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Original Article

Higher serum total bilirubin concentration is associated with lower risk of renal insufficiency in an adult population

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Abstract: *Background*: Chronic inflammation is proposed to play a central role in the pathogenesis of chronic kidney disease (CKD), and serum bilirubin has antioxidant and anti-inflammatory effects. We investigated the association between serum total bilirubin (Tb) concentration and renal function in an adult population. *Methods*: We conducted a cross-sectional study and collected anthropometric measurements, fasting blood tests, lifestyle habits and medical history of 3876 subjects attending a health examination. Renal insufficiency was defined as estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 calculated by using the CKD-EPI equation. *Results:* Serum Tb concentrations were higher in subjects without renal insufficiency than in those with renal insufficiency. Multivariable linear regression analysis showed that Tb concentration was positively associated with eGFR after adjusting for important CKD risk factors (P=0.04). Multivariable logistic regression analysis also revealed that higher Tb concentration (each increment of 1.71 μmol/L) (0.1 mg/dL) was associated with a reduced risk of renal insufficiency: odds ratios were 0.94 (P=0.005) for men and 0.90 (P=0.015) for women, respectively. When subjects were divided into quartiles of serum Tb, multivariable-adjusted odds ratios for renal insufficiency comparing the fourth to the first Tb quartile were 0.49 (P=0.001) for men and 0.35 (P=0.003) for women. A stepwise exclusion of subjects, first those with possible liver disease and second, those with CKD stage 4 and 5, showed consistent results. *Conclusion*: Higher serum Tb concentration was associated with lower risk of renal insufficiency, regardless of other conventional CKD risk factors.

Keywords: Bilirubin, kidney function, chronic kidney disease, cardiovascular disease

Introduction

Chronic kidney disease (CKD) is a relatively common disorder in the general population. It is not only associated with an increased risk of progression to end-stage renal disease [1], but also related to both a higher prevalence of cardiovascular disease (CVD) and premature death [2]. Screening studies for CKD in the general population have shown that the estimated prevalence of CKD is approximately 13.1% in the United States [3], 12.9% in Japan [4], 10.8% in China [5], and 11.93% in Taiwan [6]. In view of the high proportion of CKD in the general population, there is a need for elucidating its pathogenetic mechanisms. Diabetes, hypertension, and vascular disease have been proposed to be the most important risk factors for the development and progression of CKD [7]. However, these factors cannot completely explain the variation in the population incidence and prevalence of CKD. Several recent epidemiological studies have found that chronic inflammation also plays a central role in the pathogenesis of CKD, and several biomarkers of inflammation, including C-reactive protein

(CRP), pro-inflammatory cytokines, and white blood cell (WBC) counts, are shown to be independently associated with faster rates of kidney function loss in CKD [8, 9].

Bilirubin, an end-product of heme metabolism, is shown to have antioxidant activity. It can suppress oxidation of lipids, especially low-density lipoprotein cholesterol (LDL-C), and has anti-inflammatory activity that can inhibit tumor necrosis factor-α-induced up-regulation of E-selectin, vascular cell adhesion molecule, and intercellular adhesion molecule [10-12]. Based on these physiological effects of bilirubin, epidemiological studies have reported an inverse association between serum bilirubin concentrations and the risk of CVD, such as coronary artery disease, ischemic stroke and peripheral arterial disease [13-17]. Furthermore, cross-sectional and prospective clinical studies have found that higher serum total bilirubin concentration is associated with a reduced risk of CKD [18-20], although other studies have reported a contradictory result [21, 22]. In our previous study, higher serum total bilirubin concentrations, even within the normal range, are associated with lower total WBC counts, regardless of other classic cardiovascular risk factors [23]. Based on the potential role of inflammation in the development of CKD and the anti-inflammatory activity of bilirubin, this cross-sectional study aimed to evaluate whether serum total bilirubin concentration was associated with a change in kidney function in an adult Taiwanese population attending a physical health examination.

Materials and methods

Study population and data collection

We examined a total of 4852 participants undergoing a self-paid packaged physical examination from August 2000 to April 2002 at a medical center in central Taiwan. Medical history of hypertension, diabetes and CVD (including myocardial infarction and stroke), as well as lifestyle habits, including smoking and alcohol consumption, were collected using a structured questionnaire. Smoking was defined as current tobacco usage. Alcohol consumption was divided into less than one intake per week, habitual alcohol consumption (defined as drinking at least once per week) and excessive alcohol consumption (defined as more than 14 drinks or 210 g per week in men and more than 7 drinks or 105 g per week in women). Data on medication use, including antihypertensive, antidiabetic, anti-hyperlipidemic, and urate-lowering medications and analgesics, were also collected using the aforementioned questionnaire. All subjects were weighed in light clothing with no shoes and their height was also measured. Blood pressure (BP) in the arm was measured using standard mercury sphygmomanometers in a sitting position after 5 min of rest. After an overnight fast of at least 8 hours, a venous blood sample was collected to measure plasma glucose, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, triglyceride, and high-density lipoprotein-cholesterol (HDL-C) concentrations using a photometric enzymatic method with a chemistry analyzer (Hitachi 7600, Tokyo, Japan) at the central laboratory of the hospital. Serum creatinine (SCr) was measured using the Jaffe method (Hitachi 7600, Tokyo, Japan), which was calibrated from the isotope dilution mass spectrometry method. The internal quality control and external quality assessment of all laboratory methods were acceptable. This study was approved by the Institutional Review Board of Taichung Veterans General Hospital (protocol no: CE14268A). Of the 4832 participants aged ≥20 years, 695 did not fill out the questionnaire; an additional 259 participants with missing BP measurements and 2 with missing data on SCr and serum total bilirubin concentration were also excluded, finally allowing 3876 participants to be included in the analysis.

A diagnosis of hypertension was assigned if the subject reported a physician diagnosis of hypertension, if the subject reported taking medications for hypertension, or if the systolic BP was ≥140 mmHg or diastolic BP was ≥90 mmHg. A diagnosis of diabetes mellitus was assigned if the subject reported a physician diagnosis of diabetes, if the subject reported taking medications for diabetes, or if the fasting plasma glucose concentration was ≥7 mmol/L (126 mg/dL). Hypercholesterolemia was defined as total cholesterol concentration ≥5.18 mmol/L (200 mg/dL), hypertriglyceridemia was defined as a triglyceride concentration ≥1.70 mmol/L (150 mg/dL), and a low HDL-C concentration was defined as HDL-C <1.04 mmol/L (40 mg/dL) for men and <1.29 mmol/L

Table 1. Characteristics of subjects by eGFR (mL/min/1.73 m^2) status (n=3876)

	All	eGFR ≥60	eGFR <60	*p* value
Men				
n	2260 (100)	1892 (83.7)	368 (16.3)	
Age (years)	51.8±12.2	49.6±11.3	63.4±9.9	<0.001
BMI (kg/m^2)	24.5±3.2	24.5±3.2	24.7±3.0	0.33
Systolic BP (mmHg)	123±18	122±17	130±20	<0.001
Diastolic BP (mmHg)	77±12	76±11	79±12	<0.001
Fasting glucose (mmol/L)	5.22 (4.89-5.72)	5.22 (4.89-5.67)	5.33 (4.94-5.78)	0.09
Total cholesterol (mmol/L)	5.05±0.94	5.05±0.94	5.09±0.92	0.47
Triglyceride (mmol/L)	1.32 (0.90-1.93)	1.33 (0.90-1.95)	1.28 (0.88-1.80)	0.21
HDL-C (mmol/L)	1.34±0.34	1.35±0.33	1.33±0.37	0.42
AST (U/L)	23 (20-29)	23 (20-29)	24 (20-29)	0.78
ALT (U/L)	25 (19-37)	26 (19-38)	23 (17-32)	<0.001
Creatinine (μmol/L)	103.7±22.9	98.3±10.9	131.4±41.1	<0.001
Uric acid (μmol/L)	416.3±93.0	410.0±91.2	448.4±95.4	<0.001
Previous stroke	16 (0.7)	8 (0.4)	8 (2.2)	0.002
Previous myocardial infarction	43 (1.9)	29 (1.5)	14 (3.8)	0.007
Medication for hypertension	317 (14.0)	207 (10.9)	110 (29.9)	<0.001
Medication for diabetes	108 (4.8)	94 (5.0)	14 (3.8)	0.41
Medication for hyperlipidemia	54 (2.4)	41 (2.2)	13 (3.5)	0.17
Medication for hyperuricemia	73 (3.2)	39 (2.1)	34 (9.2)	<0.001
Analgesics	48 (2.1)	36 (1.9)	12 (3.3)	0.15
Smoking*	725 (34.3)	642 (36.0)	83 (24.9)	<0.001
Habitual alcohol consumption*	447 (22.5)	399 (23.6)	48 (16.4)	0.009
Total bilirubin (μmol/L)	14.63±6.61	14.77±6.58	13.88±6.70	0.02
Women				
n	1616 (100)	1465 (90.7)	151 (9.3)	
Age (years)	49.3±11.8	48.0±11.3	61.5±8.8	<0.001
BMI (kg/m^2)	23.4±3.4	23.3±3.3	24.8±3.8	<0.001
Systolic BP (mmHg)	119±19	117±18	135±24	<0.001
Diastolic BP (mmHg)	73±12	73±11	79±12	<0.001
Fasting glucose (mmol/L)	5.06 (4.78-5.44)	5.06 (4.72-5.44)	5.22 (4.94-5.72)	<0.001
Total cholesterol (mmol/L)	5.02±0.93	4.98±0.91	5.31±1.02	<0.001
Triglyceride (mmol/L)	0.95 (0.68-1.44)	0.93 (0.67-1.39)	1.37 (0.86-1.89)	<0.001
HDL-C (mmol/L)	1.64±0.40	1.65±0.40	1.57±0.42	0.03
AST (U/L)	20 (17-24)	20 (17-24)	22 (19-29)	<0.001
ALT (U/L)	17 (13-24)	17 (13-24)	19 (15-28)	0.01
Creatinine (μmol/L)	78.8±37.8	74.3±9.0	122.5±111.6	<0.001
Uric acid (μmol/L)	322.2±79.2	314.8±74.0	395.0±91.5	<0.001
Previous stroke	10 (0.6)	5 (0.3)	5 (3.3)	0.001
Previous myocardial infarction	12 (0.7)	8 (0.5)	4 (2.6)	0.02
Medication for hypertension	175 (10.8)	121 (8.3)	54 (35.8)	<0.001
Medication for diabetes	45 (2.8)	30 (2.0)	15 (9.9)	<0.001
Medication for hyperlipidemia	18 (1.1)	13 (0.9)	5 (3.3)	0.02
Medication for hyperuricemia	11 (0.7)	5 (0.3)	6 (4.0)	<0.001
Analgesics	54 (3.3)	48 (3.3)	6 (4.0)	0.82
Smoking*	66 (4.6)	64 (4.9)	2 (1.6)	0.14
Habitual alcohol consumption*	46 (3.7)	45 (3.9)	1 (1.0)	0.17

Total bilirubin (μmol/L)	11.42±5.08	11.53±5.02	10.41±5.55	0.01

Values are expressed as mean ± standard deviation, median (interquartile range) and number (percentage). BMI, body mass index; BP, blood pressure; HDL-C, high-density lipoprotein cholesterol. AST, aspartate aminotransferase; ALT, alanine aminotransferase. *p* values were determined by chi-square analysis, unpaired *t*-test or Mann-Whitney U test. *Some data on smoking (n=341, M:F=145:196) and alcohol consumption (n=633, M:F=277:356) were missing.

(50 mg/dL) for women, respectively [24]. Hyperuricemia was defined as a serum uric acid concentration ≥420 μmol/L (7.0 mg/dL) in men and ≥360 μmol/L (6.0 mg/dL) in women, or if the subject reported taking urate-lowering medications.

The estimated glomerular filtration rate (eGFR) was calculated by using the CKD-Epidemiology Collaboration (EPI) equation [25]: eGFR (mL/min/1.73 m^2) = 141 × min(SCr/κ, 1)$^{\alpha}$ × max(SCr/κ, 1)$^{-1.209}$ × 0.993^{age} × 1.018 [if female], where κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of SCr/κ or 1, and max indicates the maximum of SCr/κ or 1. Renal insufficiency was defined as eGFR <60 mL/min/1.73 m^2.

Statistical analysis

Body mass index (BMI, weight in kilograms/height^2 in meters) was calculated by dividing body-weight by the square of height. Continuous variables were presented as the mean value ± standard deviation for normally distributed data and as median (25^{th}-75^{th} percentile) for skewed data (fasting glucose, AST, ALT and triglyceride) and categorical variables were presented as number (percentage). Categorical and continuous variables were compared between groups by chi-square analysis and unpaired *t*-test or Mann-Whitney U test, respectively. Multivariable linear regression analysis was used to evaluate the independent correlation between eGFR and total bilirubin concentration after adjusting for age, sex, BMI, smoking, alcohol use, total bilirubin, total cholesterol, triglyceride, HDL-C concentrations, use of anti-hyperlipidemic medications, hypertension, diabetes, hyperuricemia, and history of CVD (including previous myocardial infarction and stroke).

In addition, total bilirubin concentration differs by sex, so it was separately divided into quartiles arbitrarily for men (0<Q1≤8.55, 8.55<Q2≤11.97, 11.97<Q3≤15.39 and Q4>15.39 μmol/L) and women (0<Q1≤6.84, 6.84<Q2≤10.26, 10.26<Q3≤13.68 and Q4>13.68 μmol/L). Multivariable logistic regression analysis was performed to calculate the odds ratio (OR) of renal insufficiency by quartiles of serum total bilirubin concentration, separately in men and women, after adjusting for age, BMI, smoking, alcohol use, hypercholesterolemia, hypertriglyceridemia, low HDL-C, use of anti-hyperlipidemic medications, hypertension, diabetes, hyperuricemia, and history of CVD.

To address a possible influence from liver disease, we repeated the analysis after excluding subjects with excessive alcohol consumption or laboratory evidence suggestive of possible liver disease: AST or ALT concentration > 2X upper limit of normal range (AST, 8-38 U/L; ALT, 4-44 U/L), serum total bilirubin concentration >34.2 μmol/L (2.0 mg/dL), and serum albumin concentration <35 g/L (3.5 g/dL); finally, to address a differential influence from CKD stages, we repeated the analysis after further excluding subjects with CKD stage 4 and 5 (eGFR <30 mL/min/1.73 m^2). All statistical analyses were performed using the Statistical Package for the Social Sciences statistical software for Windows, version 12.0 (SPSS Inc., Chicago, IL, USA), and a two-tailed *p* value of <0.05 was considered statistically significant.

Results

Characteristics of subjects by eGFR status

The mean age of the 3876 subjects (2260 males and 1616 females) was 50.7±12.1 years. Serum total bilirubin concentration was higher in men than in women (*P*<0.001), and it was higher in subjects without renal insufficiency than in those with renal insufficiency in both men (14.77±6.58 vs. 13.88±6.70 μmol/L, *P*=0.02) and women (11.53±5.02 vs. 10.41±5.55 μmol/L, *P*=0.01). In addition, older age, higher systolic BP, diastolic BP, uric acid level, rate of previous stroke, rate of previous myocardial infarction, and prevalence of using medications for hypertension and hyperuricemia,

Table 2. Association between eGFR (mL/min/1.73 m^2) and CKD risk factors

Variable	β	Standardized β	p value	95% CI for β
Age (years)	-0.696	-0.525	<0.001	-0.74~-0.66
Sex (male =0, female =1)	4.323	0.132	<0.001	3.21~5.44
BMI (kg/m^2)	-0.057	-0.012	0.47	-0.21~0.10
Total cholesterol (mmol/L)	-0.442	-0.026	0.12	-0.99~0.11
Triglyceride (mmol/L)	0.534	0.055	0.001	0.22~0.85
HDL-C (mmol/L)	-0.257	-0.006	0.72	-1.68~1.16
Hypertension	-1.769	-0.050	0.001	-2.85~-0.69
Diabetes mellitus	2.841	0.050	0.001	1.23~4.45
Previous CVD	-3.157	-0.028	0.046	-6.26~-0.06
Hyperuricemia	-5.737	-0.175	<0.001	-6.70~-4.78
Medication for hyperlipidemia	1.113	0.009	0.51	-2.21~4.44
Smoking	-0.170	-0.004	0.78	-1.33~0.99
Habitual alcohol consumption	1.137	0.025	0.09	-0.16~2.43
Total bilirubin (1.71 μmol/L) (0.1 mg/dL)	0.132	0.030	0.04	0.006~0.26

Multivariable linear regression adjusted for age, sex, BMI, smoking, alcohol use, total bilirubin, total cholesterol, triglyceride, HDL-C concentrations, anti-hyperlipidemic medications, hypertension, diabetes, hyperuricemia, and previous CVD (including stroke and myocardial infarction). CI, confidence interval.

Table 3. Odds ratios for renal insufficiency (eGFR<60 mL/min/1.73 m^2)

	Model 1		Model 2		Model 3	
Variable	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Men						
Age (years)	1.13 (1.11~1.15)	<0.001	1.12 (1.11~1.14)	<0.001	1.13 (1.11~1.14)	<0.001
Low HDL-C	1.73 (1.13~2.63)	0.01	1.79 (1.14~2.83)	0.01	1.67 (1.05~2.65)	0.03
Diabetes mellitus	0.47 (0.29~0.77)	0.003	0.46 (0.27~0.77)	0.003	0.48 (0.28~0.80)	0.005
Hyperuricemia	3.08 (2.25~4.21)	<0.001	3.18 (2.29~4.40)	<0.001	3.21 (2.31~4.46)	<0.001
Habitual alcohol consumption	0.66 (0.45~0.98)	0.04	0.69 (0.46~1.02)	0.06	0.67 (0.45~1.00)	0.05
Total bilirubin (μmol/L)						
Q1 (≤8.55)	1 (reference)		1 (reference)		1 (reference)	
Q2 (8.56-11.97)	0.73 (0.48~1.12)	0.15	0.77 (0.50~1.21)	0.26	0.86 (0.55~1.35)	0.50
Q3 (11.98-15.39)	0.50 (0.32~0.81)	0.004	0.50 (0.30~0.81)	0.005	0.55 (0.33~0.90)	0.02
Q4 (>15.39)	0.49 (0.32~0.74)	0.001	0.54 (0.34~0.84)	0.006	0.60 (0.38~0.94)	0.03
Women						
Age (years)	1.11 (1.08~1.14)	<0.001	1.11 (1.08~1.14)	<0.001	1.11 (1.08~1.14)	<0.001
Hypertension	2.01 (1.21~3.33)	0.007	1.94 (1.15~3.29)	0.01	1.61 (0.94~2.76)	0.08
Previous CVD	4.48 (1.36~14.71)	0.01	4.90 (1.49~16.08)	0.009	5.37 (1.63~17.69)	0.006
Hyperuricemia	4.08 (2.48~6.71)	<0.001	4.40 (2.62~7.39)	<0.001	3.91 (2.31~6.61)	<0.001
Total bilirubin (μmol/L)						
Q1 (≤6.84)	1 (reference)		1 (reference)		1 (reference)	
Q2 (6.85-10.26)	0.43 (0.23~0.78)	0.006	0.41 (0.22~0.75)	0.004	0.49 (0.26~0.91)	0.03
Q3 (10.27-13.68)	0.32 (0.16~0.66)	0.002	0.31 (0.15~0.66)	0.002	0.36 (0.17~0.78)	0.009
Q4 (>13.68)	0.35 (0.17~0.70)	0.003	0.35 (0.17~0.74)	0.006	0.42 (0.20~0.90)	0.03

Multivariable logistic regression adjusted for age, BMI, smoking, alcohol use, bilirubin quartiles, hypercholesterolemia, hypertriglyceridemia, low HDL-C, anti-hyperlipidemic medications, hypertension, diabetes, hyperuricemia, and previous CVD. OR, odds ratio. Model 1: all included participants in **Table 1**. Model 2: model 1, but excluded participants with possible liver disease (n=323, M: F=257:66). Model 3: model 2, but excluded participants with CKD stage 4 and 5 (n=12, M: F=6:6).

and lower ALT, smoking rate and habitual alcohol consumption rate were found in male subjects with renal insufficiency, compared to those without renal insufficiency; and, older

age, higher BMI, systolic BP, diastolic BP, fasting glucose, total cholesterol, triglyceride, AST, ALT, uric acid, prevalence of previous stroke and prevalence of previous myocardial infarction, and prevalence of using medications for hypertension, diabetes, hyperlipidemia and hyperuricemia, and lower HDL-C concentrations were found in female subjects with renal insufficiency, compared to those without renal insufficiency (**Table 1**).

Association between total bilirubin concentration and eGFR

Multivariable linear regression analysis showed that total bilirubin concentration was independently and positively associated with eGFR (P=0.04), after adjusting for age, sex, BMI, smoking, alcohol use, total cholesterol, triglyceride, HDL-C concentrations, use of anti-hyperlipidemic medications, hypertension, diabetes, hyperuricemia, and previous CVD (**Table 2**).

Association between total bilirubin concentration and renal insufficiency

Multivariable logistic regression analysis revealed that each increment of 1.71 μmol/L (0.1 mg/dL) in the serum total bilirubin concentration was independently associated with a reduced risk of renal insufficiency: adjusted ORs were 0.94 (95% CI 0.91-0.98, P=0.005) for men and 0.90 (95% CI 0.82-0.98, P=0.015) for women, respectively. Compared with the first total bilirubin quartile (Q1), the adjusted OR of renal insufficiency was lowest in Q4 (0.49, 95% CI 0.32-0.74, P=0.001) for men and in Q3 (0.32, 95% CI 0.16-0.66, P=0.002) for women, respectively (**Table 3**, model 1). Furthermore, additional individual adjustment of AST, ALT and use of analgesics did not significantly confound the relationship between serum total bilirubin and renal insufficiency. Therefore we did not find evidence for significant influences of AST, ALT and analgesics on our results.

When we repeated the multivariable logistic regression analysis after further excluding participants with a possible influence from liver disease (n=323; total bilirubin Q1:Q2:Q3:Q4 were 47:53:58:99 in men and 11:9:17:29 in women, respectively), the association between higher serum total bilirubin concentration (each increment of 1.71 μmol/L or 0.1 mg/dL) and lower risk of renal insufficiency remained: adjusted ORs were 0.94 (95% CI 0.89-0.98, P=0.007) for men and 0.86 (95% CI 0.77-0.95, P=0.003) for women, respectively; besides, the adjusted OR for each total bilirubin quartile (Q2, Q3, Q4) remained very much the same for both men and women (**Table 3**, model 2). Thereafter, when we further excluded subjects with CKD stage 4 and 5 (eGFR <30 mL/min/1.73 m^2) (n=12; total bilirubin Q1:Q2:Q3:Q4 were 5:1:0:0 in men and 5:0:1:0 in women, respectively), higher serum total bilirubin concentration (each increment of 1.71 μmol/L or 0.1 mg/dL) remained independently associated with lower risk of renal insufficiency: adjusted ORs were 0.94 (95% CI 0.90-0.99, P=0.016) for men and 0.88 (95% CI 0.80-0.98, P=0.016) for women, respectively; besides, the adjusted OR for each total bilirubin quartile (Q2, Q3, Q4) was slightly elevated in both men and women (**Table 3**, model 3).

Discussion

In this cross-sectional study of a Taiwanese adult population, we demonstrated the relationship between serum total bilirubin concentration and eGFR or renal insufficiency. The serum total bilirubin concentrations were higher in subjects without renal insufficiency than in those with renal insufficiency. After adjusting for several important CKD risk factors (age, sex, BMI, smoking, alcohol use, total cholesterol, triglyceride, HDL-C concentrations, anti-hyperlipidemic medications, hypertension, diabetes, hyperuricemia, and previous CVD), serum total bilirubin concentration remained positively associated with eGFR. In addition, the risk of renal insufficiency was lower among the higher quartiles of total bilirubin concentration than in the lowest quartile for both men and women after considering other conventional CKD risk factors. This suggested that a higher total bilirubin concentration was independently associated with higher eGFR and a lower risk of renal insufficiency.

Several previous studies have shown similar results. A longitudinal study in Korea found that a higher serum direct bilirubin concentration reduces the risk of CKD development [19]. Another cohort study in Japan showed lower serum total bilirubin concentrations to be a novel risk factor for CKD progression [18]. In two cross-sectional studies from Korea and Japan, there was a negative correlation

between serum total bilirubin concentration and prevalence of diabetic CKD [26], and a positive relationship between serum total bilirubin concentration and eGFR [27]. In brief, our findings were consistent with the results of those studies, indicating that a higher serum total bilirubin concentration is associated with higher eGFR and lower risk of renal insufficiency.

Some plausible mechanisms have been proposed for the relationship between total serum bilirubin concentration and kidney function [28]. First, bilirubin is an end-product of heme catabolism by heme oxygenase (HO), and it has been postulated that bilirubin can act as an endogenous antioxidant [11] and cytoprotectant [29] in the human body. However, the lack of HO-1 can lead to an increased production of reactive oxygen species that accumulate in vascular smooth muscle cells, and contribute to the pathogenesis of atherosclerosis [30]. Oxidative stress has been proposed to be an important mechanism of renal dysfunction and is very critical in progressive CKD. Therefore, higher serum bilirubin concentrations would be expected to protect against the occurrence of CKD. Second, it has been demonstrated that serum total bilirubin concentration has a negative relationship with endothelial dysfunction [13], which is also a pathogenetic factor of CKD [31]. Third, abnormalities of lipoproteins may predispose to atherosclerosis, and thus lead to the development of CKD. Bilirubin is found to be positively correlated to HDL-C, but negatively correlated to elevated triglycerides and small LDL particles [32], so a protective effect of bilirubin on CKD seems reasonable. In addition, insulin resistance is often characterized by dyslipidemia of low HDL-C concentrations and hypertriglyceridemia. In our study, triglyceride concentrations negatively correlated with serum bilirubin concentration, but there was a positive relationship between HDL-C and bilirubin concentrations in both sexes (data not shown). These findings are consistent with a previous study that found a significantly negative relationship between bilirubin concentration and incident metabolic syndrome [33].

Fourth, diabetes is one of the most important risk factors for CKD. Previous studies have shown that a higher serum total bilirubin concentration provides a protective effect against diabetes, and may predict a lower risk of development of type 2 diabetes and diabetic nephropathy [26, 34, 35]. And fifth, some epidemiological studies have found that inflammation plays a central role in the pathogenesis of CKD, and several biomarkers of inflammation, including CRP and pro-inflammatory cytokines, were shown to be independently associated with faster rates of kidney function loss [8, 9]. Studies also show that bilirubin has anti-inflammatory activities [12], and that there is an inverse relationship between circulating bilirubin and several inflammatory markers [36]. Our previous study found that higher serum total bilirubin concentrations within the normal range are associated with lower total WBC counts [23]. Therefore, it could be speculated that a higher serum total bilirubin concentration is associated with a lower risk of CKD development through its anti-inflammatory effect [13, 16, 19, 33].

However, other studies from Western countries reported contradictory results [21, 22], and showed that elevated serum concentrations of total bilirubin were independently associated with decreasing eGFR and increasing albuminuria in the US adult population. It was proposed that the discrepant results could be explained mainly by the differences in study area, ethnicity, and study methodology, as well as some unpredictable factors that may influence bilirubin concentrations and incident CKD. Besides, the bilirubin-kidney dysfunction linkage [21] may be due to the association of hyperbilirubinemia with non-alcoholic fatty liver disease (NAFLD) [37], which would promote CKD progression through the release of CRP and some inflammatory cytokines [38]. However, other studies have shown that higher bilirubin concentrations are significantly associated with a lower risk of NAFLD [39]. Our data on an adult population in Taiwan showed consistent results, whether participants with possible liver diseases were excluded or not.

Conventional risk factors for CKD included older age, smoking, obesity, elevated BP, diabetes, dyslipidemia and vascular diseases. Alcohol consumption, especially mild consumption, was reported to be protective against the development of proteinuria [40]. Our study showed that habitual alcohol consumption had similar beneficial effects in countering the risk of renal insufficiency in men; this may possibly be attributed to the anti-atherogenic effects of mild alcohol consumption [41]. However, less

attention has been paid to the role of serum total bilirubin in the development of CKD. In our study, smoking was correlated to lower serum total bilirubin concentrations in both sexes; therefore, smoking cessation [42] might be a clinical strategy to increase serum total bilirubin concentrations in therapy and prevent CKD progression. In addition, both unconjugated and conjugated bilirubin has been shown to be effective in preventing oxidation of human LDL [10], so administration of HO-1 inducers, supplementation with bilirubin or biliverdin, and administration of drugs which can decrease the efficacy of hepatic bilirubin conjugation may possibly prevent atherogenic disease by increasing serum bilirubin concentrations [43]. Besides, an induction of HO-1, which can increase bilirubin/biliverdin production [44], has been shown to ameliorate renovascular hypertension in the renin-angiotension system in hypertensive rats [45]. Taken together, it is postulated that developing some ways to increase serum total bilirubin concentration might be conducive to the prevention and treatment of CKD [46].

Finally, studies have shown that elevated uric acid concentrations increase the risk of development and progression of CKD [47]. Our study also found that the uric acid concentration was higher in subjects with renal insufficiency than in those without renal insufficiency, and that hyperuricemia was independently associated with lower eGFR and a higher risk of renal insufficiency in both sexes after adjusting for important CKD risk factors.

Our study has some strengths. Participants were apparently healthy community dwellers, and when we repeated the analysis after excluding subjects with abnormal liver function and excessive alcohol consumption, which may exist in some liver diseases, and then further excluded subjects with advanced CKD (stage 4 and 5), the results were consistent.

However, our study had several limitations. First, this cross-sectional study lacked time-dependent evidence to determine the temporal association of total bilirubin with kidney function or risk of CKD. Second, we lacked data on the serum direct bilirubin concentration; some studies have shown a differentially protective effect of direct bilirubin on CKD and metabolic syndrome [19, 48]. Third, we performed only a single measurement of serum bilirubin and creatinine, which might have within-subject variation; however, all subjects were examined in the morning after an overnight fast (>8 h). Fourth, we assessed kidney function by calculating eGFR rather than directly measuring GFR of 24-h urine creatinine clearance. However, studies have recommended current GFR estimates for the detection and evaluation of kidney function, although the greater inaccuracy of GFR estimates still needs to be considered [25]. In addition, we defined renal insufficiency as eGFR <60 mL/min/1.73 m^2 rather than the CKD definition by KDIGO, in which CKD is classified based on cause, GFR category and albuminuria category [49]. Presumably some of the subjects with eGFR above 60 mL/min/1.73 m^2 who were classified as without renal insufficiency might have stage 1 or 2 of CKD. Finally, our subjects consisted of participants who attended a self-paid packaged physical check-up at our hospital; although large in number, this sample might differ from the general population in terms of socioeconomic factors, race and culture.

In conclusion, our cross-sectional study suggested that higher serum total bilirubin concentration was independently associated with increased eGFR and lower risk of renal insufficiency, regardless of other conventional CKD risk factors. Large prospective research endeavors are required to further investigate the beneficial role and mechanisms of bilirubin in kidney function in the general population.

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Disclosure of conflict of interest

None.

Abbreviations

ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BMI, Body mass index; BP, Blood pressure; CKD, Chronic kidney disease; CRP, C-reactive protein; CVD, Cardiovascular disease; eGFR, Estimated glomerular filtration

rate; EPI, Epidemiology Collaboration; HDL-C, High-density lipoprotein-cholesterol; HO, Heme oxygenase; LDL-C, Low-density lipoprotein-cholesterol; NAFLD, Non-alcoholic fatty liver disease; OR, Odds ratio; SCr, Serum creatinine; WBC, White blood cell.

Author contributions

WDC and YYW-conception, design, sample collection and assembly of data. ATL, WDC, SYL, YMS and WHS-analysis and interpretation of the data. ATL-the first draft of the manuscript. JTL-the accuracy of the laboratory methods. YMS-contribution equal to corresponding author. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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