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Original Article

Association between serum bilirubin levels and progression of albuminuria in Taiwanese with type 2 diabetes mellitus

Wai Kin Chan [a,c], Sung-Sheng Tsai [a,c], Yan-Rong Li [a,c], Wei-Yu Chou [a,c], Hsiao-Lien Chen [b], Szu-Tah Chen [a,c,*]

[a] Division of Endocrinology and Metabolism, Department of Internal Medicine, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan

[b] Division of Endocrinology and Metabolism, Department of Internal Medicine, Lo-Hsu Medical Foundation, Lotung Poh-Ai Hospital, Yilan, Taiwan

[c] College of Medicine, Chang Gung University, Taoyuan, Taiwan

ARTICLE INFO

Article history:
Received 12 March 2018
Accepted 8 December 2019
Available online 5 May 2021

Keywords:
Albuminuria
Bilirubin
Chronic kidney diseases
Diabetic nephropathy

ABSTRACT

Background: To investigate the association between serum bilirubin (BIL) levels and the progression of albuminuria in type 2 diabetic Taiwanese.

Methods: Longitudinal data from January 2001 to June 2015 were retrospectively reviewed from Chang Gung Memorial Hospital in Taiwan. A total of 2877 type 2 diabetic patients with normal total BIL levels were divided into 4 groups according to BIL, with the highest BIL in the fourth group. The urinary albumin/creatinine ratio (UACR) trend and progression, as well as other laboratory measurements, were evaluated among the four groups. The cumulative incidence and Cox proportional hazard model analysis were performed to examine the relationship between BIL and the risk of albuminuria progression (AUPr).

Results: The mean duration of follow-up was 1.5 years (±1.37 years). The mean patient age, glycosylated hemoglobin level, and duration of diabetes were 62.52 years, 7.9%, and 3.94 years, respectively. A significant correlation was observed between BIL and both the UACR at baseline ($P < 0.001$) and the cumulative incidence of AUPr (log–rank test, $P = 0.031$). Hazard ratio (HR) analysis revealed that patients in the fourth BIL quartile had the lowest HR risk of AUPr among the four groups (adjusted HR = 0.70; 95% Confidence Interval = 0.56 –0.89, $P < 0.05$).

Conclusions: Higher serum BIL levels are associated with a lower risk of AUPr in type 2 diabetes patients in Taiwan.

* *Corresponding author*. Division of Endocrinology and Metabolism, Department of Internal Medicine, Chang Gung Memorial Hospital at Linkou, 5, Fusing St., Gueishan, Taoyuan, 333, Taiwan.
E-mail address: stc1105@cgmh.org.tw (S.-T. Chen).

Peer review under responsibility of Chang Gung University.
https://doi.org/10.1016/j.bj.2019.12.004

At a glance commentary

Scientific background on the subject

Being an important endogenous antioxidant, bilirubin has been regarded as a potential nephrotoxic protector base on the findings that serum bilirubin level is negatively and positively correlated with albuminuria and the estimated glomerular filtration rate; respectively, in both diabetic and non-diabetic populations.

What this study adds to the field

This study suggests that the serum bilirubin level may be a surrogate marker of diabetic nephropathy progression in Asian population. Nevertheless, the potential benefits of antioxidants in the protection and treatment of diabetic microvascular complications requires further research.

Albuminuria (ABU) is defined as the daily excretion of urinary albumin > 30 mg/day, and/or a urinary albumin/creatinine ratio (UACR) > 30 mg/g [1]. ABU is a recognized risk factor and a surrogate marker for microvascular disease, such as diabetic nephropathy (DN) [2]. Clinically, ABU can be divided into micro- or macro-ABU (mABU or MABU) according to the daily amount of albumin (30–299 mg/day or ≧ 300 mg/day) [1]. In addition, ABU is also regarded as an independent risk factor for major cardiovascular events [3,4].

DN frequently leads to end-stage renal disease, even in developed countries, including Taiwan [5]. With its unique glomerular change, DN is often associated with ABU during the course of uncontrolled diabetes. Treatment guidelines recommend renin-angiotensin system blockers (RASBs) as a first-line treatment for type 2 diabetic patients with both hypertension and ABU, especially MABU [6,7].

An interesting finding of markedly reduced vascular complications, including nephropathy, in diabetic patients with Gilbert syndrome inspired the notion of the possible beneficial effect of bilirubin (BIL) on DN [8]. BIL has been shown to be an important endogenous antioxidant [9]. For example, Fujii et al. [10] demonstrated the protective effect of BIL/biliverdin against DN in a rodent model via down-regulation of renal nicotinamide adenine dinucleotide phosphate oxidase (NOX). Several clinical observation studies have reported that the serum BIL level is negatively and positively correlated with ABU and the estimated glomerular filtration rate (eGFR), respectively, in type 1 [11] and type 2 [12–14] diabetic patients, as well as in non-diabetic populations [15,16]. Of note, most of these studies were either cross-sectional or < 1-year cohort studies. We therefore conducted a retrospective study to assess the correlation between serum BIL levels and subsequent development or progression of DN in a cohort of Taiwanese type 2 diabetic patients.

Methods

Source of data

Data were obtained from the Chang Gung Research Database, which pools data from the branches of Chang Gung Memorial Hospital (CGMH) located in the northern, southern, and central parts of Taiwan. This registry data bank includes all patients who sought evaluation in the Outpatient Department of CGMH between January 2001 and June 2015. The present study was approved by the Institutional Review Board of the Chang Gung Medical Foundation (IRB No. 201601266B0). The requirement for informed consent was waived because of the retrospective nature of this study. Patients diagnosed with type 2 diabetes according to the 9th version of the International Classification of Diseases, Clinical Modification (ICD-9 CM; code 250.xx) were selected from the database, while patients with type 1 diabetes (ICD-9 CM codes 250.01, 250.03, 250.21, 250.23, 250.31, 250.33, 250.41, 250.43, 250.51, 250.53, 250.61, 250.63, 250.71, 250.73, 250.81, 250.83, 250.91, and 250.93) were excluded. To better ensure that only patients with type 2 diabetes were included, we enrolled patients > 30 years of age. Patients with hepatic diseases (ICD-9 CM code 571), nephrotic syndrome (ICD-9 CM codes 581 and 583), urinary tract infection (ICD-9 CM code 599), amyloidosis (ICD-9 CM code 277.3), systemic lupus erythematosus (ICD-9 CM code 710), gout (ICD-9 CM code 274), pregnancy (ICD-9 CM code v22 and 646), cancer, and an eGFR < 60 mL/min/1.73 m^2 were excluded. Patients with no measurements of serum BIL within 12 months of the index date or <2 UACR measurements during the follow-up period were also excluded. Furthermore, although patients with confirmed liver disease were excluded, patients with serum BIL levels above the normal range were also excluded. The index date was defined as the date of the first UACR measurement.

Data collection

Demographic and clinical data, including age, gender, duration of diabetes, medications for hypertension, hyperlipidemia, and diabetes (including RASBs, calcium channel blockers [CCBs], other anti-hypertensive drugs, statins, insulin, and oral anti-diabetic drugs) were collected. Laboratory tests, including hemoglobin (Hb), glycosylated hemoglobin (HbA1c), lipid profile (total cholesterol, low-density lipoprotein [LDL], high-density lipoprotein [HDL], and triglycerides [TG]), renal function (blood urea nitrogen [BUN] and creatinine [Cr]), sodium (Na), potassium (K), calcium (Ca), phosphorus (P), liver function (total BIL, albumin, aspartate aminotransferase [AST], alanine aminotransferase [ALT], γ-glutamyltransferase [GGT], and alkaline phosphatase [ALP]), uric acid (UA), and spot UACR, were retrieved. The surrogate renal function eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) equation with the Taiwanese coefficient [17].

Outcomes

Stages of DN were classified as follows: normoalbuminuria (UACR < 30 mg/g); mABU (UACR ≧ 30–299 mg/g); and MABU

(UACR ≥ 300 mg/g). Events were defined as albuminuria progression (AUPr), i.e., progression of the stage and/or persistent MABU.

Statistical analysis

Continuous variables were presented as the mean and standard deviation (SD), as indicated. Categorical data were expressed by actual frequencies and percentages. The trends in categorical variables were compared using the Cochran-Armitage trend test. For statistical analyses, Student's t-test and analysis of variance (ANOVA) were applied. The cumulative incidence of AUPr was estimated by the Kaplan–Meier method, and the statistical differences among groups were determined by the log–rank test. Hazard ratios (HRs) and the corresponding 95% confidence intervals (CIs) for reaching AUPr in each serum total BIL group were compared to the reference category (the first quartile of the BIL group) by univariate and multivariate Cox proportional hazard model analyses. In the multivariate Cox model, the following parameters were considered potential covariates: gender, age, duration of diabetes, HbA1c, eGFR, Cr, use of RASBs, CCBs and oral anti-diabetic drugs. P-values < 0.05 were considered significant. All statistical analyses were carried out using the SAS suite of analytics software, version 9.4 (SAS Institute, Cary, NC, USA).

Results

Study population

A total of 449,111 adult patients aged 30 or above with type 2 diabetes were initially identified (Fig. 1). Among these, 249,553 were excluded because of hepatic diseases (n = 116,778), nephrotic syndrome (n = 11,700), urinary tract infection (n = 71,566), amyloidosis (n = 257), systemic lupus erythematosus (n = 2031), gout (n = 21,217), pregnancy (n = 2695), cancer (n = 16,718), and an eGFR < 60 mL/min/1.73 m^2 (n = 6591). Patients with no measurements of serum BIL within 12 months of the index date or < 2 UACR measurements during the follow-up period (n = 196,503) were also excluded. Furthermore, patients with serum BIL levels above the normal range (n = 178) were also excluded. As a result, between January 2001 and June 2015, a total of 2877 patients who met the criteria were enrolled in this cohort study.

Demography

The patients were divided into four groups according to total serum BIL level (Table 1). The trend in UACR change was evaluated and compared among the four groups (Tables 1 and 2). Table 1 shows the demographics and laboratory data of the four groups of patients according to the serum BIL levels. Most of the patients were ranked into the second and third group as a result of the positively-skewed distribution of BIL. A higher proportion of male patients (62.67%) were enrolled. The mean duration of follow up was 1.5 years (±SD 1.37 years). The mean patient age, HbA1c level, and duration of diabetes were 62.52 years, 7.9%, and 3.94 years, respectively. Males predominated at all serum BIL levels, with higher ratios as the BIL levels increased. Although significantly lower HbA1c levels were observed in patients with higher BIL levels, no difference in ante cibum or post cibum glucose levels was observed among these groups. Patients with higher serum BIL levels had a shorter duration of diabetes ($p = 0.031$), a higher concentration of Hb ($p < 0.001$), and a higher eGFR ($p = 0.041$). Higher levels of BIL were accompanied by an increasing levels of liver function tests, including albumin, AST, and ALT ($p < 0.001$), but not ALP and GGT. Significant lower BUN, Cr ($p < 0.001$), and K ($p = 0.001$), and higher Ca ($p < 0.001$), were observed, but no difference in Na and P existed (Table 1). In groups with higher BIL levels, significant lower TG ($p = 0.002$), higher HDL ($p < 0.001$), but not total cholesterol ($p = 0.619$) or LDL ($p = 0.346$) were also observed. No difference existed in UA level between the four groups. Except for statins and antihypertensive medications, all other medications were prescribed to a lesser extent to patients with higher serum BIL levels. A significant association was observed between the UACRs at baseline and the serum BIL groups ($p < 0.001$). Gender associated analysis showed significant correlation among BIL and increasing eGFR ($p < 0.001$) and lowering log UACR ($p < 0.001$) in female patients; nevertheless, only the change of log UACR was significantly correlated ($p = 0.009$) with BIL in male patients (Table 2).

Incidence and hazard ratio of developing albuminuria

Although a trend of lower HR for AUPr as BIL level increased was observed across groups, only patients in the fourth quartile BIL group had a significantly lower HR for AUPr (p < 0.05), based on multivariate Cox regression hazard analysis (Table 3). The adjusted AUPr HR for patients in the fourth group was 0.70; 95% CI = 0.56–0.89, P < 0.05, which was the lowest HR of all four groups. The association between BIL levels and AUPr events is shown in Fig. 2. BIL level groups differed significantly in the cumulative incidence of events (P = 0.031, log–rank test). Thus, higher BIL was associated with a significantly lower risk of AUPr.

Discussion

The renal protective effect of BIL has recently attracted much attention. Higher serum BIL within the normal range is associated with less ABU and less deterioration in glomerular function in both type 1 and type 2 diabetic patients [11–14,18–20]. Because most of the relevant studies have been reported in Japanese populations, a large-scale single-center database cohort study from Taiwan was conducted to evaluate a possible ethnic difference in results and to assess the relationship between serum BIL and AUPr in type 2 diabetes.

As summarized in Table 1, although all patients had similar chronic kidney disease (CKD) stages (CKD stages 1–2) and HbA1c levels (7.89% ± 1.78%), the relatively better renal function and HbA1c control observed in patients with higher BIL levels may be partly explained by the shorter duration of diabetes in these groups. In agreement with the pathophysiology of liver diseases, higher levels of BIL were accompanied with elevated albumin, AST, and ALT (although confirmed

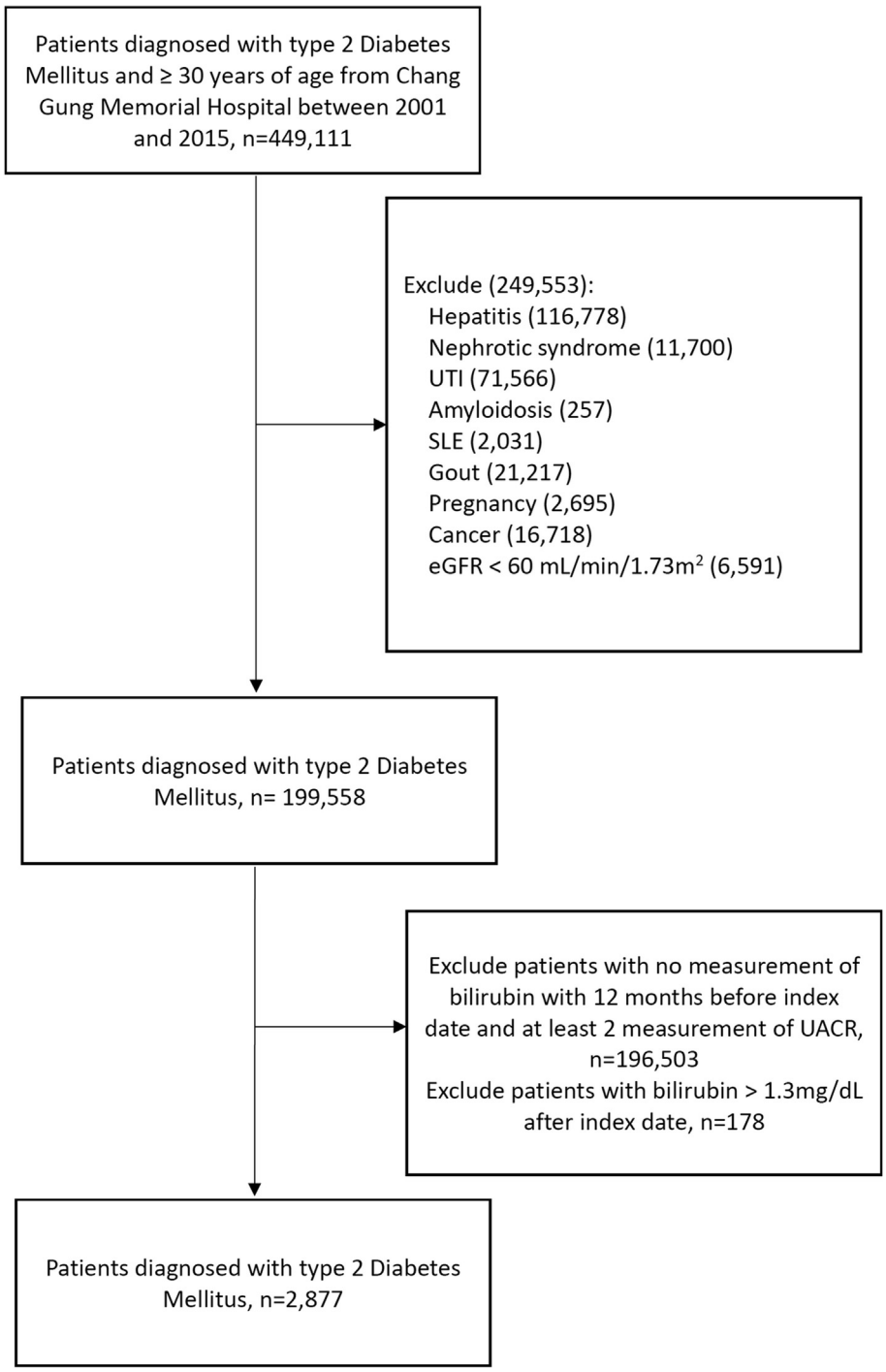


Fig. 1 Flowchart of cohort study. Patient collection flowchart of the cohort study analyzing progression of albuminuria in type 2 diabetic Taiwanese with normal bilirubin levels.

hepatitis has been excluded, the possibility of co-existing undiagnosed liver diseases, such as viral hepatitis, alcoholic liver disease, non-alcoholic steatohepatitis, and autoimmune hepatitis cannot be ruled out [21] in this study). Our finding of better lipid profiles, including lower TG and higher HDL, in patients with higher BIL levels was consistent with previous studies reporting a similar relationship between the lipoprotein spectrum and Gilbert syndrome [22–26]. Albeit its mechanism remained obscured, an *in vivo* study reporting BIL-regulated cholesterol, adipokines, and peroxisome proliferator-activated receptor gamma metabolism [27] might explain both HbA1c and lipid profiles in higher BIL groups in the current study. Finally, although total cholesterol and LDL did not differ significantly, it may simply be explained by the fact that we did not check atherogenic lipids, especially oxidized LDL.

Table 1 Baseline characteristics based on total serum bilirubin concentration groups.

	Entire cohort n = 2877	Serum total bilirubin groups				
		Group 1 n = 306	Group 2 n = 1155	Group 3 n = 916	Group 4 n = 500	P
Range of serum total bilirubin (mg/dL)	0.1–1.3	0.1–0.39	0.4–0.69	0.7–0.99	1.0–1.3	–
Male (%)	1803 (62.67)	174 (56.86)	691 (59.83)	576 (62.88)	362 (72.4)	<0.001
Age (years), mean (SD)	62.52 ± 12.31	60.83 ± 11.98	62.83 ± 12.41	62.81 ± 12.4	62.31 ± 12.07	0.066
DM duration (years), mean (SD)	3.94 ± 4.41	3.94 ± 4.46^{A,B}	4.12 ± 4.39^{A}	4 ± 4.47^{A,B}	3.42 ± 4.3^{B}	0.031
Laboratory data, mean (SD)						
HbA_{1C} (%)	7.9 ± 1.77	8.39 ± 1.93^{A}	7.93 ± 1.77^{B}	7.76 ± 1.73^{B}	7.81 ± 1.73^{B}	<0.001
Hb (g/L)	13 ± 2.02	11.98 ± 1.8^{A}	12.81 ± 1.96^{B}	13.26 ± 1.92^{C}	13.61 ± 2.15^{C}	<0.001
Albumin (g/dL)	4.09 ± 0.54	3.89 ± 0.56^{A}	4.07 ± 0.53^{B}	4.13 ± 0.52^{B}	4.17 ± 0.54^{B}	<0.001
Glucose (AC) (mg/dL)	148.6 ± 49.11	152.9 ± 61.15	147.5 ± 49.78	148.2 ± 45.83	149 ± 44.91	0.386
Glucose (PC) (mg/dL)	213.2 ± 48.96	211.4 ± 48.06	214.9 ± 52.87	212.3 ± 47.14	211.9 ± 43.06	0.496
ALP (U/L)	79.75 ± 41.75	81.87 ± 34.89	78.06 ± 32.78	79.99 ± 46.31	81.9 ± 53.58	0.259
AST (U/L)	36.77 ± 79.75	29.59 ± 32.39^{A}	32.1 ± 45.5^{A}	37.77 ± 67.6^{A}	50.09 ± 150.3^{B}	0.001
ALT (U/L)	35.21 ± 58.77	26.6 ± 26.18^{A}	31.34 ± 44.18^{A,B}	36.61 ± 48.93^{B}	46.88 ± 101.79^{C}	<0.001
GGT (U/L)	42.75 ± 47.14	44.79 ± 42.22	42.64 ± 40.85	42.3 ± 59.29	42.59 ± 37.52	0.880
Blood urea nitrogen (mg/dL)	18.02 ± 11.23	21.37 ± 15.59^{A}	18.24 ± 10.95^{B}	17.45 ± 10.69^{B}	16.47 ± 9.03^{B}	<0.001
Creatinine (mg/dL)	1.03 ± 0.83	1.39 ± 1.85^{A}	1.03 ± 0.72^{B}	0.96 ± 0.49^{B}	0.97 ± 0.43^{B}	<0.001
eGFR (mL/min/1.73m^2)	82.91 ± 33.31	78.89 ± 39.23^{A}	82.17 ± 34.98^{A,B}	84.85 ± 31.69^{B}	83.5 ± 27.63^{A,B}	0.041
Uric acid (mg/dL)	6.04 ± 1.5	6.15 ± 1.57	6.04 ± 1.46	6 ± 1.52	6.02 ± 1.5	0.474
Sodium (mEq/L)	138.3 ± 4.02	137.8 ± 4.14	138.3 ± 3.95	138.4 ± 4.15	138.3 ± 3.85	0.218
Potassium (mEq/L)	4.11 ± 0.57	4.18 ± 0.44^{A}	4.11 ± 0.54^{A}	4.12 ± 0.69^{A}	4.03 ± 0.42^{B}	0.001
Calcium (mg/dL)	8.96 ± 0.49	8.83 ± 0.56^{A}	8.96 ± 0.45^{B}	8.98 ± 0.46^{B}	8.99 ± 0.58^{B}	<0.001
Phosphorus (mg/dL)	3.89 ± 1.24	3.9 ± 0.61	3.86 ± 0.58	3.85 ± 0.55	4.02 ± 2.7	0.066
Total cholesterol (mg/dL)	107.5 ± 36.45	106.9 ± 39.63	108.6 ± 37.34	106.6 ± 34.63	106.9 ± 35.62	0.619
HDL-cholesterol (mg/dL)	45.46 ± 12.55	42.46 ± 12.2^{A}	45.16 ± 12.19^{B}	46.71 ± 12.97^{B}	45.68 ± 12.49^{A,B}	<0.001
Triglycerides (mg/dL)	166.8 ± 198.89	202.6 ± 258.24^{A}	170.4 ± 218.68^{A,B}	158 ± 185.48^{B}	152.7 ± 111.36^{B}	0.002
LDL-cholesterol (mg/dL)	105.3 ± 30.62	103.5 ± 30.36	104.6 ± 29.04	106.6 ± 32.33	105.4 ± 31.11	0.347
UACR (mg/g) at baseline	277.3 ± 931.95	680 ± 617.7^{A}	281.4 ± 926.89^{B}	214.5 ± 764.81^{B}	136.6 ± 468.94^{C}	<0.001
Log UACR at baseline	3.52 ± 1.88	4.42 ± 2.09^{A}	3.57 ± 1.86^{B}	3.34 ± 1.82^{C}	3.16 ± 1.68^{C}	<0.001
Co-medication						
RASB use (%)	1448 (50.33)	181 (59.15)	587 (50.82)	442 (48.25)	238 (47.6)	0.002
CCB use (%)	716 (24.89)	109 (35.62)	298 (25.8)	201 (21.94)	108 (21.6)	<0.001
Other anti-hypertensive drug use (%)	1066 (37.05)	118 (38.56)	435 (37.66)	337 (36.79)	176 (35.2)	0.272
Statin use (%)	1065 (37.02)	126 (41.18)	435 (37.66)	328 (35.81)	176 (35.2)	0.071
Insulin use (%)	990 (34.41)	146 (47.71)	407 (35.24)	283 (30.9)	154 (30.8)	<0.001
Oral anti-diabetic drug use (%)	2142 (74.45)	241 (78.76)	885 (76.62)	650 (70.96)	366 (73.2)	0.006

Continuous data are expressed as the mean and SD.
Abbreviations: SD: standard deviation; DM: Diabetes mellitus; HbA1c: glycosylated hemoglobin; Hb: hemoglobin; AC: ante cibum; PC: post cibum; ALP: alkaline phosphatase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: g-glutamyltransferase; eGFR: estimated glomerular filtration rate; HDL: high-density lipoprotein; LDL: low-density lipoprotein; UACR: urinary albumin creatinine ratio; RASB: renin-angiotensin system blocker; CCB: calcium channel blocker.
Other anti-hypertensive drug includes diuretics, alpha-blocker, beta-blocker and centrally acting antihypertensive drugs. Oral anti-diabetic drug includes sulfonylurea, biguanide, dipeptidyl peptidase-4 inhibitor, thiazolidinedione and alpha-glucosidases inhibitor. A, B and C indicate significant difference between groups in multiple comparison.

Although a significant correlation was found between BIL levels and baseline UACR, HR analysis showed statistical significance in the highest, but not in the second or third, quartile groups. The reason for the fluctuating HRs in the second and third groups was unclear. Similar results were seen with respect to the cumulative incidence of AUPr (Fig. 2); however, it is unusual that cumulative incidence to develop albuminuria occurred within the first 2 years and then became stable between 2 and 6 years, the higher rate of development of albuminuria in the early years of our follow-up period is not often observed in most clinical courses. These findings were most consistent with previous cohort studies [12–14].

Additionally, although the baseline eGFR of patients enrolled in the current study had already declined from the early ultrafiltration stage, the trend toward a better baseline eGFR in the higher BIL groups may also imply a protective effect of BIL on glomerular filtration. Nevertheless, this factor should be adjusted for the duration of diabetes and the degree of glucose control. Both genders had similar results with respect to eGFR and log UACR change, with the exception of an equivocal eGFR variation in male patients (Table 2). In contrast to other studies [12–14], the lower prescription rate of RASBs and CCBs to patients with higher BIL levels may explain the possible protective effect of BIL against micro- and macro-vascular diseases, although the association between BIL levels and the rate of eGFR decline was not assessed in the current study due to the short duration of follow up.

Table 2 Gender associated distribution of eGFR and log UACR in various BIL groups.

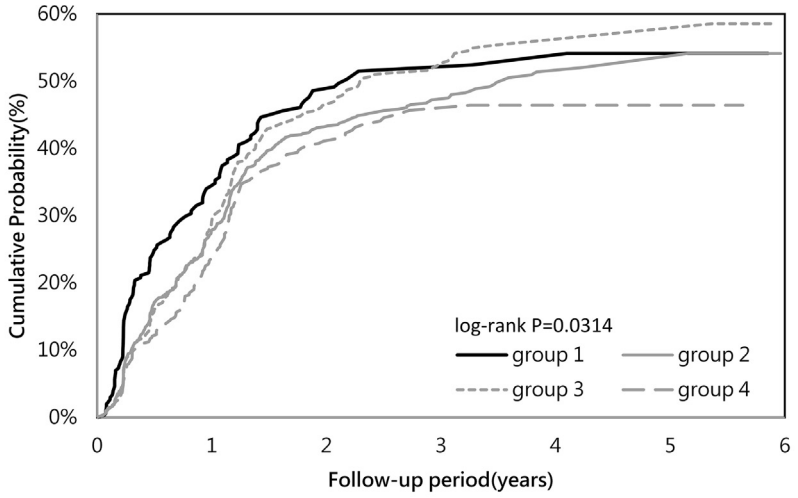
	Gender	Entire cohort	Group 1	Group 2	Group 3	Group 4	P
eGFR (mL/min/1.73m^2)	**Female**	85.72 ± 35.83	80.66 ± 36.35	83.11 ± 38.40	90.47 ± 33.90	87.59 ± 29.34	0.009
	Male	81.23 ± 31.60	77.55 ± 41.34	81.54 ± 32.50	81.53 ± 29.84	81.93 ± 26.82	0.448
Log UACR at baseline	**Female**	3.61 ± 1.82	4.40 ± 2.02	3.70 ± 1.85	3.29 ± 1.66	3.34 ± 1.63	<0.001
	Male	3.46 ± 1.91	4.43 ± 2.15	3.49 ± 1.86	3.38 ± 1.91	3.09 ± 1.70	<0.001

Data are expressed as the mean and SD.
Abbreviations: SD: standard deviation; eGFR: Estimated glomerular filtration rate; UACR: urinary albumin creatinine ratio.

Table 3 Incidence and hazard ratio of development of albuminuria.

	N	Event	Person-years	Incidence per 100 person-years (95% CI)	Crude hazard ratio (95% CI)		Adjusted hazard ratio[a] (95% CI)	
Group 1	306	134	482.58	27.77 (23.07–32.47)	1	Reference	1	Reference
Group 2	1155	447	1864.62	23.97 (21.75–26.20)	0.83	(0.68–1.00)	0.80	(0.65–0.97)
Group 3	916	380	1396.62	27.22 (24.48–29.96)	0.91	(0.74–1.10)	0.88	(0.71–1.08)
Group 4	500	178	812.39	21.91 (18.69–25.13)	0.74	(0.59–0.93)[b]	0.70	(0.56–0.89)[b]

[a] Adjustment covariates: gender, age, duration of diabetes, HbA1c, eGFR, Cr, use of RASBs, CCBs and oral anti-diabetic drugs.
[b] $p < 0.05$



Number at risk							
Group 1	306	154	97	63	28	9	0
Group 2	1155	647	370	211	99	29	0
Group 3	916	505	260	134	63	25	0
Group 4	500	290	164	81	36	9	0

Fig. 2 Cumulative incidence by different bilirubin groups. Comparison of cumulative incidence among different bilirubin groups. Each group is represented by dotted or broken lines of different lengths, as shown in the figure. Significant differences among the groups was determined using the log–rank test (P = 0.031).

Endothelial dysfunction is believed to be induced by oxidative stress and hyperglycemia plays a central role in the pathogenesis of micro- and macro-vascular diseases [28]. Oxidative stress-induced reactive oxygen species (ROS) may activate cellular stress pathways by damaging DNA and signal amplifying proteins [29]. ROS may be produced in the kidney by various cells, including fibroblasts, endothelial cells, vascular smooth muscle cells, mesangial cells, tubular cells, and podocytes. ROS are produced by the enzyme NOX, and NOX-4 is most abundantly expressed in the kidney [30]. NOX

activation may be a major cause of increased oxidative stress in diabetic renal and other vascular diseases [29,30]. Fujii et al. [10] reported that BIL protects DN progression through NOX-4 suppression in diabetic rats with hereditary hyperbilirubinemia. Even so, further studies are warranted to prove and explore the mechanisms which underlie BIL protection against DN progression in humans.

Mechanisms other than ROS production, such as preservation of renal blood flow and glomerular filtration through calcium handling in vascular smooth muscle cells to affect intracellular storing and release, may also explain the protective role of BIL against DN [31]. Whether or not mechanisms other than ROS production may explain the higher Ca levels in the higher BIL groups remains unclear. To summarize, the radical-scavenging effect of BIL may be one of the most important mechanisms by which BIL protects diabetic patients from progression of DN.

This study has several limitations because of the retrospective design. First, individual blood pressures and body weights were not available in the data bank. Second, the intervals for laboratory measurements, including UACR and BIL, were irregularly arranged. Besides, not all tests were performed on every patient. Third, factors such as diet, alcohol use, cigarette smoking, physical activity, and socioeconomic status were not available. Fourth, total, but not direct or indirect, BIL levels were analyzed. Fifth, information on the co-medications was obtained at the index date and during the follow-up period without acknowledging prescription duration or the medications received from other local clinics. Finally, data collected from a single medical center may not represent the general population of type 2 diabetic patients in Taiwan. However, the data includes patients from all areas of Taiwan.

Conclusions

In conclusion, our analysis from this large-scale single-center cohort study has provided evidence that higher serum BIL levels are associated with a lower risk of AUPr in type 2 diabetic patients, which suggests that the serum BIL level may be a surrogate marker of DN progression.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest

The authors declare no conflicts no interest.

Acknowledgments

The authors wish to acknowledge the support of CORPG3F0791 from Chang Gung Memorial Hospital and the Maintenance Project for the Center for Big Data Analytics and Statistics (Grant CLRPG3D0043) for assistance with study design, monitoring, analysis, and interpretation of data. Special thanks to Alfred Hsing-Fen Lin for providing helpful suggestions.

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