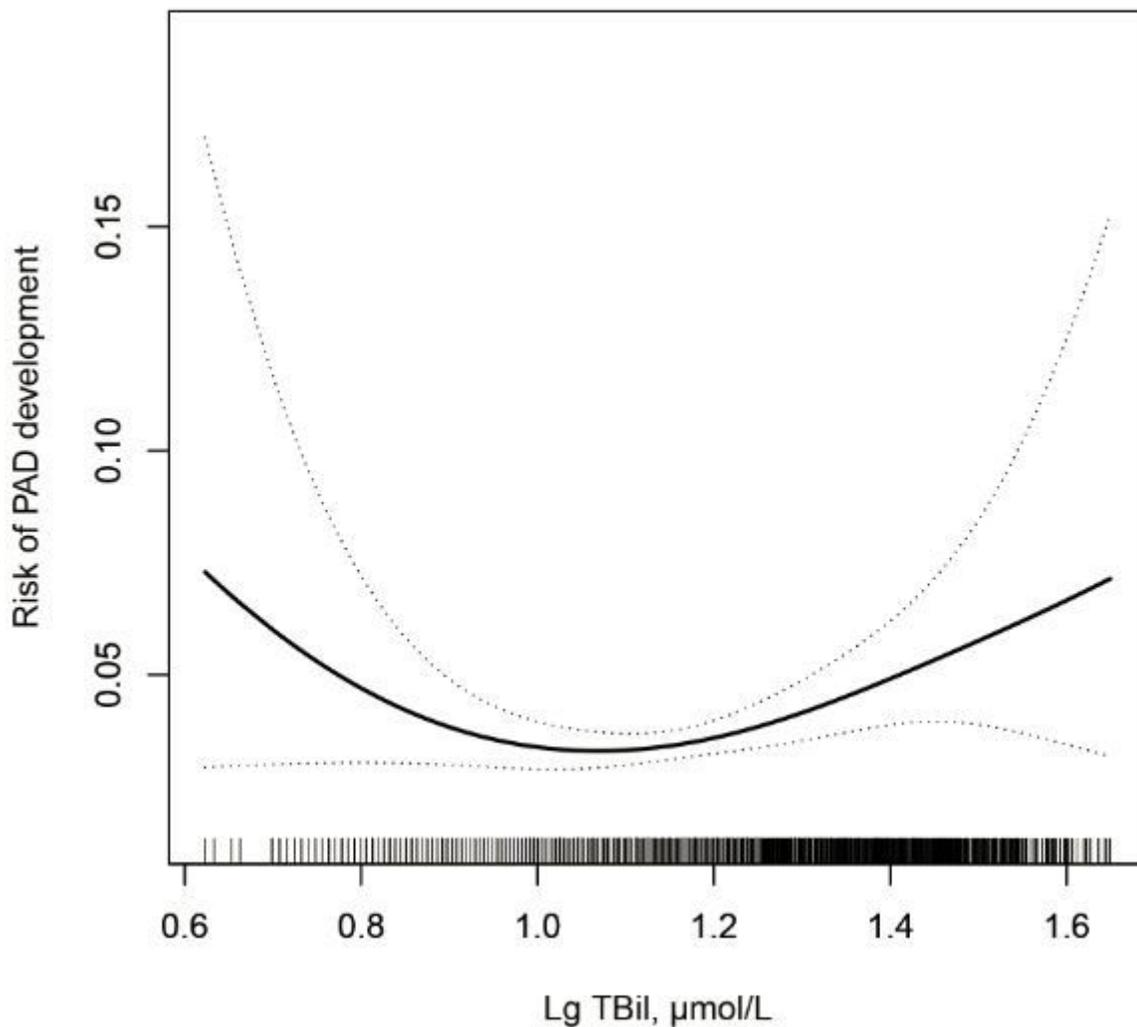


Figure 1

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.

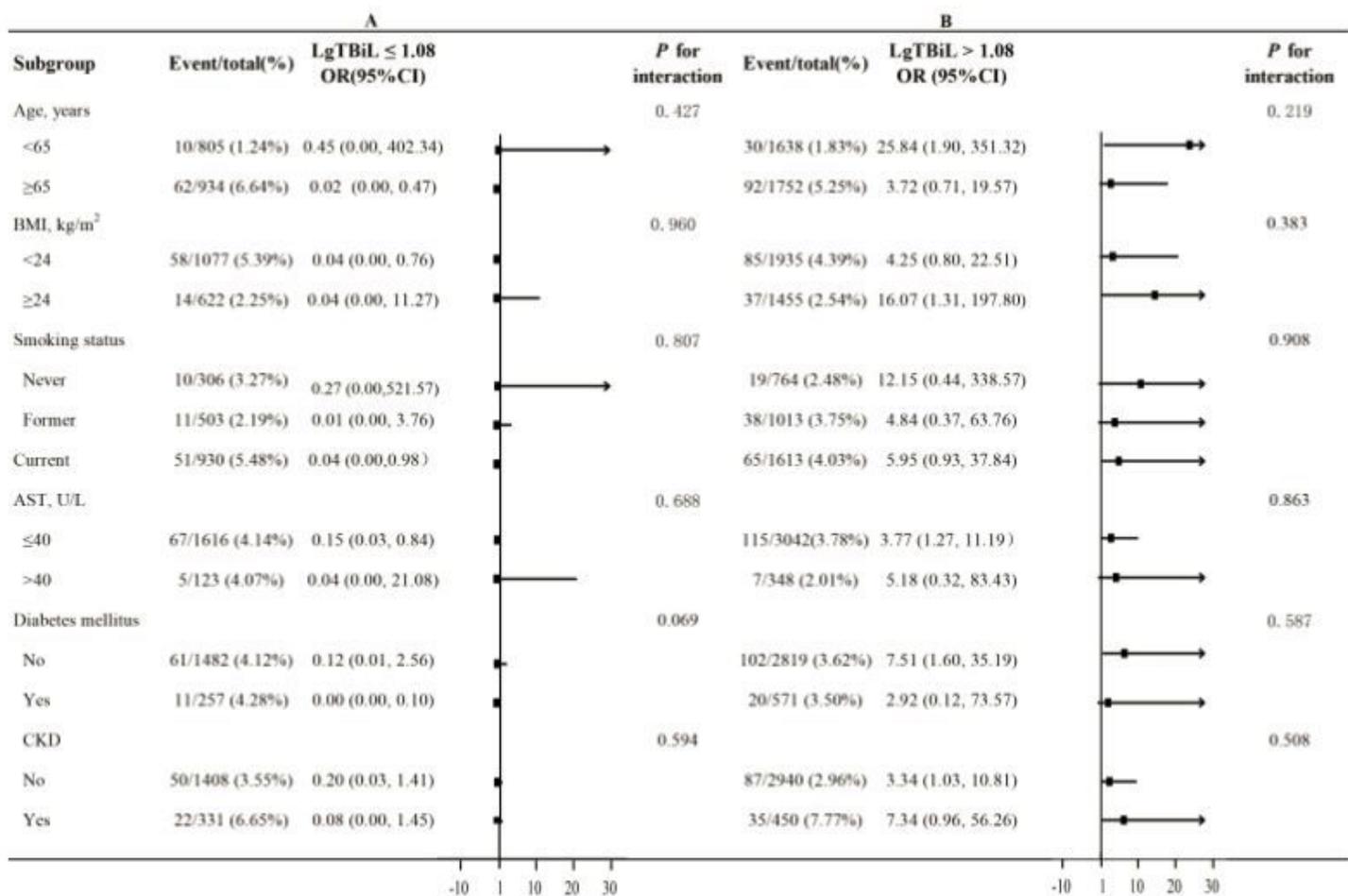


Figure 2

Stratified analysis for the PAD and LgTBil in various subgroups divided by 1.08. (A: LgTBil ≤1.08 μmol/L, B: LgTBil >1.08 μmol/L). *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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