Relationships between serum total bilirubin levels and hypercholesterolemia in Chinese men and women.

Xuemei Zhang  
Tianjin Medical University General Hospital

Chao Huang  
University of Hull

Yaguang Fan  
Tianjin Medical University General Hospital

Ke Xu (✉ ke_xu@hotmail.com)  
Tianjin Medical University General Hospital

Xue Li  
Tianjin Medical University General Hospital

Ming Liu  
Tianjin Medical University General Hospital

Xiaojun Ren  
Tianjin Medical University General Hospital

Mei Zhu  
Tianjin Medical University General Hospital

Qing He  
Tianjin Medical University General Hospital

Qing Zhang  
Tianjin Medical University General Hospital

Kun Song  
Tianjin Medical University General Hospital

Qiyu Jia  
Tianjin Medical University General Hospital

Chunmei Zhang  
Tianjin Medical University General Hospital

Xiaoran Wang  
Tianjin Medical University General Hospital

Zhaowei Meng (✉ jamesmencius@163.com)  
Department of Nuclear Medicine, Tianjin Medical University General Hospital
Abstract

Objective

Bilirubin, an antioxidant, is reported to relate with metabolic syndrome and cardiovascular diseases. The current study is to assess the relationship between total bilirubin (TB) and hypercholesterolemia in the Chinese population.

Methods

This cross-sectional study involved 48971 males and 31327 females who were in good health. Physical examinations and laboratory tests including TB and total cholesterol (TC) were performed. Subjects were divided into the following groups according to age: subjects younger than 30 years, subjects aged between 30 to 60 years and subjects older than 60 years. Binary logistic regression models examining factors independently related to TB was performed for males and females in different age groups.

Results

TB was negatively related to most parameters in males and females, such as body mass index (BMI), blood urea nitrogen (BUN), TC, triglycerides (TG), low-density lipoprotein (LDL) and glucose (G). The incidence of high TC was higher in females than in males and decreased with the increasing of TB in both genders. People older than 30 years demonstrated a similar trend. When there were no other covariates, TB appeared to be an important risk factor for high TC for men and women. However, this relationship disappeared when other covariates were added in the binary logistic regression models. Similar results also existed in men younger than 60 years and women younger than 30 years.

Conclusions

Our findings implied that serum TB concentration was not an independent risk predictor for hypercholesterolemia. TB was thought to be a covariate factor in the development of dyslipidemia.

Background

Hypercholesterolemia, a metabolic disorder characterized by the elevated level of serum total cholesterol, is a common form of hyperlipidemia. Relationship between hypercholesterolemia and cardiovascular disease mortality was a research hotspot in recent years [1, 2]. Literatures showed that hypercholesterolemia was associated with increased cardiac oxidative stress [3, 4]. In mammalian tissues, TB was probably the most abundant endogenous anti-oxidant, accounting for the majority of human serum anti-oxidant activity[5]. Compared to other anti-oxidants, TB exhibited strong superoxide and peroxy radical scavenger activity [6]. Increasing in vivo evidences have found that TB played an
important role in preventing many diseases (such as cardiovascular disease [7], Gilbert syndrome [8]) and reducing cancer mortality [9].

Recent studies have been committed to the relationship between diseases and their risk factors such as TB and oxidative stress. However, information is lacking on the interaction between TB and dyslipidemia. Previous studies reported a negative relationship between TB and TC. However, these studies have some shortcomings, such as narrow population distribution [10, 11] and age range [12], gender restrictions [13], no further statistical analysis and the exclusion of other factors [9]. Opposite opinion was also existed. A cross-sectional analysis of 594 Indigenous Australians reported a positive correlation between TB and TC [14]. However, their research population was too small to be demographically representative. Other articles found no relationship exist between TB and TC in healthy people [15–17]. The current study was performed to access the association between TB and TC in Chinese population.

**Materials And Methods**

**Participants**

Participants were recruited from a community-based health survey carried out by our multidisciplinary team in General Hospital Affiliated to Tianjin Medical University. Our previous articles had reported the protocol of that survey [18-22]. Participants were required to complete a detailed health questionnaire. On the basis of their responses, they attended a screening examination including taking blood after an overnight fast (time ≥ 8h). To control the confounding factors, subjects met the following situations were excluded: subjects with a medical history of blood, liver, kidney, stomach, thyroid inflammation, infection, cancer or autoimmune diseases; subjects taking any medicine which might affect TB and TC; subjects who had abnormal liver function tests, defined as a alanine aminotransferase (ALT) level greater than 100 U/L or a serum TB level > 40 μmol/L; subjects who had excessive drinking or pregnancy. Visual hemolysis tests were performed on the centrifuged samples to avoid increased TB due to hemolysis. Finally, a total of 48971 males and 31327 females were collected from September 2010 to September 2015.

**Measures**

Anthropometric measurements and fasting blood glucose tests were performed for participants during their visitation to our institution. Measurements of body height (BH), body weight (BW), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were collected; BMI was calculated by dividing the subjects’ weight in kilograms by their height in meters squared. An auto-analyzer (Hitachi Corporation, Tokyo, Japan) was used to measure the following biochemical indicators: uric acid (UA), ALT, TB, BUN, creatinine (Cr), TG, TC, high-density lipoprotein (HDL) and LDL.

All parameters had laboratory standards, including: ALT 5–40 U/L; TB 3.4–20 μmol/L; BUN 1.7–8.3 mmol/L; Cr 44–115 μmol/L; TG 0.57–1.71 mmol/L; TC 3.59–5.17 mmol/L; HDL 0.8–2.2 mmol/L; LDL 1.33–3.36 mmol/L; Glucose (G) 3.6-5.8 mmol/L. High TC was defined as TC ≥ 5.18 mmol/L. Subjects
were divided into the following groups according to age: subjects younger than 30 years, subjects aged between 30 to 60 years and subjects older than 60 years.

**Statistical analysis**

All statistical analyses were performed separately by gender and age groups with the software of Statistical Package for Social Sciences (SPSS version 17.0, Chicago, IL, USA). Statistically significance was defined as $P < 0.05$. Most of the factors could be described using a normal distribution except ALT, TB TG, HDL and G. Independent sample's $t$ test was used to compare the differences between the indicators for normal distributed variables, and the results were expressed as mean ± standard deviation (SD). Continuous variables with a skewed distribution, analyzed by Mann-Whitney $U$ test, were described with median and interquartile range. Spearman bivariate correlations were performed between TB and other variables in different genders. The concentration of TB was divided into four quartiles. Chi-square test was used to compare the inter-group prevalence differences of high TC. At last, binary logistic regression models were performed to analyze the crude and adjusted odds ratios for high TC with 95% confidence intervals.

**Results**

**Characteristics of the participants in different genders**

Males were younger than females. Most of the parameters had higher levels in males than in females, except TC, HDL and LDL. Differences also existed between high TC group and normal TC group (Table 1). All of the parameters were higher in high TC group than in normal TC group in both genders except ALT, TG, HDL and G. In high TC group, females were older than males. Males had higher levels of all parameters than females while HDL and LDL were higher in females. In normal TC group, females were younger than males. The levels of most parameters were higher in males than in females. Only HDL was higher in females than in males.
### Table 1
Participant characteristics

| Parameters    | Males            | Females           | P    |
|---------------|------------------|-------------------|------|
|               | 48971            | 31327             |      |
| Age (years)   | 46.57 ± 12.19    | 47.72 ± 13.13     | < 0.001 |
| BMI (kg/m²)   | 25.91 ± 3.22     | 24.00 ± 3.48      | < 0.001 |
| SBP (mmHg)    | 125.41 ± 16.05   | 121.35 ± 18.34    | < 0.001 |
| DBP (mmHg)    | 81.04 ± 11.08    | 74.82 ± 10.26     | < 0.001 |
| ALT (U/L)     | 23.00 (17.00 ~ 33.00) | 16.00 (12.00 ~ 21.00) | < 0.001 |
| TB (µmol/L)   | 12.80 (9.90 ~ 16.60) | 10.40 (8.10 ~ 13.50) | < 0.001 |
| BUN (mmol/L)  | 5.01 ± 1.24      | 4.43 ± 1.22       | < 0.001 |
| Cr (µmol/L)   | 79.29 ± 11.34    | 59.84 ± 9.58      | < 0.001 |
| UA (µmol/L)   | 362.23 ± 74.75   | 264.72 ± 59.84    | < 0.001 |
| TG (mmol/L)   | 1.48 (1.04 ~ 2.14) | 1.06 (0.75 ~ 1.55) | < 0.001 |
| TC (mmol/L)   | 5.13 ± 0.95      | 5.23 ± 1.03       | < 0.001 |
| HDL (mmol/L)  | 1.23 (1.06 ~ 1.45) | 1.53 (1.30 ~ 1.80) | < 0.001 |
| LDL (mmol/L)  | 3.09 ± 0.83      | 3.11 ± 0.91       | 0.013 |
| G (mmol/L)    | 5.10 (4.70 ~ 5.50) | 4.90 (4.60 ~ 5.30) | < 0.001 |

BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, ALT = alanine aminotransferase, TB = total bilirubin, BUN = blood urea nitrogen, Cr = creatinine, UA = uric acid, TG = triglycerides, TC = total cholesterol, HDL = high-density lipoprotein, LDL = low-density lipoprotein, G = glucose

Data are shown as the mean ± SD for normally distributed variables and median (inter-quartile range) for skewed distributed variables (analyzed by independent sample’s t test and Mann-Whitney U test).

### Correlations between TB and other key variables

Spearman bivariate correlations were analyzed between TB and other key variables. For males, TB illustrated obviously negative relationships to BMI, BUN, TC, UA, TG, LDL and G. No relationships were found between TB and age, SBP, DBP and ALT. On the contrary, TB was significantly positively correlated...
with Cr and HDL. For females, significantly negative relationships were found between TB and BMI, SBP, DBP, BUN, TC, TG, LDL and G. TB showed no relationships with age and UA. Positive relationships also existed between TB and ALT, Cr and HDL.

**Incidence of high TC according to TB quartiles**

Incidence of high TC was compared between genders according to the quartiles of serum TB levels. Females showed significantly higher overall incidence of high TC than males in the second to the fourth TB quartiles. The incidence decreased in higher TB quartiles in both genders (Fig. 1A). Incidence of high TC was also compared between men and women of different age groups. For people younger than 30 years, men showed a significantly higher incidence of high TC than women. The incidence decreased with the increasing of TB quartiles in men, while women showed a decreasing trend in the second to the fourth TB quartiles. For people aged from 30 to 60 years, women had a significantly higher incidence of high TC than men only in the second and the fourth TB quartiles. For people older than 60 years, the incidence of high TC was higher in women than in men. There was almost no obvious decreasing of the incidence from the first to the last TB quartiles in women. Men showed a mildly decreasing of the incidence from the first to the last TB quartiles (Fig. 1B, 1C and 1D).

**Risks of high TC in different genders**

High TC risks for different genders were calculated by binary logistic regression models. All participants were analyzed in different genders (Table 2). Model 2 showed a protective effect of TB on high TC in both genders; for instance, the adjusted odd ratio (OR) of TB showed such a role (males: 0.985; females: 0.974). After adjusting for age, BMI, SBP, DBP, ALT, BUN, Cr, UA, TG, HDL, LDL and G, TB showed no association with TC both in women and men. This relationship was also not changed with the rising of TB quartiles in both genders. In addition, the appeal results were not changed in the binary logistic regression analysis of different age groups (Table 3).
Table 2  
Participant characteristics in High TC

| Parameters | Males                      | Females                      | T value |
|------------|----------------------------|------------------------------|---------|
|            | Normal TC                  | High TC                      |         |
|            | Case number                | 26946                        |         |
|            | Age (years)                | 45.18 ± 12.58                |         |
|            | BMI (kg/m2)                | 25.65 ± 3.26                 | < 0.001 |
|            | SBP (mmHg)                 | 123.67 ± 15.42               |         |
|            | DBP (mmHg)                 | 79.71 ± 10.83                | < 0.001 |
|            | ALT (U/L)                  | 25.00(18.00 ~ 35.00)         | < 0.001 |
|            | TB (µmol/L)                | 12.60(9.70 ~ 16.30)          | < 0.001 |
|            | BUN (mmol/L)               | 4.92 ± 1.21                  | < 0.001 |
|            | Cr (µmol/L)                | 79.00 ± 11.19                | < 0.001 |
|            | UA (µmol/L)                | 354.39 ± 71.98               | < 0.001 |
|            | TG (mmol/L)                | 1.74(1.26 ~ 2.49)            | < 0.001 |
|            | HDL (mmol/L)               | 1.28(1.10 ~ 1.50)            |         |
|            | LDL (mmol/L)               | 2.55 ± 0.51                  |         |
|            | G (mmol/L)                 | 5.20(4.80 ~ 5.70)            |         |
|            | Normal TC                  | 15991                        |         |
|            | High TC                    | 52.62 ± 12.13                | < 0.001 |
|            | BMI (kg/m2)                | 25.36 ± 3.39                 |         |
|            | SBP (mmHg)                 | 127.54 ± 16.53               | < 0.001 |
|            | DBP (mmHg)                 | 82.66 ± 11.17                | < 0.001 |
|            | ALT (U/L)                  | 22.00(16.00 ~ 30.00)         | < 0.001 |
|            | TB (µmol/L)                | 12.90(10.00 ~ 16.80)         | < 0.001 |
|            | BUN (mmol/L)               | 5.11 ± 1.25                  | < 0.001 |
|            | Cr (µmol/L)                | 79.64 ± 11.51                | < 0.001 |
|            | UA (µmol/L)                | 371.82 ± 76.92               | < 0.001 |
|            | TG (mmol/L)                | 1.28(0.91 ~ 1.84)            | < 0.001 |
|            | HDL (mmol/L)               | 1.20(1.03 ~ 1.41)            | < 0.001 |
|            | LDL (mmol/L)               | 3.75 ± 0.66                  |         |
|            | G (mmol/L)                 | 5.00(4.60 ~ 5.40)            |         |

BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, ALT = alanine aminotransferase, TB = total bilirubin, BUN = blood urea nitrogen, Cr = creatinine, UA = uric acid, TG = triglycerides, TC = total cholesterol, HDL = high-density lipoprotein, LDL = low-density lipoprotein, G = glucose

Data are shown as the mean ± SD for normally distributed variables and median (inter-quartile range) for skewed distributed variables (analyzed by independent sample's t test and Mann-Whitney U test).
| Parameters | Correlation coefficients for males | Correlation coefficients for females |
|------------|-----------------------------------|-------------------------------------|
|            | r       | P       | r       | P       |
| Age        | -0.004  | 0.345   | 0.000   | 0.891   |
| BMI        | -0.048  | < 0.001 | -0.081  | < 0.001 |
| SBP        | -0.004  | 0.355   | -0.020  | 0.001   |
| DBP        | 0.002   | 0.701   | -0.017  | 0.003   |
| ALT        | 0.000   | 0.852   | 0.042   | < 0.001 |
| BUN        | -0.050  | < 0.001 | -0.034  | < 0.001 |
| Cr         | 0.060   | < 0.001 | 0.048   | < 0.001 |
| UA         | -0.009  | 0.038   | 0.000   | 0.947   |
| TC         | -0.050  | < 0.001 | -0.016  | 0.005   |
| TG         | -0.085  | < 0.001 | -0.078  | < 0.001 |
| HDL        | 0.099   | < 0.001 | 0.054   | < 0.001 |
| LDL        | -0.059  | < 0.001 | -0.017  | 0.003   |
| G          | -0.095  | < 0.001 | -0.105  | < 0.001 |

BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, ALT = alanine aminotransferase, TB = total bilirubin, BUN = blood urea nitrogen, Cr = creatinine, UA = uric acid, TG = triglycerides, TC = total cholesterol, HDL = high-density lipoprotein, LDL = low-density lipoprotein, G = glucose

r: correlation coefficient
Table 4
The risks of high TC according to TB quartiles in different genders

| TB Quartiles | Males          |               | Females         |               |
|--------------|----------------|---------------|-----------------|---------------|
| Model 1      | TB values      | Adjusted OR (CI)^ | TB values      | Adjusted OR (CI)^ |
| TB Quartile 1| TB < 9.90 (µmol/L, reference) | 1.005 (0.896–1.127) | TB < 8.10 (µmol/L, reference) | 0.948 (0.774–1.161) |
| TB Quartile 2| 9.90 ≤ TB < 12.80 | 1.094 (0.974–1.227) | 8.10 ≤ TB < 10.40 | 0.857 (0.698–1.054) |
| TB Quartile 3| 12.80 ≤ TB < 16.60 | 1.009 (0.898–1.134) | 10.40 ≤ TB < 13.50 | 0.935 (0.752–1.163) |
| TB Quartile 4| TB ≥ 16.60      | 0.985 (0.982–0.988)** | TB ≥ 13.50      | 0.974 (0.968–0.980)** |
| Model 2      |                | 1.004 (0.996–1.011) |                | 0.991 (0.973–1.009) |
| Model 3      |                |                |                |               |

TB = total bilirubin, OR = odds ratio, CI = confidence interval.

^Logistic regression model 1 with TB Quartile 1 as reference, including age, body mass index, blood pressure, alanine aminotransferase, blood urea nitrogen, creatinine, uric acid, triglycerides, high-density lipoprotein, low-density lipoprotein and glucose as covariates.

Model 2 and 3 analyzed TB as a continuous variable. Model 2 included TB as a covariate and model 3 included age, body mass index, blood pressure, alanine aminotransferase, blood urea nitrogen, creatinine, uric acid, triglycerides, high-density lipoprotein, low-density lipoprotein and glucose as covariates.

* P < 0.05, ** P < 0.01.
Table 5
The risks of high TC according to TB quartiles among people in different age groups

| TB quartile | Male | Female |
|-------------|------|--------|
|             | TB values | Adjusted OR (CI)^ | TB values | Adjusted OR (CI)^ |
| Age ≤ 30    |       |        |          |        |
| Model 1     |       |        |          |        |
| TB Quartile 1 | TB < 10.0 (µmol/L, reference) | | TB < 8.10 (µmol/L, reference) | |
| TB Quartile 2 | 10.00 ≤ TB < 13.10 | 1.602 (1.001–2.565) | 8.10 ≤ TB < 10.80 | 0.920 (0.375–2.256) |
| TB Quartile 3 | 13.10 ≤ TB < 17.40 | 1.282 (0.784–2.095) | 10.80 ≤ TB < 14.40 | 0.700 (0.303–1.618) |
| TB Quartile 4 | TB ≥ 17.40 | 1.410 (0.861–2.307) | TB ≥ 14.40 | 0.927 (0.402–2.137) |
| Model 2     | 0.976 (0.966–0.987)** | | 0.993 (0.978–1.008)** |
| Model 3     | 1.009 (0.978–1.041) | | 0.978 (0.925–1.034) |
| 30 < age ≤ 60 |       |        |          |        |
| Model 1     |       |        |          |        |
| TB Quartile 1 | TB < 9.80 (µmol/L, reference) | | TB < 8.00 (µmol/L, reference) | |
| TB Quartile 2 | 9.80 ≤ TB < 12.70 | 0.999 (0.881–1.132) | 8.00 ≤ TB < 10.30 | 1.176 (0.942–1.469) |
| TB Quartile 3 | 12.70 ≤ TB < 16.40 | 1.106 (0.975–1.254) | 10.30 ≤ TB < 13.30 | 1.121 (0.898–1.400) |
| TB Quartile 4 | TB ≥ 16.40 | 1.030 (0.907–1.170) | TB ≥ 13.30 | 1.022 (0.812–1.286) |
| Model 2     | 0.987 (0.983–0.991)** | | 0.990 (0.985–0.996)** |
| Model 3     | 1.004 (0.996–1.012) | | 0.999 (0.981–1.018) |
| Age > 60    |       |        |          |        |
| Model 1     |       |        |          |        |
| TB Quartile 1 | TB < 10.20 (µmol/L, reference) | | TB < 8.60 (µmol/L, reference) | |

^Adjusted OR with 95% CI
| TB quartile | Male | Female |
|-----------|------|--------|
|           | TB values | Adjusted OR (CI)^ | TB values | Adjusted OR (CI)^ |
| TB Quartile 2 | 10.20 ≤ TB < 13.00 | 0.812 (0.551–1.198) | 8.60 ≤ TB < 10.70 | 1.021 (0.599–1.739) |
| TB Quartile 3 | 13.00 ≤ TB < 16.60 | 0.970 (0.657–1.431) | 10.70 ≤ TB < 13.60 | 1.092 (0.647–1.843) |
| TB Quartile 4 | TB ≥ 16.60 | 0.691 (0.465–1.027) | TB ≥ 13.60 | 1.014(0.595–1.729) |
| Model 2 | | 0.992 (0.983–1.002) | | 0.996 (0.982–1.010) |
| Model 3 | | 0.993 (0.967–1.020) | | 1.006 (0.964–1.050) |

TB = total bilirubin, OR = odds ratio, CI = confidence interval.

^Logistic regression model 1 with TB Quartile 1 as reference, including age, body mass index, blood pressure, alanine aminotransferase, blood urea nitrogen, creatinine, uric acid, triglycerides, high-density lipoprotein, low-density lipoprotein and glucose as covariates.

Model 2 and 3 analyzed TB as a continuous variable. Model 2 included TB as a covariate and model 3 included age, body mass index, blood pressure, alanine aminotransferase, blood urea nitrogen, creatinine, uric acid, triglycerides, high-density lipoprotein, low-density lipoprotein and glucose as covariates.

* P < 0.05, ** P < 0.01.

**Discussion**

Our study found no relationship between TB and TC in both genders of all age groups. Recent articles have described different opinions. J.T. Hughes et al. [14] found that serum TB concentration was positively related with TC in 594 Indigenous Australians. Recent reports found the use of statins have a low negative effect on TB concentration [23]. So they thought this reverse causality could explain the positive correlation between TB and TC. On the contrary, negative relationship between TB and TC was also reported in several articles. Pearson correlation analysis suggested a significantly negative correlation between TC and TB in some researches [12, 13], yet their Pearson correlation coefficients were in low value ranges, similar to our results. No further research was expended to prove their conclusions. Some articles reported that TB treatment decreased the levels of TC and TG in Gunn rats [24, 25]. However, the results of animal studies are not completely applicable to human. In a cross-sectional analysis of 1,711 subjects with type 2 diabetes, TB was inversely associated with age, TC and TG levels in men [13]. The narrow participants ranging and the impact of diabetes make the results of the study less representative of healthy populations. Jenko-Praznikar, Z. et al. [12] proposed a negative relationship between TB and TC in overweight asymptomatic middle-aged adults (64 women and 32 men). Another study on TB and cardiovascular disease found inverse correlations between TB and BMI, LDL and TC.
This study also lacked demographic representation (868 asymptomatic individuals). They speculated that other cardiovascular risk factors caused a negative correlation between TB and TC.

The negative relationship between TB and TC showed by our Spearman correlation study can be explained next. Firstly, it might be the effect of oxidative stress. Heme oxygenase catalyzes the cleavage of the heme ring, which forms ferrous iron, carbon monoxide and bilirubin. Biliverdin reductase (BVR) rapidly reduces biliverdin to bilirubin [26]. BVR physiologically regenerates bilirubin in the catalytic cycle, thereby providing the cytoprotective effect of anti-oxidants. Bilirubin had been reported to be an important chain-breaking anti-oxidant that prevents lipid peroxidation [27] and was also a physiological lipid-lowering agent [28]. As an anti-oxidant, the level of TB in the body might be changed with its anti-oxidant effect. Therefore, the association between TB and TC might be caused by the occurrence of oxidative stress [12]. Animal studies could also confirm this speculation. In diabetes mellitus type I Sprague-Dawley rats models [29], serum TC and both serum and hepatic TG were decreased by TB. In the meanwhile, this effect accompanied by suppression of oxidative stress, as well as liver X receptor-α and Sterol regulatory element binding protein-1 which involved in regulating lipid synthesis and metabolism [30]. Secondly, insulin resistance could influence their relationship. BVR was both a substrate for insulin receptor (IR) tyrosine kinase activity and an IR substrate 1 (IRS-1) serine phosphorylated kinase [31]. And IRS-1 serine phosphorylation was thought to be a mechanism of insulin resistance [32]. Therefore, the increased incidence of insulin resistance would be accompanied by an increase in BVR-catalyzed redox reactions, which would affect the level of TB. In a cross-sectional study [33], 12342 Korean adults aged 20 years and over were involved to investigate the negative relationship between TB and metabolic syndrome. Oxidative stress accompanied the development of metabolic syndrome which was associated with insulin resistance and dislipidemia [33]. They suggested that TB levels were influenced by the insulin resistance-related dyslipidemic status, which could explain the inverse association between TB and TC.

Our study suggested that TB was not a risk predictor for high-TC in a large range of Chinese after excluding the confounding factors. There were no differences in both genders of different age groups. Several studies were consistent with our results. A previous study has showed no relationship existed between TB and blood lipids in 327 heart failure patients with a preserved left ventricular ejection fraction [16]. Jaeseong Jo et. al. [17] proposed that direct bilirubin had a stronger relationship with metabolic syndrome than TB in Korean population. In a cross-sectional and longitudinal study of healthy population [34], the authors found no association between baseline TB and the incidence of dislipidemia in men. No causal relationship existed between TB and lipid status. A previous study [15] reported that the reduction of TB in diabetic patients was accompanied by increased levels of TG, LDL, TC and decreased levels of HDL. But this phenomenon was not found in the healthy control group. However, their sample size was too small (53 type 2 diabetes mellitus patients and 53 non-diabetic subjects) to meaningfully explore potential interactions. Our previous research also showed that TB could not be a risk factor of hypertriglyceridemia in a healthy Chinese population [35]. In summary, our results imply that there is no direct connection between TB and dyslipidemia, and TB is probably just a covariate in the development of dyslipidemia.
There are several limitations in our study. Firstly, it is a cross-sectional study, further longitudinal studies was needed to explore the causal relationship between TB and TC. Secondly, the direct bilirubin and indirect bilirubin were not obtained. Thirdly, failure to detect sex hormone levels in participants, which might affect the level of TB. Fourthly, it was difficult to identify corroborating details of other factors which conduce to the balance of anti-oxidant and oxidant stress among participants including poor eating habits, physical inactivity and a comprehensive assessment of tobacco.

**Conclusions**

In accordance with previous data on a general population, our findings suggested that serum TB was not a risk factor of high TC in a healthy Chinese population. Longitudinal studies are necessary to determine whether serum TB levels have predictive value for high TC in both genders.

**Abbreviations**

TB: total bilirubin; TC:total cholesterol; BMI:body mass index; BUN: blood urea nitrogen; TG: triglycerides; LDL:low-density lipoprotein; G:glucose; Cr: creatinine, HDL: high-density lipoprotein; ALT: alanine aminotransferase; BH: body height; BW: body weight; SBP: systolic blood pressure; DBP: diastolic blood pressure; UA: uric acid; G: glucose; SD: standard deviation; OR: odd ratio; BVR: Biliverdin reductase; IR: insulin receptor; IRS-1: insulin receptor substrate 1

**Declarations**

**Ethics approval and consent to participate**

The organizational review committee and the ethics committee of General Hospital Affiliated to Tianjin Medical University approved the ethics, methods and protocols of the survey. Signed informed consents were collected from all participants. We confirm that all methods were carried out in the light of the relevant guidelines and rules.

**Consent for publication**

Not applicable.

**Availability of data and materials**

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

**Competing interests**

The authors declare that they have no competing interests.
Funding

Not applicable.

Author contributions statement

Zhaowei Meng, Ming Liu and Ke Xu designed the investigation.

Xuemei Zhang, Zhaowei Meng, Chao Huang, YaGuang Fan, Ke Xu, Xue Li, Xiaojun Ren, Mei Zhu, Qing He, Kun Song, Qiyu Jia, Chunmei Zhang and Xiaoran Wang conducted the investigation and collected data.

Xuemei Zhang and Zhaowei Meng performed the statistics.

Xuemei Zhang, Chao Huang and Zhaowei Meng wrote the main manuscript.

All authors reviewed the manuscript.

Acknowledgments

The National Key Clinical Specialty Project supported this study and awarded it to the Departments of Nuclear Medicine and Radiology.

This study was supported by Tianjin Medical University General Hospital New Century Excellent Talent Program; Young and Middle-aged Innovative Talent Training Program from Tianjin Education Committee; and Talent Fostering Program (the 131 Project) from Tianjin Education Committee, Tianjin Human Resources and Social Security Bureau (awarded to Zhaowei Meng).

This study was supported by China National Natural Science Foundation grant number 81571709 and 81971650, Key Project of Tianjin Science and Technology Committee Foundation grant 16JCZDJC34300 (awarded to Zhaowei Meng).

This study was also supported by Tianjin Science and Technology Committee Foundation grants 11ZCGYSY05700, 12ZCZDSY20400, and 13ZCZDSY20200 (awarded to Qing Zhang, Qiyu Jia and Kun Song).

References

1. Hoogendoorn A, den Hoedt S, Hartman EMJ, Krabbendam-Peters I, Te Lintel Hekkert M, van der Zee L, van Gaalen K, Witberg KT, Dorst K, Ligthart JMR, et al. Variation in Coronary Atherosclerosis Severity Related to a Distinct LDL (Low-Density Lipoprotein) Profile: Findings From a Familial Hypercholesterolemia Pig Model. Arterioscler Thromb Vasc Biol. 2019;39:2338–52.

2. Mattina A, Giammanco A, Giral P, Rosenbaum D, Carrie A, Cluzel P, Redheuil A, Bittar R, Beliard S, Noto D, et al. Polyvascular subclinical atherosclerosis in familial hypercholesterolemia: The role of cholesterol burden and gender. Nutr Metab Cardiovasc Dis. 2019;29:1068–76.
3. Csont T, Bereczki E, Bencsik P, Fodor G, Gorbe A, Zvara A, Csonka C, Puskas LG, Santha M, Ferdinandy P. Hypercholesterolemia increases myocardial oxidative and nitrosative stress thereby leading to cardiac dysfunction in apoB-100 transgenic mice. Cardiovasc Res. 2007;76:100–9.

4. Varga ZV, Kupai K, Szucs G, Gaspar R, Paloczi J, Farago N, Zvara A, Puskas LG, Razga Z, Tiszlavicz L, et al. MicroRNA-25-dependent up-regulation of NADPH oxidase 4 (NOX4) mediates hypercholesterolemia-induced oxidative/nitrative stress and subsequent dysfunction in the heart. J Mol Cell Cardiol. 2013;62:111–21.

5. Gopinathan V, Miller NJ, Milner AD, Rice-Evans CA. Bilirubin and ascorbate antioxidant activity in neonatal plasma. FEBS Lett. 1994;349:197–200.

6. Farrera JA, Jauma A, Ribo JM, Peire MA, Parelldata PP, Roques-Choua S, Bienvenue E, Seta P. The antioxidant role of bile pigments evaluated by chemical tests. Bioorg Med Chem. 1994;2:181–5.

7. Djousse L, Levy D, Cupples LA, Evans JC, D’Agostino RB, Ellison RC. Total serum bilirubin and risk of cardiovascular disease in the Framingham offspring study. Am J Cardiol. 2001;87:1196–200. A1194, 1197.

8. Vitek L, Jirsa M, Brodanova M, Kalab M, Marecek Z, Danzig V, Novotny L, Kotal P. Gilbert syndrome and ischemic heart disease: a protective effect of elevated bilirubin levels. Atherosclerosis. 2002;160:449–56.

9. Temme EH, Zhang J, Schouten EG, Kesteloot H. Serum bilirubin and 10-year mortality risk in a Belgian population. Cancer Causes Control. 2001;12:887–94.

10. Chin HJ, Cho HJ, Lee TW, Na KY, Oh KH, Joo KW, Yoon HJ, Kim YS, Ahn C, Han JS, et al. The mildly elevated serum bilirubin level is negatively associated with the incidence of end stage renal disease in patients with IgA nephropathy. J Korean Med Sci. 2009;24(Suppl):22–9.

11. McArdle PF, Whitcomb BW, Tanner K, Mitchell BD, Shuldiner AR, Parsa A. Association between bilirubin and cardiovascular disease risk factors: using Mendelian randomization to assess causal inference. BMC Cardiovasc Disord. 2012;12:16.

12. Jenko-Praznikar Z, Petelin A, Jurdana M, Ziberna L. Serum bilirubin levels are lower in overweight asymptomatic middle-aged adults: an early indicator of metabolic syndrome? Metabolism 2013, 62:976–985.

13. Kim ES, Mo EY, Moon SD, Han JH. Inverse association between serum bilirubin levels and arterial stiffness in Korean women with type 2 diabetes. PLoS One. 2014;9:e109251.

14. Hughes JT, Barzi F, Hoy WE, Jones GRD, Rathnayake G, Majoni SW, Thomas MAB, Sinha A, Cass A, MacIsaac RJ, et al. Bilirubin concentration is positively associated with haemoglobin concentration and inversely associated with albumin to creatinine ratio among Indigenous Australians: eGFR Study. Clin Biochem. 2017;50:1040–7.

15. Dullaart RP, de Vries R, Lefrandt JD. Increased large VLDL and small LDL particles are related to lower bilirubin in Type 2 diabetes mellitus. Clin Biochem. 2014;47:170–5.

16. Zheng H, Li Y, Xie N. Association of serum total bilirubin levels with diastolic dysfunction in heart failure with preserved ejection fraction. Biol Res. 2014;47:7.
17. Jo J, Yun JE, Lee H, Kimm H, Jee SH. Total, direct, and indirect serum bilirubin concentrations and metabolic syndrome among the Korean population. Endocrine. 2011;39:182–9.
18. Wang S, Zhang J, Zhu L, Song L, Meng Z, Jia Q, Li X, Liu N, Hu T, Zhou P, et al: Association between liver function and metabolic syndrome in Chinese men and women. Scientific Reports 2017, 7.
19. Zhou PP, Meng ZW, Liu M, Ren XJ, Zhu M, He Q, Zhang Q, Liu L, Song K, Jia Q, et al: The associations between leukocyte, erythrocyte or platelet, and metabolic syndrome in different genders of Chinese. Medicine 2016, 95.
20. Ren XJ, Meng ZW, Liu M, Zhu M, He Q, Zhang Q, Liu L, Song K, Jia QY, Jia Q, et al: No associations exist between mean platelet volume or platelet distribution width and thyroid function in Chinese. Medicine 2016, 95.
21. Meng ZW, Liu M, Zhang Q, Liu L, Song K, Tan J, Jia Q, Zhang GZ, Wang RF, He YJ, et al: Gender and Age Impact on the Association Between Thyroid-Stimulating Hormone and Serum Lipids. Medicine 2015, 94.
22. Zhang Q, Lou SS, Meng ZW, Ren XJ. Gender and age impacts on the correlations between hyperuricemia and metabolic syndrome in Chinese. Clin Rheumatol. 2011;30:777–87.
23. Ong KL, Wu BJ, Cheung BM, Barter PJ, Rye KA. Association of lower total bilirubin level with statin usage: the United States National Health and Nutrition Examination Survey 1999–2008. Atherosclerosis. 2011;219:728–33.
24. Boon AC, Hawkins CL, Bisht K, Coombes JS, Bakrania B, Wagner KH, Bulmer AC. Reduced circulating oxidized LDL is associated with hypocholesterolemia and enhanced thiol status in Gilbert syndrome. Free Radic Biol Med. 2012;52:2120–7.
25. Fu YY, Kang KJ, Ahn JM, Kim HR, Na KY, Chae DW, Kim S, Chin HJ. Hyperbilirubinemia reduces the streptozotocin-induced pancreatic damage through attenuating the oxidative stress in the Gunn rat. Tohoku J Exp Med. 2010;222:265–73.
26. Dore S, Takahashi M, Ferris CD, Zakhary R, Hester LD, Guastella D, Snyder SH. Bilirubin, formed by activation of heme oxygenase-2, protects neurons against oxidative stress injury. Proc Natl Acad Sci U S A. 1999;96:2445–50.
27. Maines MD. New insights into biliverdin reductase functions: linking heme metabolism to cell signaling. Physiology (Bethesda). 2005;20:382–9.
28. Bulmer AC, Verkade HJ, Wagner KH. Bilirubin and beyond: a review of lipid status in Gilbert’s syndrome and its relevance to cardiovascular disease protection. Prog Lipid Res. 2013;52:193–205.
29. Xu J, Lee ES, Baek SH, Ahn SY, Kim S, Na KY, Chae DW, Chin HJ. Effect of bilirubin on triglyceride synthesis in streptozotocin-induced diabetic nephropathy. J Korean Med Sci. 2014;29(Suppl 2):155–63.
30. Yao DW, Luo J, He QY, Xu HF, Li J, Shi HB, Wang H, Chen Z, Loor JJ. Liver X receptor alpha promotes the synthesis of monounsaturated fatty acids in goat mammary epithelial cells via the control of stearoyl-coenzyme A desaturase 1 in an SREBP-1-dependent manner. J Dairy Sci. 2016;99:6391–402.
31. Lerner-Marmarosh N, Shen J, Torno MD, Kravets A, Hu Z, Maines MD. Human biliverdin reductase: a member of the insulin receptor substrate family with serine/threonine/tyrosine kinase activity. Proc Natl Acad Sci U S A. 2005;102:7109–14.

32. Tanti JF, Gremeaux T, Van Obberghen E, Le Marchand-Brustel Y. Insulin receptor substrate 1 is phosphorylated by the serine kinase activity of phosphatidylinositol 3-kinase. Biochem J. 1994;304(Pt 1):17–21.

33. Choi SH, Yun KE, Choi HJ. Relationships between serum total bilirubin levels and metabolic syndrome in Korean adults. Nutrition Metabolism Cardiovascular Diseases. 2013;23:31–7.

34. Oda E. Cross-sectional and longitudinal associations between serum bilirubin and dyslipidemia in a health screening population. Atherosclerosis. 2015;239:31–7.

35. Zhang X, Meng Z, Li X, Liu M, Ren X, Zhu M, He Q, Zhang Q, Song K, Jia Q, et al. The association between total bilirubin and serum triglyceride in both sexes in Chinese. Lipids Health Dis. 2018;17:217.

**Figures**

![Figure A](image1.png)

![Figure B](image2.png)

![Figure C](image3.png)

![Figure D](image4.png)
Figure 1

Incidence of high TC in different genders by TB quartiles. Picture (A) analyzed all participants, while pictures (B, C, D) separately analyzed participants in different age groups. Participants were divided into three groups according to age (first group: age ≤ 30; second group: 30 < age ≤ 60; third group: age > 60). For picture (A), TB quartiles 1 to 4 referred to the followings respectively: male: TB ≤ 9.9 umol/L, 9.9 umol/L < TB ≤ 12.8 umol/L, 12.8 umol/L < TB ≤ 16.6 umol/L, TB ≥ 16.6 umol/L; female: TB ≤ 8.1 umol/L, 8.1 umol/L < TB ≤ 10.4 umol/L, 10.4 umol/L < TB ≤ 13.5 umol/L, TB ≥ 13.5 umol/L. For pictures (B)(C) and (D), TB quartiles were also calculated separately in each gender with different age groups. * P < 0.05, ** P < 0.01.