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Inverse Relationship Between Serum Bilirubin Levels and Diabetic Foot in Chinese Patients with Type 2 Diabetes Mellitus

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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Background: Several studies demonstrated that bilirubin, a potent endogenous antioxidant, is a strong protective factor for many diabetic complications such as nephropathy, retinopathy, neuropathy, and vasculopathy. The purpose of this study was to assess the association between serum bilirubin levels and diabetic foot (DF) in Chinese patients with type 2 diabetes mellitus (T2DM).

Material/Methods: The present cross-sectional study of bilirubin levels in relation to DF was conducted in 1,269 T2DM patients with (n=578) and without (n=691) DF. Blood test results were obtained on hospital admission, including total bilirubin (T-BIL), direct bilirubin (D-BIL), and indirect bilirubin (I-BIL). Data on Wagner classification and amputation procedure in patients with DF were collected by reviewing electronic medical records. Univariate or multivariate analysis were performed to explore the association between bilirubin and DF.

Results: Serum I-BIL levels were shown to play a protective role regarding the presence and severity of DF (OR=0.75, p=0.029 and OR=0.90, p=0.021, respectively). In addition, in a comparison of the lowest and highest tertiles of serum bilirubin concentration, the highest tertile of serum T-BIL (OR=0.51, p=0.011) and I-BIL (OR=0.28, p<0.001) was significantly related with a lower Wagner grade of DF. Patients with DF in the highest tertiles of T-BIL carried a significantly lower risk of amputation events than those in the lowest tertiles (OR=0.47, p=0.025).

Conclusions: The present study provided evidence that decreased serum bilirubin levels were independently associated with the presence and severity of DF and amputation events in patients with DF.

MeSH Keywords: Amputation • Bilirubin • Diabetes Mellitus • Diabetic Foot • Diabetic Neuropathies • Peripheral Arterial Disease

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Background

Diabetes mellitus (DM) is one of the most important chronic diseases and poses a great threat to human health [1]. According to the International Diabetes Federation, the global number of diabetes cases reached 415 million in 2015 [2]. One of the common and severe complications of DM is diabetic foot (DF), which has an estimated lifetime incidence as high as 25% [3]. In low-income countries, the cost of treating a complex DF ulcer can be equivalent to 5.7 years of annual income [4]. Furthermore, the majority of DF patients will ultimately face amputation, decreasing quality of life and increasing risk of heart disease-related mortality [5]. The International Working Group on the Diabetic Foot (IWGDF) points out that the prevention and treatment of DF ulcer can significantly reduce the amputation rate [6]. The identification of novel risk factors could provide new information about the pathogenesis of DF and thus provide important information to aid in development of effective interventions.

Bilirubin, the end product of heme catabolism, has been considered merely as a potentially damaging waste product. However, recent studies report that serum bilirubin level is a strong protective factor against and possible candidate biomarker for many complications of diabetes, such as nephropathy [7], retinopathy, neuropathy, and vasculopathy. Additionally, total bilirubin level was negatively related with the incidence of amputation [8] and arterial stiffness [9] in T2DM. However, few studies have analyzed the potential value of bilirubin in patients with DF. The present study evaluated the relationship between serum bilirubin concentration and the presence and severity of DF in Chinese individuals. In addition, this study aimed to explore whether bilirubin levels were in association with amputation in patients with DF.

Material and Methods

Participants

A total of 1,269 patients previously diagnosed with T2DM were enrolled from the First Affiliated Hospital of Wenzhou Medical University between January 2007 and March 2013 for this cross-sectional study. The diagnosis of diabetes mellitus was based on 1999 World Health Organization criteria [10]. Patients with renal dysfunction (creatinine >176.8 μmol/L), abnormal liver function (alanine aminotransferase (ALA) >100 U/L and/or aspartate aminotransferase (AST) >100 U/L), liver cirrhosis, systemic inflammatory disease, progressive malignancy, and all bladder diseases were excluded.

The present study was approved by the appropriate ethics committees and was conducted in accordance with Declaration of Helsinki.

Methods

Electronic patient medical records were reviewed, and data on age, duration of DM, sex, smoking and drinking habit, body mass index (BMI), heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), hypertension, hyperlipidemia, microvascular complication (diabetic retinopathy (DR) or diabetic peripheral neuropathy (DPN) or diabetic nephropathy (DN), macrovascular complication and management of hyperglycemia (oral hypoglycemic agents and/or insulin) were collected.

Baseline biochemical variables were obtained on hospital admission including serum white blood cell (WBC), total bilirubin (T-BIL), direct bilirubin (D-BIL), indirect bilirubin (I-BIL), ALA, AST, γ-glutamyl transpeptidase (GGT), creatinine, uric acid (UA), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), glycated hemoglobin (HbA1c), and hemoglobin (Hb).

Body mass index (BMI) was calculated by dividing the patient's weight in kilograms by height in meters squared. Blood pressure was measured using a mercury sphygmomanometer after 20 minutes rest. Hypertension was defined as SBP ≥140 mm Hg, DBP ≥90 mm Hg, or the administration of antihypertensive therapy. According to the criteria set by the National Cholesterol Education Program-Adult Treatment Panel III, hyperlipidemia was defined as TG ≥2.26 mmol/L, TC ≥6.19 mmol/L, HDL <0.91 mmol/L in women or HDL <1.14 mmol/L in men, LDL ≥4.14 mmol/L, or the administration of lipid-lowering agents. DR was diagnosed when non-proliferative or proliferative retinopathy was observed during fundus examination and stereo fundus photography after pupillary dilation by the same ophthalmologist. The diagnosis of DPN was based on evidence of the neuropathy total symptom score ≥6, vibration perception threshold >15 volts or at least two abnormal nerve conduction velocity tests. DN was defined on the basis of persistent microalbuminuria detected during at least two examinations with a high urine albumin-to-creatinine ratio (ACR >30 mg/g) or overt albuminuria (ACR >300 mg/g), with or without eGFR <60 mL/min/1.73 m^2 and in the presence of DR. Macrovascular complications were defined as previous or present myocardial infarction, coronary revascularization, stroke, carotid artery stenosis, or limb artery stenosis. Estimation of the glomerular filtration rate (eGFR) was performed using the Chronic Kidney Disease Epidemiology Collaboration formula [11].

Eligible patients with T2DM were categorized into two groups: the non-DF group (NDF; n=691) and the DF group (n=578) according to the 2015 IWGDF diagnostic criteria [12].

To describe the severity of DF, we used Wagner classification (grade 0-5) [13] which were collected when patients with DF were admitted to hospital. The 578 patients with DF were

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divided into three groups: low grade group (n=40), patients with Wagner grade level 0 or 1; middle grade group (n=425), patients with Wagner grade level 2 or 3, and high-grade group (n=113), patients with Wagner grade level 4 or 5.

The lower extremity amputation (LEA) procedure information was also collected through medical records. DF patients with severe symptom and poor therapeutic effect suffered from LEA events a few days after patients' admission. The 578 patients with DF were divided into two groups: Non-amputation group (n=446) and amputation group (n=132).

Statistical analysis

The statistical analysis was performed using SPSS version 19.0. Continuous data with normal distribution are shown as mean ± standard deviation and data with a non-normal distribution are shown as median (quartile interval). Categorical data are expressed as number (percentage). The distributions of our data were measured by Kolmogorov-Smirnov. In univariate analysis, the comparison of continuous data was accomplished using Student's *t*-test, one-way ANOVA, Mann-Whitney U test, or Kruskal-Wallis H test, and the comparison of categorical variables was accomplished using the χ^2-test. The pairwise comparison after the univariate analysis was performed using the Bonferroni correction. All binary or ordinal multivariate logistic regression analyses were performed to determine the risk factors for DF in patients with DM, with classification by Wagner grade and amputation status. After adjusting for multiple confounding variables, the adjusted odd ratio (OR) and 95% confidence interval (CI) was calculated; Factors with $p<0.05$ were considered as statistically significant risk factors.

Results

Baseline characteristics

The baseline characteristics of the study patients in the NDF and DF groups are shown in Table 1. Patients in the DF group were older than those in the NDF group ($p<0.001$). The proportion of patients with a positive smoking status and drinking consumption was significantly higher in the DF group than that in the NDF group ($p<0.001$). Patients with DF showed increased levels of WBC, creatinine, and SBP compared with those in the NDF group ($p<0.005$). Patients with DF exhibited lower T-BIL, I-BIL, ALT, AST, GGT, UA, TC, TG, HDL, LDL, Hb, and HbA1c levels than those in the NDF group (all $p<0.05$). There were fewer patients with hyperlipidemia and more patients with macrovascular complications in the DF group than those in the NDF group ($p<0.001$). The treatment before admission between two groups has significantly different. But the insulin doses were no significant difference between the two groups.

Associations between serum bilirubin levels and the presence of DF in patients with type 2 DM

We divided the patients into two equal groups and the cutoff value of T-BIL, I-BIL, and D-BIL was 9 μmol/L, 3 μmol/L, and 6 μmol/L, respectively. After adjusting for risk factors including age, sex, smoking, drinking, BMI, HbA1c, WBC, ALT, AST, GGT, Hb, and TG, we found that the serum I-BIL level had a negative association with the presence of DF as a categorized variable (OR=0.75, 95% CI=0.57–0.98, $p=0.029$, Table 2). However, serum T-BIL and D-BIL levels showed no significant differences between the two groups ($p>0.05$, Table 2).

Associations between serum bilirubin concentration and the severity of DF

The concentrations of serum T-BIL and I-BIL differed significantly among the three grade groups ($p=0.001$ and $p<0.001$, respectively, Table 3). Furthermore, after categorizing the serum bilirubin concentration in tertiles, the data showed that the highest tertile of serum T-BIL level (>10 μmol/L, T3 group) was associated with lower Wagner grade score compared with the lowest tertile of serum T-BIL level (<7 μmol/L, T1 group, $p=0.007$, Table 3). This association persisted after adjustment for age, sex, duration of DF, smoking, drinking, BMI, WBC, ALT, GGT, creatinine, TC, HDL, LDL, HbA1c, DBP, hypertension, DPN, and macrovascular complications; this was true when serum T-BIL level was considered either as a continuous variable (OR=0.933, 95% CI=0.889–0.980, $p=0.005$, Table 3) or when categorized in tertiles (highest versus lowest tertile: OR=0.506, 95% CI=0.298–0.857, $p=0.011$, Table 3). A similar association was found between Wagner grade groups and tertiles of serum I-BIL levels ($p<0.001$, Table 3); this association also persisted after adjustment for the aforementioned compound factors and serum I-BIL levels when considered either as a continuous variable (OR=0.902, 95% CI=0.826–0.994, $p=0.021$ Table 3) or when categorized in tertiles (highest versus lowest tertile: OR=0.28, 95% CI=0.147–0.538, $p<0.001$, Table 3).

Associations between serum bilirubin concentration and LEA in patients with DF

Serum T-BIL and I-BIL concentrations were significantly inversely associated with LEA (all $p<0.001$). The inverse association between T-BIL concentration and LEA persisted after adjustment for age, sex, duration of DF, BMI, WBC, ALT, AST, GGT, UA, TC, HDL, LDL, HbA1c, heart rate, hypertension, hyperlipidemia, and macrovascular complications (OR=0.910, 95% CI=0.847–0.977, $p=0.009$, Table 4), but not for I-BIL ($p=0.233$, Table 4).

In further analyses according to T-BIL and I-BIL tertiles, patients in the highest tertiles of T-BIL and I-BIL carried a significantly lower risk of LEA than patients in the lowest tertiles

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Table 1. The clinical characteristics of the subjects between NDF and DF group.

Variables	NDF		DF		P-value
N	691		578		
Age (years)[a]	62	(53–70)	66	(58–75)	<0.001*
Duration of diabetes (months)[b]	108	(48–150)	120	(60–156)	0.189
Male (%)[c]	374	(54.12%)	327	(56.57%)	0.382
Smoking(%)[c]	158	(22.22%)	462	(79.93%)	<0.001*
Drinking (%)[c]	127	(18.38%)	460	(79.58%)	<0.001*
BMI (kg/m^2)[b]	24.1	(22.2–26.4)	23.8	(21.5–26.3)	0.073
WBC (10^9/L)[b]	6.6	(5.6–8.0)	7.1	(5.6–9.0)	<0.001*
T-BIL (μmol/L)[b]	9	(7–12)	9	(6–11)	0.005*
D-BIL (μmol/L)[b]	3	(2–4)	3	(2–4)	0.174
I-BIL (μmol/L)[b]	6	(4–8)	5	(4–7)	<0.001*
ALT (U/L)[b]	20	(14–30)	16	(11–24)	<0.001*
AST (U/L)[b]	20	(17–26)	19	(15–25)	<0.001*
GGT (U/L)[b]	27	(19–46)	25	(16–43)	0.012*
Creatinine (μmol/L)[b]	62	(50–77)	66	(52–83)	0.005*
UA (μmol/L)[b]	300	(249–366)	263	(206–335)	<0.001*
TC (mmol/L)[b]	4.7	(3.88–5.53)	4.235	(3.53–5.04)	<0.001*
TG (mmol/L)[b]	1.54	(1.03–2.22)	1.16	(0.82–1.68)	<0.001*
HDL (mmol/L)[b]	1.05	(0.9–1.28)	1.04	(0.86–1.25)	0.044*
LDL (mmol/L)[b]	2.7	(2.1–3.4)	2.5	(1.9–3.2)	0.001*
HbA1C (%)[b]	9.4	(7.8–10.9)	8.85	(7.3–10.8)	0.001*
Hb (g/L) b	128.4	(126.9–129.9)	117.6	(116.1–119.0)	<0.001*
HR (bpm)[b]	82	(76–90)	84	(76–90)	0.087
SBP (mmHg)[b]	140	(126–158)	144	(130–160)	0.004*
DBP (mmHg)[b]	80	(70–88)	80	(70–88)	0.057
Hypertension (%)[c]	402	(58.2%)	349	(60.4%)	0.426
Hyperlipidemia (%)[c]	236	(34.2%)	81	(14%)	<0.001*
DR (%)[c]	337	(48.8%)	300	(51.9%)	0.266
DPN (%)[c]	351	(50.8%)	314	(54.3%)	0.210
DN (%)[c]	160	(23.2%)	131	(22.7%)	0.836
Macrovascular complication (%)[c]	427	(61.8%)	452	(78.2%)	<0.001*

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Table 1 continued. The clinical characteristics of the subjects between NDF and DF group.

Variables	NDF		DF		P-value
Treatment					
Newly diagnose	90	(13%)	162	(28%)	
OHA	304	(44%)	87	(15%)	
Insulin	200	(29%)	121	(21%)	<0.001*
OHA & Insulin	97	(14%)	208	(36%)	
Insulin dose (U)	31.4	(28.7–34.0)	31.7	(27–36.3)	0.905

[a] – Continuous variables of normal distribution was expressed with mean ±SD and P-values were obtained by Student's t-test; [b] – continuous variables of non-normal distribution was median (quartile interval) and P-values were obtained by Mann-Whitney U test; [c] – categorized data was expressed with number (percentage) and P-values were obtained by Pearson's Chi-square test. * P<0.05. BMI – body mass index; Hb – hemoglobin; WBC – white blood cell; T-BIL – total bilirubin; D-BIL – direct bilirubin; I-BIL – indirect bilirubin; ALT – alanine aminotransferase; AST-aspartate aminotransferase; GGT – γ-glutamyl transferase; UA – uric acid; TC – total cholesterol; TG – triglyceride; HDL – high-density lipoprotein; LDL – low density lipoprotein; HbA_1C – hemoglobin A_1C; HR – heart rate; SBP – systolic blood pressure; DBP – diastolic blood pressure; DR – diabetic retinopathy; DPN – diabetic peripheral neuropathy; DN – diabetic nephropathy; OHA – oral hypoglycemic agents.

Table 2. Logistic regression analysis for the presence of DF in T2DM patients.

Variables	OR(95%CI)	P-value
T-BIL (μmol/L)[a]		
<9 μmol/L	1 (ref)	0.058
≥9 μmol/L	0.81 (0.64–1.01)	
D-BIL (μmol/L)[b]		
<3 μmol/L	1 (ref)	0.452
≥3 μmol/L	1.10 (0.85–1.43)	
I-BIL (μmol/L)[c]		
<6 μmol/L	1 (ref)	0.029*
≥6 μmol/L	0.75 (0.57–0.98)	

[a] – Adjusted for risk factors including age, sex, smoking, drinking, BMI, HbA_1C, WBC, ALT, AST, GGT, and TG; [b] – adjusted the variable in a and I-BIL; [c] – adjusted the variable in a and D-BIL. * P<0.05.

of T-BIL and I-BIL (p=0.017 and p<0.001, respectively). This association with LEA also persisted for T-BIL (OR=0.47, 95% CI=0.243–0.921, p=0.025, Table 4), but not for I-BIL (p=0.157, Table 4) after adjustment for the aforementioned confounding factors and D-BIL.

Discussion

The mechanisms of DF are complex and involves a number of possible pathogenic factors, including DPN, peripheral artery disease (PAD) [14], infection [15], and wound healing such as vascular endothelial growth factor-mediated angiogenesis [16]. The interaction and combination of the varying contribution of pathogenic factors may lead to the different degrees and variable outcomes of DF. Despite progress regarding the pathophysiology and management strategy of DF, up to 8–21% of patients with DF still undergo LEA [17,18]. LEA is associated not only with a significantly reduced quality of life, but also with increased economic burden and mortality [19,20]; hence, an improved understanding of potential risk factors in the severity of DF and predictors for LEA events with DF ulcer is urgently required. Such understanding may be helpful for clinicians to initiate more effective strategies for the diagnosis, follow-up, and treatment of DF.

The present study documented novel associations of serum bilirubin levels with the presence and severity of DF in patients with type 2 DM; decreased serum I-BIL levels were most pronounced in patients with DF. Furthermore, we found an

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Table 3. Correlations of serum bilirubin concentration with Wagner grade in the DF group.

Variables	Low grade group (N=40)	Middle grade group (N=425)	High grade group (N=113)	P_1	P_2	OR (95%CI)
T-BIL (μmol/L)	11 (8–13)	9 (7–11)	8 (5–11)	0.001*	0.005*[d]	0.933 (0.889–0.980)
By tertiles				0.012*		
T1 (<7 μmol/L)	10 (25%)	159 (37.4%)	56 (49.6%)	0.104[a]	1 (ref)	1 (ref)
T2 (7–10 μmol/L)	10 (25%)	133 (31.3%)	28 (24.8%)	0.222[b]	0.149[d]	0.686 (0.411–1.145)
T3 (>10 μmol/L)	20 (50%)	133 (31.3%)	29 (25.6%)	0.007*[c]	0.011*[d]	0.506 (0.298–0.857)
I-BIL (μmol/L)	7 (5–9)	5 (4–7)	4 (3–6)	<0.001*	0.021*[e]	0.902 (0.826–0.994)
By tertiles				<0.001*		
T1 (<4 μmol/L)	10 (25%)	153 (36%)	61 (54.0%)	0.240[a]	1 (ref)	1 (ref)
T2 (4–6 μmol/L)	9 (22.5%)	126 (29.6%)	34 (30.1%)	<0.005*[b]	0.214[e]	0.7 (0.403–1.22)
T3 (>6 μmol/L)	21 (52.2%)	146 (34.4%)	18 (15.9%)	<0.001*[c]	<0.001*[e]	0.28 (0.147–0.538)

P_1 – calculate by one-way ANOVA or χ^2 test; [a] – comparing T1 with T2; [b] – comparing T2 with T3; [c] – comparing T3 with T1; P_2 – calculate by ordinal logistic regression; [d] – adjusted for age, sex, duration of DF, smoking, drinking, BMI, WBC, ALT, GGT, creatinine, TC, HDL, LDL, HbA_1C, DBP, hypertension, DPN and macrovascular complication; [e] – adjusted the variable in d and D-BIL. * P<0.05 in regression or P is lower than adjusted P value in the pairwise comparison by Bonferroni correction.

Table 4. Association between serum bilirubin concentration and amputation in the DF group.

Variables	Non-amputation (N=446)	Amputation (N=132)	P_1	P_2	OR (95%CI)
T-BIL (μmol/L)	9 (7–12)	8 (5–10)	<0.001*	0.009*[d]	0.910 (0.847–0.977)
By tertiles			0.044*		
T1 (<7 μmol/L)	168 (37.7%)	59 (44.7%)	0.794[a]	1 (ref)	1 (ref)
T2 (7–10 μmol/L)	127 (28.5%)	43 (32.6%)	0.044[b]	0.783	1.045 (0.573–1.903)
T3 (>10 μmol/L)	151 (33.8%)	30 (22.7%)	0.017*[c]	0.025*	0.474 (0.243–0.921)
I-BIL	5 (4–7)	4 (3–6)	<0.001*	0.233[e]	0.89 (0.711–1.113)
By tertiles			<0.001*		
T1 (<4 μmol/L)	159 (35.6%)	67 (50.8%)	0.259[a]	1 (ref)	1 (ref)
T2 (4–6 μmol/L)	126 (28.3%)	42 (31.8%)	0.003*[b]	0.794	1.09 (0.569–2.088)
T3 (>6 μmol/L)	161 (36.1%)	23 (17.4%)	<0.001*[c]	0.157	0.559 (0.25–1.25)

P_1 – calculate by Mann-Whitney U test or χ^2 test; [a] – T1 compare with T2; [b] – T2 compare with T3; [c] – T3 compare with T1; P_2 – calculate by binary logistic regression; [d] – adjusted for age, sex, duration of DF, BMI, WBC, ALT, AST, GGT, UA, TC, TG, HDL, LDL, HbA_1C, HR, hypertension, hyperlipidemia and macrovascular complication; [e] – adjusted the variable in d and D-BIL. * P<0.05 in regression or P is lower than adjusted P value in the pairwise comparison by Bonferroni correction.

independent association between decreased levels of serum I-BIL and the presence of DF. Moreover, serum T-BIL and I-BIL levels were independent risk factors for the severity of DF. Recent studies have demonstrated a significant inverse relationship between serum bilirubin levels and diabetes [21], diabetic nephropathy [8], retinopathy [22], and neuropathy [23]. The present study extends this relationship to the presence of DF.

The higher Wagner grades of ulcers, HbA1c ≥8%, PAD, older age, and hypertension have been recognized as the predictors

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of LEA in patients with DF. Our study revealed that serum T-BIL levels were inversely associated with the risk of LEA with DF; this risk was independent of numerous other known contributory factors. Patients with DM and low T-BIL levels (<7 μmol/L) have a 2.1-fold increased risk of LEA compared with high T-BIL levels (>10 μmol/L). The role of serum T-BIL levels as a predictor of LEA events is in accordance with the previous FIELD study in a subsidiary analysis by Chan et al. [9]. The study showed a protective role of bilirubin in the severity of DF. PAD is present in up to half of patients with DF and is an independent risk factor for the severity of DF as well as amputation [24]. Previous studies consistently found that elevated serum bilirubin levels were negatively associated with PAD [25–27], arterial stiffness [10], and carotid intima media thickness [28] in patients with DM. This inverse association is strongly supported by evidence arising from experimental studies. For instance, bilirubin has been demonstrated to block smooth muscle cell proliferation via inhibition of the p38 MAPK signaling pathway [29] and to promote angiogenesis through Akt-eNOS-dependent endothelial cell activation [30]. Bilirubin has been also shown to reduce arterial stiffness by suppressing expression of cell adhesion molecules [21], an essential step in atherosclerosis. In addition, bilirubin is an atheroprotective candidate due to its potent antioxidant and anti-inflammatory properties; this includes both D-BIL [31] and I-BIL [32].

Our findings are further supported by studies [23] showing an inverse relationship between bilirubin levels and DPN, one of the main pathogenic factors of DF. Accordingly, Chung et al. [33] reported a significant inverse relationship between bilirubin levels and cardiovascular autonomic neuropathy in patients with T2DM. Bilirubin, which was recently reported to demonstrate effective antioxidant and anti-inflammatory activity *in vitro*, might exert a neuroprotective effect by lowering oxidative stress and inhibiting protein kinase C activity [34] and advanced glycation end-product formation [35]. Hence, evidence from studies about the atheroprotective and neuroprotective effect of bilirubin might support a strong inverse link of serum bilirubin levels with the presence and severity of DF and incidence of LEA in patients with DF.

Notably, many studies primarily concentrated on T-BIL function and did not differentiate D-BIL and I-BIL from T-BIL. However, three types of bilirubin (T-BIL, D-BIL, and I-BIL) were analyzed separately in the current study. Interestingly, we found that I-BIL, but not D-BIL or T-BIL, acted as an independent risk factor for the incidence of DF and that I-BIL and T-BIL, but not D-BIL, were independent risk factors for severity of DF. T-BIL consists of two subfractions: principally I-BIL and D-BIL. Both D-BIL and I-BIL have antioxidant and anti-inflammatory properties; however, observations by Sano et al. [34] revealed that bilirubin, especially I-BIL, exerts an irreversible inhibitory effect on protein kinase C. Several lines of evidence demonstrate that higher I-BIL is associated with the development of coronary heart disease (CHD) [36] and PAD [25] in T2DM and nephropathy in type 1 DM [37]. However, Wang et al. [38] reported that D-BIL levels, but not I-BIL, were associated with increased risk of type 2 DM. Accordingly, further studies are needed to reveal the different effects of the three types of bilirubin.

The present study had several limitations. First, participants in the DF group were inpatients from an urban university hospital. Among the 578 DF patients enrolled in this study, 113 patients (19.6%) were classified as Wagner 4 or 5 grade, and the LEA rate (22.8%), including major and minor amputation, was overestimated. Therefore, our results may not be applicable to general DF populations. Second, medications used for hypertension and dyslipidemia prior to admission in many patients lowered blood pressure and serum lipid levels, thereby potentially confounding our results. Third, although our data preformed a positive result, in consideration of the significant different number of sample subgroups in Table 3, the result also needs prospective study to verify it. Fourth, in consideration of unavoidable missing of some relative data in the cross-sectional study, we could not analyze the information of drug compliance or exercise therapy in a suitable statistical efficiency. But we would record the missing information in further follow-up study and discuss the relationship between them and serum bilirubin levels.

In conclusion, the present study provides the first evidence that decreased serum bilirubin levels are independently associated with the severity of DF. It also suggests an independently negative association between serum bilirubin levels and amputation events in patients with DF. Further longitudinal studies are necessary to identify whether serum bilirubin levels, and which types of bilirubin, have predictive value for DF and LEA in patients with DM.

Conclusions

The present study provides the first evidence that decreased serum bilirubin levels are independently associated with the severity of DF. It also suggests an independent negative association between serum bilirubin levels and amputation events in patients with DF.

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Conflict of interests

None.

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