Int J Clin Exp Med 2018;11(11):12621-12628
www.ijcem.com /ISSN:1940-5901/IJCEM0077678

Original Article

Decreased indirect bilirubin is associated with the prevalence of peripheral neuropathy in Chinese female patients with type 2 diabetes mellitus

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Received September 6, 2017; Accepted July 2, 2018; Epub November 15, 2018; Published November 30, 2018

Abstract: *Aims:* To investigate the association between serum bilirubin levels in the physiological range and diabetic peripheral neuropathy (DPN) in Chinese patients with type 2 diabetes. *Methods:* This cross-sectional study involved 1,402 patients with type 2 diabetes and 344 healthy controls. The subjects were stratified into sex-specific groups, and the relationship between bilirubin and DPN was analyzed. Binary logistic regression analysis was used to estimate the potential risk factors for DPN. Comparison of the prevalence of DPN and other clinical parameters across indirect bilirubin quartiles was conducted. Pearson correlation analysis was used to examine the association between the serum indirect bilirubin and other clinical parameters, and linear regression analysis was conducted to explore the influencing factor for indirect bilirubin. *Results:* The total bilirubin, direct bilirubin and indirect bilirubin concentrations were significantly lower in individuals with DPN than in those without DPN in women and healthy controls. The binary logistic regression analysis showed that serum indirect bilirubin was associated with the prevalence of DPN in women. Subjects were further assigned to quartiles based on serum indirect bilirubin levels. The prevalence of DPN in women was significantly lower among persons in the highest indirect bilirubin quartile than among those in the lowest quartile. Linear regression analysis showed that the indirect bilirubin level was negatively correlated with WBC, HB and RBC in women. *Conclusions:* Serum indirect bilirubin levels within the physiological range were inversely associated with DPN in Chinese women with type 2 diabetes.

Keywords: Bilirubin, diabetic peripheral neuropathy, type 2 diabetes

Introduction

The prevalence of diabetes mellitus (DM) and its complications is increasing rapidly due to an aging population. Although the exact mechanism is not clear, oxidative stress has been implicated in the pathogenesis of type 2 diabetes and its chronic complications [1]. Bilirubin might antagonize oxidative stress by acting as an antioxidant and cytoprotectant, which may have beneficial effects on diseases related to oxidative stress [2]. Recently, serum bilirubin levels have gained particular attention for the possible protective role against the development of diabetes and its chronic complications. There is a body of evidence indicating that elevated serum bilirubin levels are inversely associated with diabetes [3-6], diabetic retinopathy (DR) [7-10], diabetic nephropathy (DN) [11-20], diabetic cardiovascular autonomic neuropathy [21], diabetic peripheral neuropathy (DPN) [22], and limb amputation [23, 24].

However, the association between serum bilirubin levels and DPN in patients with type 2 diabetes mellitus in China has not been fully established. The aim of this study was to investigate the correlation between the serum bilirubin levels within the physiological range and DPN in Chinese patients with type 2 diabetes mellitus (T2DM).

Materials and methods

Subjects

We retrospectively analyzed 1,402 patients with type 2 diabetes who were hospitalized in the Lianyungang First People's Hospital from October 2010 to September 2014. T2DM was biochemically confirmed according to the World Health Organization diagnostic criteria for the classification of diabetes. A diagnosis of hypertension was assigned if the subject reported a physician diagnosis of hypertension, if the subject reported taking prescription medications for hypertension, or if the systolic blood pressure (SBP) was >140 mmHg or the diastolic blood pressure (DBP) was >90 mmHg. A history of cardiovascular disease or cerebrovascular disease (CVD) was defined as a history of angina, myocardial infarction and/or cerebrovascular incidents. Subjects with a urine albuminuria/creatinine ratio (ACR) >30 mg/g were regarded as having DN.

Patients with alcohol abuse, uremia, viral hepatitis, other hemolytic, autoimmune, drug-induced liver diseases associated with hyperbilirubinemia, hypothyroidism/hyperthyroidism, vitamin B12 deficiency, peripheral arterial disease, trauma, cancer, compression due to vertebral disk herniation, inflammatory and infectious diseases, HIV infections, unexplained weight loss and neurotoxic drugs, severe cardiovascular diseases, severe liver disease (e.g., aspartate aminotransferase or alanine aminotransferase greater than two times the normal level), severe renal disease (Scr >115 μmol/l), and central nervous system disorders were excluded.

A total of 344 healthy controls from the individuals who visited our hospital for check-up were enrolled.

The study was approved by the institutional review board of the Lianyungang First Affiliated Hospital to Nanjing Medical University.

Neuropathy assessment and physical examination

All patients took a complete history of neurological symptoms and received a physical examination. The definition of DPN required the presence of both clinically evident DPN and abnormal nerve conduction velocity.

Peroneal nerve conduction velocities (NCVs) were conducted on both sides of each individual using EMG (Keypoint 4; Medtronic, Minneapolis, MN, USA). Electrophysiological examination was considered abnormal if one abnormal attribute (among conduction velocity, amplitude, distal latency or F-wave latency) appeared in no less than two nerves.

Clinical feature measurement

Demographic and clinical data were recorded. Blood pressure measurements were obtained from the subject in a seated position by using a standard manual sphygmomanometer. The retained values were the average of the two readings (left and right arm). Height and weight were measured with the participants standing without shoes and lightly clothed. BMI was calculated as the ratio of weight divided by height squared (kg/m^2). Retinal conditions were assessed by ophthalmologists using a combination of clinical examination, stereoscopic retinal photographs, and/or fluorescein angiography.

Blood samples were measured for metabolic parameters. Plasma glucose, total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), BUN, serum creatinine (Scr), serum acid (UA), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and r-glutamine aminotransferase (r-GTT) were measured with an automatic analyzer (UniCel DxH 800), and glycosylated hemoglobin (HbA1c) was determined by a BIO-RAD D-10TM kit (USA). ACR was measured using a fluorescent immunoassay (Sequoia-Turner model 450 digital fluorometer, Block Scientific, Holbrook, NY, USA).

Statistics

All data was expressed as the means ± SD or number (percentages). Comparisons of two continuous variables were performed using Student's t-test, and categorical variables were analyzed using the Chi-square statistical test. A binary logistic regression analysis was performed with 'presence of DPN' as the dichotomous dependent variable and other independent covariates. Pearson correlation analyses were performed to examine the association between indirect bilirubin and various parameters, and linear regression analysis was con-

Table 1. Comparison of baseline characteristics in healthy control and diabetic patients with and without DPN by sex

Parameters	Men			Women		
	Health control	NDPN	DPN	Health control	NDPN	DPN
Number (n)	168	370	312	176	516	204
Age (y)	55.48±11.94	54.42±14.18	62.48±11.94[b,c]	60.45±11.73	55.52±10.93[a]	61.48±12.50[b]
Duration (y)	-	7.97±6.35	12.40±8.00[b]	-	8.28±6.77	13.07±7.75[b]
BMI (Kg/m^2)	25.13±3.79	26.02±4.51	25.13±3.79	24.62±4.51	25.98±3.58	25.50±4.39
SBP (mmHg)	125.89±20.29	128.95±14.52[a]	132.89±20.29[b,c]	123.07±16.43	126.69±19.70	135.07±16.47[b,c]
DBP (mmHg)	81.23±9.07	80.64±8.58	81.23±9.07	74.79±9.62	75.83±10.02	80.79±9.74[b,c]
HbA1c (%)	-	7.31±2.17	9.36±2.30[b]	-	8.90±2.12	9.48±2.19[b]
UA1b/Cr (mg/g)	13.84±25.00	29.92±84.38[a]	79.70±12.86[b,c]	24.03±25.99	35.59±17.89[a]	126.99±37.93[b,c]
TB (umol/l)	14.56±4.69	15.58±11.10	14.33±6.47	12.17±3.99	12.28±5.45	11.82±3.96[b,c]
DBIL (umol/l)	2.66±0.69	2.66±2.55	2.37±1.17	2.28±0.72	2.15±2.66	1.90±0.93[b,c]
IBIL (umol/l)	11.90±4.12	12.66±6.34	11.96±5.50	9.88±3.18	10.18±3.95	9.31±3.43[b,c]
TP (g/l)	73.41±3.03	67.32±7.28[a]	66.23±5.92[c]	73.08±3.29	67.91±5.32[a]	66.88±8.04[c]
ALB (g/l)	55.09±5.57	42.77±5.09[a]	40.68±4.79[b,c]	45.75±2.09	40.88±4.66	39.59±4.63[c]
GLO (g/l)	26.65±2,43	25.00±4.20	25.56±4.88	27.33±2.81	26.87±3.61	27.63±4.35
ALT (U/L)	23.08±10.19	29.43±46.99	22.32±12.19	15.50±7.47	18.75±6.98	17.79±7.11
AST (U/L)	24.28±4.95	20.11±9.15[a]	19.87±12.19[c]	19.06±4.15	20.29±17.32	24.26±46.92
r-GGT (U/L)	23.71±11.18	36.20±26.90[a]	36.28±52.15[c]	17.17±13.52	28.23±57.27[a]	30.70±52.59[c]
ALP (U/L)	79.57±25.50	82.97±64.53	79.97±35.65	80.65±24.54	89.18±40.29[a]	84.53±29.69[c]
BUN (mmol/l)	5.38±1.14	5.85±1.61	6.07±1.57[c]	4.44±0.95	5.38±1.60[a]	5.68±1.97
Scr (umol/l)	84.84±9.46	78.65±15.04[a]	79.08±15.38	70.48±7.56	68.00±13.00[a]	67.43±17.79
UA (mmol/l)	268.03±76.47	296.99±89.54[a]	306.05±106.35[b,c]	257.63±51.31	261.45±83.93[a]	273.92±93.58[b]
TC (mmol/l)	4.06±0.91	4.91±1.62	4.72±1.69	4.40±0.95	4.93±1.14	4.86±1.32
TG (mmol/l)	1.19±0.53	2.56±4.07[a]	3.25±9.81[c]	1.10±0.66	2.29±2.02[a]	2.17±1.21
HDL-c (mmol/l)	1.26±0.26	0.97±0.27[a]	0.98±0.26[c]	1.41±0.22	1.13±0.42[a]	1.05±0.25
LDL-c (mmol/l)	2.70±0.40	2.71±0.82	2.64±0.96	2.45±0.42	2.80±0.94[a]	2.81±1.31
FPG (mmol/l)	5.38±0.34	10.75±3.72[a]	12.87±4.03[b,c]	5.23±0.41	10.22±0.42[a]	13.28±4.37[b]
FCP (ng/ml)	-	3.64±1.56	2.63±1.83[b]	-	2.51±1.50	2.08±1.26[b]
DR (n)	-	27	79[b]	-	12	110[b]
DN (n)	-	15	51[b]	-	12	60[b]
Hypertension (n)	-	85	203[b]	-	52	264[b]
Statin use	-	33	101[b]	-	48	196[b]
ACEI/ARB use	-	58	129[b]	-	49	213[b]
WBC (10*^{9}/l)	6.51±1.89	6.42±1.77	6.95±3.15	6.13±1.47	6.14±1.67	6.69±1.83
RBC (10*^{12}/l)	4.62±0.78	4.79±0.53	4.46±0.75[b]	4.28±0.42	4.29±0.43	4.21±0.54
Hb (g/l)	142.45±15.67	146.78±14.28	137.87±19.62[b]	126.32±13.42	127.74±12.58	125.15±15.32
PLT (10*^{9}/l)	194.23±52.41	194.00 ±50.59	195.45±67.92	218.45±54.78	222.04±53.77	214.52±63.48

Data were shown as mean ± SD. NDPN: Non-diabetic peripheral neuropathy; DPN: Diabetic peripheral neuropathy; BMI: Body mass index; DBP: Diastolic blood pressure; SBP: Systolic blood pressure; HbA1c: Glycosylated hemoglobin A1c; ACR: Urine albuminuria/creatinine ratio; TB: Total bilirubin; IBIL: indirect bilirubin; DBIL: direct bilirubin; TP: Total protein; ALB: Albumin: GLO: globuin; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; r-GGT: r–Glutamine aminotransferase; Scr: serum Creatinine; BUN: blood urea nitrogen; UA: serum uric acid; TC: Total Cholesterol; HDL-C: high density lipoprotein cholesterol; TG: Triglycerides; LDL-C: Low-density lipoprotein cholesterol; FPG: Fasting plasma glucose; FCP: Fasting C-peptide; DR: Diabetic retinopathy; DN: diabetic nephropathy. CVD: cardiovascular disease or cerebrovascular disease. [a]P<0.05, NDPN group *vs.* healthy control group; [b]P<0.05, DPN group *vs.* NDPN group. [c]P<0.05, DPN group *vs.* healthy control group.

ducted to explore the influence factor for indirect bilirubin. All above tests were considered significant at P<0.05 (two-tailed). Data were analyzed using SPSS version 22.0.

Results

Statistical analysis of patient characteristics

Of the 1,402 subjects, 516 (38.8%) were diagnosed with DPN. **Table 1** shows the clinical and biochemical characteristics of the subjects stratified by healthy control and patients with and without DPN for both sexes. For both sexes, the patients with DPN were older, had longer durations, higher glycemia, higher ACR, UA and SBP, lower C peptide, higher statin and ACER/ARB use, and greater prevalences of hypertension, DN, and DR than the patients without DPN. For women, the serum total bilirubin and indirect bilirubin levels were significantly lower in patients with DPN than in those without DPN.

Table 2. Analysis of binary logistic regression of DPN risk factors in patients with female patients

Variable	β	β'	Wald	P	OR	95% CI
Constant	-13.68	7.16	3.64	0.04	0.000	
Duration	0.08	0.02	5.99	0.00	1.18	1.021-2.32
HbA1c	0.12	0.05	5.76	0.02	1.12	1.09-1.32
UA1b/Cr	0.03	0.01	9.31	0.00	2.41	1.17-4.97
UA	0.37	0.17	5.01	0.03	1.68	1.49-1.95
IBIL	-0.05	0.31	2.37	0.04	0.87	0.65-092
SBP	0.02	0.01	2.14	0.04	1.17	0.54-5.54
DR	1.49	0.32	4.32	0.00	2.50	2.34-8.65
DN	1.03	0.52	4.04	0.03	2.78	1.03-6.82

However, for men, there was no significant difference in the serum bilirubin levels between the DPN and non-DPN groups. It should be noted that subjects with DPN had higher diastolic blood pressure (DBP) than those without DPN in women.

Final model of binary logistic regression analysis (stepwise) with DPN as dependent variables in female patients

We performed a binary logistic regression analysis (stepwise method), considering the presence/absence of DPN as the dependent variable, in order to evaluate the contribution of bilirubin and clinical variables to the risk of developing DPN in female patients. The analysis showed that the independent variables associated with DPN were a longer duration of diabetes, higher glycemia, higher ACR, higher SBP, higher UA, higher prevalences of DR and DN, and lower indirect bilirubin. Therefore, this analysis confirmed the involvement of indirect bilirubin in the susceptibility to DPN in women (**Table 2**). However, bilirubin levels were unrelated to the presence of DPN in men (data not shown).

Correlation analysis between indirect bilirubin and other clinical parameters in female patients

To further examine the correlation between indirect bilirubin levels and the prevalence of DPN in female patients, subjects were categorized into four quartiles (Q1-Q4) according to indirect bilirubin levels. Their clinical and biochemical characteristics according to bilirubin quartile categories are shown in **Table 3**. For women, the women with higher indirect bilirubin levels had a shorter duration of diabetes, lower HbA1c, lower ACR, better lipid profiles and higher WBC and HB than the patients within the lowest indirect bilirubin quartiles. Moreover, as the bilirubin quartile increased, the prevalence of diabetic microvascular complications and hypertension decreased.

Correlations between indirect bilirubin levels and various parameters

The Pearson correlation coefficients between the serum indirect bilirubin levels and other variables were calculated. The serum indirect bilirubin was negatively correlated with duration, ARC, UA, HbA1c, DBP, FCP, BUN, TG, HDL-c and LDL-c levels and positively associated with WBC, RBC and HB levels. Furthermore, linear regression analysis showed that the WBC, RBC and HB levels were the influencing factors for indirect bilirubin in female patients (data not shown).

Expression levels of indirect bilirubin among mild, moderate and severe DPN groups

From **Tables 1**, **2**, we knew that there was a significant association between indirect bilirubin and DPN in women. To explore whether there was a link between qualified indirect bilirubin and DPN disease severity, the analysis of the relationship between the indirect levels and DPN disease severity was further performed. According to TCSS, DPN subjects were divided into mild (6-8 points), moderate (9-11 points), and severe (12 to 19 points) groups. The expression levels of indirect bilirubin decreased with the neuropathy stages (2.09± 1.13 *vs.* 1.41±0.98 *vs.* 0.98±0.25, $P<0.05$). Compared with Q4 quartiles, there was a significant increase in the prevalence of patients in the Q2 quartiles (**Table 4**).

Discussion

The major finding of our study is that a lower serum indirect bilirubin level is a risk factor for the development of DPN in Chinese women. Individuals in the lower quartiles of indirect bilirubin levels had increasing prevalences of neuropathy and other microvascular complications, such as diabetic retinopathy and nephropathy. However, there was no association between bilirubin levels and DPN in men.

Table 3. Baseline clinical and biochemical characteristics of the studied based on serum indirect bilirubin quartiles in female patients

IBIL	Women			
	Q1	Q2	Q3	Q4
Number (n)	178	184	172	186
Age (y)	60.71±12.71	61.47±14.10	60.40±12.58	59.16±10.04
Duration (y)	11.32±6.96	11.15±6.78	10.76±7.13	9.04±6.49
BMI (Kg/m^2)	25.43±3.72	25.31±4.96	25.27±5.13	25.30±3.33
SBP (mmHg)	132.99±14.63	131.90±16.75	130.74±16.20	130.70±17.24
DBP (mmHg)	78.48±9.95	78.26±8.46	79.12±12.13	80.68±8.74
HbA1c (%)	9.45±2.00	9.43±2.18	9.21±2.53	8.83±2.04
ACR (mg/g)	79.99±22.83	59.65±28.26	38.20±9.85	26.45±10.52
TP (g/l)	64.78±6.88	66.72±6.74	67.13±5.15	67.52±8.28
ALB (g/l)	38.45±5.67	40.61±4.54	40.89±5.14	42.44±3.98
GLO (g/l)	26.35±4.60	26.15±4.84	25.68±3.82	26.06±4.03
ALT (U/L)	18.36±7.32	17.29±7.11	17.81±6.83	18.40±7.18
AST (U/L)	16.88±5.26	16.24±5.08	16.35±4.67	15.84±4.12
GGT (U/L)	24.10±18.79	21.85±14.63	20.94±12.80	21.78±15.04
ALP (U/L)	75.96±26.66	84.55±30.25	76.26±21.66	76.57±23.72
BUN (mmol/l)	5.79±1.73	5.38±1.68	5.29±1.50	5.24±1.56
Scr (umol/l)	66.92±17.24	69.36±16.64	70.08±19.79	66.77±17.28
UA (mmol/l)	282.55±86.94	277.56±100.01	264.22±75.74	260.70±100.33
TC (mmol/l)	5.33±1.24	4.94±1.08	4.78±1.02	4.56±1.09
TG (mmol/l)	2.88±2.10	2.06±1.21	1.84±1.27	1.73±0.91
HDL-c (mmol/l)	1.07±0.59	1.10±0.25	1.11±0.43	1.10±0.24
LDL-c (mmol/l)	2.97±0.96	2.89±1.09	2.71±0.85	2.62±0.87
FPG (mmol/l)	9.61±4.37	10.02±4.52	10.41±4.32	11.44±3.67
FCP (ng/ml)	2.07±0.94	2.22±1.41	1.90±0.90	2.08±1.17
WBC	6.15±1.78	6.41±1.73	6.29±1.68	6.89±1.85
RBC	3.96±0.42	4.37±0.41	4.23±0.46	4.41±0.40
HB	118.06±11.91	127.10±13.21	126.15±12.40	133.11±11.71
PLT	233.87±74.51	213.68±44.89	216.80±59.96	221.68±57.20
DR (n)	42 (23.6)	36 (19.56)	26 (15.11)	18 (9.68)
DN (n)	28 (15.73)	22 (11.96)	14 (8.14)	8 (4.30)
DPN (n)	94 (52.80)	84 (45.65)	68 (39.53)	50 (26.88)
Hypertension (n)	88 (49.44)	84 (45.65)	80 (46.51)	64 (34.41)
CVD (n)	8 (4.49)	16 (8.70)	16 (9.30)	8 (4.30)

Data were shown as mean ± SD.

Therefore, the present study demonstrated that indirect bilirubin levels in the physiological range are significantly and inversely associated with DPN in the Chinese female population. Individuals with lower indirect bilirubin concentrations had a higher risk for the development of DPN.

It is well known that DPN is one of the most common complications of diabetes and causes severe morbidity and mortality, resulting in a considerable economic burden for diabetes care. It is documented that DPN is caused primarily by microvascular injury and the duration of DM, hyperglycemia, hypertension, and hyperlipidemia. Chronic hyperglycemia-associated reactive oxygen species stress and low-grade inflammation might be one of the important mechanisms underlying the onset and exacerbation of diabetic neuropathy. Bilirubin may exhibit strong antioxidant and anti-inflammatory properties on the vasculature [25] and has

Table 4. Odds risk for DPN in different IBIL quartiles in female patients

	B	SE	Walds	P	OR (95% CI)
Constant	0.07	0.17	0.17	0.68	1.07
IBIL group (4)			9.83	0.02	
IBIL group (1)	0.59	0.24	5.08	0.02	1.82 (1.12, 1.91)
IBIL group (2)	0.05	0.24	0.04	0.83	1.05 (0.66, 1.68)
IBIL group (3)	0.54	0.24	5.00	0.03	1.72 (1.07, 2.76)

recently drawn much interest particularly in relation to oxidative stress-related diseases, including diabetes and its chronic complications. However, there are limited studies on the relationship between bilirubin and DPN. Kim *et al.* [22] have reported that low serum total bilirubin levels are significantly associated with DPN in both sexes, independent of classic risk factors and other microvascular complications. However, the comparisons in their study did not include total, direct or indirect bilirubin, which is the best predictor of the development of DPN. In our analyses, it was shown that indirect bilirubin was a significant explanatory variable for type 2 diabetic peripheral neuropathy in Chinese female patients, similar to the of known factors, such as HbA1c and the duration of diabetes in multiple regression analysis. Moreover, the patients with more severe diabetic retinopathy showed lower indirect bilirubin levels. Taken together, these findings suggest the possibility that lower indirect bilirubin levels may be involved in the onset and/or deterioration of DPN in Chinese female patients with type 2 diabetes mellitus. The discrepancy between the current results and those of Kim *et al.* may have resulted from the differences in the populations' characteristics. However, we cannot explain the lack of association between bilirubin and DPN in diabetic men. Hepatic uridine diphosphate glucuronyl transferase (UGT1A1), which encodes hepatic urine diphosphate-glucuronyl transferase and is a major enzyme in bilirubin conjugation and in the regulation of bilirubin levels, could explain 18% of the variation in serum total bilirubin levels. Ali *et al.* reported that total bilirubin levels are causally associated with a lower risk of new-onset T2D by implementation of Mendelian randomization and examined the UGT1A1 rs-6742078 (Bilirubin as a potential causal factor in type 2 diabetes risk: a Mendelian randomization study). Further studies are warranted to explore and confirm this sex-based divergence and the role of UGT1A1 rs6742078 in the development of DPN.

The precise mechanisms underlying the protective effects of bilirubin on the incidence of DPN are largely unknown. There are two possible explanations. First, bilirubin has anti-inflammatory and antioxidant properties. Because indirect bilirubin usually comprises the majority of total bilirubin, indirect bilirubin could be important for the antioxidative stress anti-inflammatory effects. On the basis of *in vitro* and *in vivo* studies, bilirubin has been recognized as a potent antioxidant under physiological conditions, which suppresses the oxidation of lipids and lipoproteins, especially low-density lipoprotein cholesterol [26, 27]. Second, bilirubin is also an endogenous tissue protector by virtue of its involvement in immune reactions and inflammatory processes. Further investigations are necessary on the underlying mechanism that links bilirubin to neuroprotection and the prognostic significance of serum bilirubin in DPN.

There are some limitations in our study that are worth noting. First, owing to the retrospective nature of the present study, we are not able to establish a causal or resultant relationship between indirect bilirubin and DPN. Second, there were missing data, especially on the history of ever-smoking and ever-drinking. However, it has been documented that there were no significant interactions between smoking status or serum bilirubin levels. Third, the present study was carried out in an urban university hospital in an ethnically homogenous population in China, and the patients were admitted to the hospital because of uncontrolled hyperglycemia, which might not be generalized to other type 2 diabetic patient populations.

However, most of the other studies in the literature have compared only total bilirubin as the parameter for the prediction of diabetic complications. Our study is the first to consider all of the three parameters of bilirubin stratified by sex in a Chinese population.

In summary, we have observed that low indirect bilirubin levels were significantly associated with the incidence of DPN in Chinese women. Further experimental and longitudinal studies

are expected to elucidate the role of serum indirect bilirubin in the pathogenesis of DPN.

Acknowledgements

This study was supported by the Lianyungang Health Bureau program, Jiangsu Provincial in china (bureau 1402, 201510) and the Health International (Regional) Exchange Support Program of Jiangsu Province. However, The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Here, we are grateful to Dr. Yang Tao for the technical assistance.

Disclosure of conflict of interest

None.

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