Serum total bilirubin concentration in patients with type 2 diabetes as a possible biomarker of polyvascular disease

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Abstract

Aims The aim of this study was to investigate the association between serum total bilirubin concentration and complicated macrovascular diseases, such as cerebrovascular disease (CBVD), cardiovascular disease (CAD), and peripheral arterial disease (PAD), in patients with type 2 diabetes.

Methods We performed a retrospective cross-sectional study in 674 patients with type 2 diabetes. Serum total bilirubin concentration was compared between patients with and without CBVD, CAD, and PAD. Logistic regression analyses were performed to identify risk factors for CBVD, CAD, and PAD. Associations between total bilirubin concentration and the number of complicated macrovascular diseases were analyzed.

Results Patients with CBVD and PAD showed significantly lower serum total bilirubin concentrations than did those patients without those diseases. However, the bilirubin concentration did not differ between patients with and without CAD. Total bilirubin concentration was an independent predictor of CBVD, but not of CAD or PAD. There was a statistically significant trend for a decrease in bilirubin concentration in the presence of an increasing number of macrovascular diseases.

Conclusion The presence of more than one macrovascular disease, called polyvascular disease, carries a high risk for cardiovascular mortality. Serum total bilirubin concentration may be useful as a clinical biomarker of polyvascular disease.

Keywords Total bilirubin · Atherosclerosis · Polyvascular disease · Cerebrovascular disease · Coronary artery disease · Peripheral arterial disease

Introduction

Atherosclerotic cardiovascular disease (ASCVD) is a leading cause of morbidity and mortality worldwide [1, 2]. Atherosclerosis is a progressive systemic disease resulting in cerebrovascular disease (CBVD), coronary artery disease (CAD), and peripheral arterial disease (PAD) [3]. In diabetic patients with poor glycemic control, the process of atherosclerosis is accelerated. Besides hyperglycemia, hypertension and dyslipidemia are established as independent risk factors for ASCVD. Numerous studies have shown the beneficial effects of controlling these traditional risk factors for the prevention of ASCVD. However, even with control of these traditional risk factors, a substantial risk of ASCVD remains in some cases [4], which suggests the existence of additional residual risk factors [5].

The presence of more than one affected vascular bed in the cerebrovascular, coronary, or peripheral arterial systems is called polyvascular disease. Polyvascular disease carries a high risk for cardiovascular mortality [2, 6, 7], and its prevalence is well established, especially among older individuals [8–10]. It has also been shown that ASCVD risk increases substantially with an increasing number of affected arterial beds [2, 6, 11]. The increase in

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cardiovascular event rates with an increasing number of affected vascular beds is suggestive of a progressive, extensive, and systemic disease [12, 13].

Bilirubin has recently drawn attention as a physiological modulator of oxidative stress in diabetic patients [14]. Bilirubin exerts potent antioxidant properties by scavenging reactive oxygen species [15, 16]. In addition, it exerts anti-inflammatory effects on the vasculature [17–19]. Both oxidative stress and chronic inflammation are essential to the onset and progression of atherosclerosis [20, 21]. Therefore, bilirubin, once considered simply the useless end product of heme degradation, has emerged as a beneficial endogenous inhibitor of atherosclerosis. Consistent with this notion, there are numerous reports that bilirubin concentration is inversely correlated with CBVD [22–25], CVD [24, 26–28], and PAD [29–32]. Hypobilirubinemia can now be recognized as a possible residual risk factor for the development of macrovascular disease in diabetic patients.

To the best of our knowledge, there have been no reports that examined the association of bilirubin concentration with the simultaneous presence of atherosclerotic disease of these three vascular beds (CBVD, CAD, and PAD) in the same patient. In addition, there have been no reports concerning the relationship between bilirubin concentration and the number of vascular beds affected by ASCVD. Therefore, we conducted this study to investigate the association between serum bilirubin concentration and the number of macrovascular diseases present in patients with type 2 diabetes.

Materials and methods

Study subjects

We performed a retrospective cross-sectional study of 674 consecutive Japanese patients (446 males and 228 females) with type 2 diabetes mellitus who were admitted to Keio University Hospital for blood glucose control from January 2008 to December 2013. The following patients were excluded from the study: patients receiving hemodialysis or peritoneal dialysis, patients <20 years old, pregnant patients, patients with malignant disease or hematological disease under treatment, patients with liver cirrhosis, patients with liver function test abnormalities [i.e., serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT) level >3 times the upper limit of the normal range], and patients with an unstable hemodynamic condition.

Data collection

Basic demographic data from all patients were collected from medical records, including gender, age, height, weight,

duration of diabetes, blood pressure, serum hemoglobin A1c (HbA1c) levels, serum total bilirubin levels, serum AST and ALT levels, estimated glomerular filtration rate (eGFR), and serum lipid levels. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Subjects were evaluated for the presence of diabetic retinopathy by an ophthalmologist using fundoscopy after pupillary dilation. Diabetic nephropathy was defined as a urinary albumin-to-creatinine ratio (ACR) ≥ 30 mg/g creatinine (Cr) or an eGFR <30 ml/min/1.73 m². CBVD and CAD were defined by the presence of a personal history of these diseases. PAD was defined as a history of PAD or current intermittent claudication associated with an ankle brachial index (ABI) <0.9 . Hypertension was defined as a systolic blood pressure ≥ 140 mmHg, a diastolic blood pressure ≥ 90 mmHg, and/or prior treatment for hypertension. Dyslipidemia was defined as a serum low-density-lipoprotein (LDL) cholesterol level ≥ 120 mg/dl, a serum high-density-lipoprotein (HDL) cholesterol level <40 mg/dl, a serum triglyceride level ≥ 150 mg/dl, and/or prior treatment for dyslipidemia. Alcohol drinking was defined as a prior or current habit of drinking alcohol. Smoking was defined as prior or current tobacco usage. HbA1c was presented as the equivalent National Glycohemoglobin Standardization Program (NGSP) value. eGFR was calculated using the following formula established by the working group of the Japanese Chronic Kidney Disease Initiative: $\text{eGFR (ml min}^{-1} \text{ 1.73 m}^{-2}) = 194 \times (\text{serum creatinine})^{-1.094} \times (\text{age})^{-0.287}$ (in females: $\times 0.739$) [33].

Statistical analysis

Serum total bilirubin concentration was compared between patients with and without CBVD, CAD, and PAD, between patients taking and not taking insulin, insulin sensitizer (biguanide and/or thiazolidinedione), renin-angiotensin-aldosterone system blockers [angiotensin II receptor blocker (ARB) or angiotensin-converting enzyme inhibitor (ACEI)], and statin using the nonparametric Mann-Whitney *U* test. Logistic regression analyses were performed to examine the effects of various factors on the presence of CBVD, CAD, and PAD, and the following factors were considered as independent variables: total bilirubin concentration, BMI, age, gender, duration of diabetes, serum HbA1c levels, eGFR, presence of dyslipidemia, hypertension, and smoking habit. Associations between total bilirubin concentration and the number of complicated macrovascular diseases (CBVD, CAD, and PAD) were analyzed using the nonparametric Jonckheere-Terpstra test. Because only two patients had all three macrovascular diseases, they were combined with those patients with two macrovascular diseases for the Jonckheere-Terpstra test. All statistical analyses were conducted using the Statistical

Package for the Social Sciences (version 17.0; SPSS, Chicago, IL, USA). Patient age, duration of diabetes, BMI, and serum HbA1c levels were expressed as mean $\pm$ standard deviation. On the other hand, total bilirubin, AST, ALT, eGFR, LDL cholesterol, HDL cholesterol, and triglyceride levels were expressed as the median and interquartile range (IQR) because these parameters were not normally distributed. Values of $p < 0.05$ (two-sided) were considered statistically significant.

Results

Patients characteristics

Table 1 shows the patient characteristics and laboratory data. The serum total bilirubin concentration of the 674 patients with type 2 diabetes was 0.7 mg/dl (IQR 0.5–0.9 mg/dl). The mean $\pm$ SD of age, duration of diabetes, BMI, and serum HbA1c levels were 64.5 ± 14.1 years, 13.9 ± 10.9 years, 25.5 ± 6.3 kg/m², and $9.2 \pm 2.2\%$, respectively. Many patients were

complicated by microvascular (retinopathy and nephropathy) and macrovascular (CBVD, CAD, and PAD) diseases. Of 674 patients, 456 had no macrovascular complications. The number of patients with 1, 2, or 3 macrovascular diseases was 166, 50, and 2, respectively. Many patients had hypertension, dyslipidemia, and a smoking habit, all of which are classical ASCVD risk factors.

Of 674 patients, 537 were taking diabetes medication. As oral hypoglycemic agents, 198, 159, 107, 104, 50, and 31 patients were taking sulphonylurea, alfa-glucosidase inhibitor, dipeptidyl-peptidase IV inhibitor, biguanide, thiazolidinedione, and glinide, respectively. As injection therapy, 257 and 6 patients were taking insulin and glucagon-like peptide-1 receptor agonist, respectively. As antihypertensive agents, 287, 269, and 34 patients were taking ARB, calcium channel blocker, and ACEI, respectively. As antidyslipidemic agent, 264 patients were taking statin.

Total bilirubin concentration and macrovascular diseases

Patients with CBVD showed a significantly lower serum total bilirubin concentration than those without CBVD [0.6 mg/dl (IQR 0.5–0.8 mg/dl) vs. 0.7 mg/dl (IQR 0.5–0.9 mg/dl), respectively; $p = 0.012$]. Similarly, patients with PAD showed a significantly lower serum total bilirubin concentration than those without PAD [0.6 mg/dl (IQR 0.4–0.75 mg/dl) vs. 0.7 mg/dl (IQR 0.5–0.9 mg/dl), respectively; $p = 0.013$]. However, there was no significant difference between the patients with [0.7 mg/dl (IQR 0.5–0.8 mg/dl)] and without [0.7 mg/dl (IQR 0.5–0.9 mg/dl)] CAD ($p = 0.094$).

Patients taking ARB or ACEI showed a significantly lower serum total bilirubin concentration than those not taking these drugs [0.6 mg/dl (IQR 0.5–0.8 mg/dl) vs. 0.8 mg/dl (IQR 0.5–0.9 mg/dl), $p < 0.0001$]. Similarly, patients taking insulin showed a significantly lower bilirubin concentration than those not taking insulin [0.6 mg/dl (IQR 0.5–0.8 mg/dl) vs. 0.7 mg/dl (IQR 0.5–0.9 mg/dl), $p = 0.011$]. On the other hand, there were no significant differences between the patients taking biguanide and/or thiazolidinedione [0.7 mg/dl (IQR 0.5–0.9 mg/dl)] and those not taking these medications [0.7 mg/dl (IQR 0.5–0.9 mg/dl), $p = 0.13$]. No significant difference was detected between the patients taking [0.7 mg/dl (IQR 0.5–0.83 mg/dl)] and not taking statin [0.7 mg/dl (IQR 0.5–0.9 mg/dl), $p = 0.086$], either.

As shown in Table 2, the total bilirubin concentration [odds ratio (OR) 0.360, 95% confidence interval (CI) 0.149–0.868, $p = 0.023$], age (OR 1.066, 95% CI 1.040–1.093, $p < 0.0001$), and presence of hypertension (OR 2.087, 95% CI 1.154–3.773, $p = 0.015$) were shown

Table 1 Patient demographics and laboratory data

Number of patients	674
Age (years)	64.5 ± 14.1
Gender (male/female)	446/228
Duration of diabetes (years)	13.9 ± 10.9
BMI (kg/m ²)	25.5 ± 6.3
HbA1c (%)	9.2 ± 2.2
Total bilirubin (mg/dl)	0.7 (IQR 0.5–0.9)
AST (IU/l)	22 (IQR 17–29)
ALT (IU/l)	20 (IQR 14–32)
eGFR (ml/min/1.73 m ²)	63.3 (IQR 42.7–81.9)
LDL cholesterol (mg/dl)	105 (IQR 84–130)
HDL cholesterol (mg/dl)	45 (IQR 37–54)
Triglyceride (mg/dl)	125 (IQR 88–183)
Smoking, n (%)	324 (48.1)
Alcohol, n (%)	234 (34.7)
Hypertension, n (%)	422 (62.6)
Dyslipidemia, n (%)	301 (44.7)
Diabetic retinopathy, n (%)	279 (41.4)
Diabetic nephropathy, n (%)	323 (47.9)
Cerebrovascular disease, n (%)	101 (15.0)
Coronary artery disease, n (%)	128 (19.0)
Peripheral arterial disease, n (%)	43 (6.4)

Values are expressed as number (%), mean $\pm$ SD, or median (interquartile range)

BMI body mass index, HbA1c hemoglobin A1c, IQR interquartile range, AST aspartate aminotransferase, ALT alanine aminotransferase, eGFR estimated glomerular filtration rate, LDL low-density lipoprotein, HDL high-density lipoprotein

to be independent determinants of CBVD. As for CAD, age (OR 1.035, 95% CI 1.012–1.058, $p = 0.0024$), male gender (OR 0.379, 95% CI 0.212–0.676, $p = 0.001$), and presence of dyslipidemia (OR 4.683, 95% CI 2.916–7.519, $p < 0.0001$) were shown to be independent determinants. As for PAD, duration of diabetes (OR 1.059, 95% CI 1.026–1.093, $p < 0.0001$), eGFR (OR 0.977, 95% CI 0.960–0.994, $p = 0.0067$), and smoking habit (OR 2.402, 95% CI 1.063–5.428, $p = 0.035$) were demonstrated to be independent determinants. As for CAD and PAD, total

bilirubin concentration was not a significant explanatory factor.

As shown in Table 3, there was a statistically significant trend for a decrease in bilirubin concentration with the presence of an increasing number of macrovascular diseases ($p = 0.0495$). Patients with two ($n = 50$) or three ($n = 2$) macrovascular diseases showed the lowest bilirubin concentrations [0.6 mg/dl (IQR 0.5–0.7 mg/dl)], and those without macrovascular disease showed the highest concentrations [0.7 mg/dl (IQR 0.5–0.9 mg/dl)].

Table 2 Logistic-regression analysis of macrovascular disease

	OR	95% CI	<i>p</i> value
Cerebrovascular disease			
Total bilirubin	0.360	0.149–0.868	0.023
BMI	1.016	0.974–1.059	0.47
Age	1.066	1.040–1.093	<0.0001
Gender (female = 1, male = 0)	1.195	0.671–2.129	0.55
Duration of diabetes	1.000	0.977–1.022	0.97
HbA1c	0.918	0.798–1.056	0.23
eGFR	1.007	0.998–1.017	0.14
Dyslipidemia	0.999	0.992–1.006	0.77
Hypertension	2.087	1.154–3.773	0.015
Smoking	1.536	0.890–2.651	0.12
Coronary artery disease			
Total bilirubin	0.724	0.341–1.537	0.40
BMI	1.035	0.997–1.075	0.072
Age	1.035	1.012–1.058	0.0024
Gender (female = 1, male = 0)	0.379	0.212–0.676	0.001
Duration of diabetes	1.006	0.984–1.028	0.61
HbA1c	1.024	0.901–1.163	0.72
eGFR	0.993	0.984–1.003	0.17
Dyslipidemia	4.683	2.916–7.519	<0.0001
Hypertension	1.398	0.795–2.459	0.25
Smoking	1.373	0.846–2.228	0.20
Peripheral arterial disease			
Total bilirubin	0.394	0.093–1.663	0.20
BMI	0.941	0.866–1.022	0.15
Age	1.014	0.978–1.052	0.45
Gender (female = 1, male = 0)	1.72	0.718–4.098	0.22
Duration of diabetes	1.059	1.026–1.093	<0.0001
HbA1c	1.211	0.996–1.473	0.055
eGFR	0.977	0.960–0.994	0.0067
Dyslipidemia	1.693	0.825–3.472	0.15
Hypertension	0.987	0.411–2.371	0.98
Smoking	2.402	1.063–5.428	0.035

OR odds ratio, CI confidence interval, BMI body mass index, HbA1c hemoglobin A1c, eGFR estimated glomerular filtration rate, LDL low-density lipoprotein

Discussion

This study demonstrated that diabetic patients with CBVD and PAD had lower total bilirubin concentrations than those without these vascular complications. Our findings are in agreement with several previous reports [22–25, 29–32]. In addition, we demonstrated a significant decrease in the total bilirubin concentration in those patients with an increased number of affected arterial beds. Patients with polyvascular disease, having at least two of the three macrovascular diseases studied (CBVD, CAD, and PAD), showed a lower serum total bilirubin concentration than those with one or no macrovascular disease. A low total bilirubin concentration may be a residual risk factor for ASCVD and cardiovascular mortality. Further studies are warranted to examine whether the inclusion of bilirubin concentration can improve the performance of existing ASCVD risk scores used in routine practice, such as the Framingham risk score [34].

In this study, the patients taking ARB, ACEI, or insulin showed lower bilirubin concentration than those not taking these medications. The use of insulin sensitizers (biguanide and thiazolidinedione) and statin did not affect bilirubin concentration. Considering that the bilirubin concentration might be regulated at least in part by the state of oxidative

Table 3 Comparison of serum total bilirubin concentration according to the number of complicated macrovascular diseases present in the patient

	<i>N</i>	Total bilirubin (mg/dl)	J-T, <i>p</i> value
0	456	0.7 (IQR 0.5–0.9)	0.0495
1	166	0.6 (IQR 0.5–0.8)	
2,3	52	0.6 (IQR 0.5–0.7)	

Values are expressed as number or median (interquartile range)

J-T Jonckheere-Terpstra test, IQR interquartile range

0, 1, 2, and 3 correspond to the number of complicated macrovascular diseases. The three macrovascular diseases are cerebrovascular disease, coronary artery disease, and peripheral arterial disease

stress and chronic inflammation, it was expected that these medications might increase the bilirubin concentration. The reason for our observations is not clear. One possible explanation is that ARB, ACEI, or insulin might be administered to the patients whose bilirubin concentration was originally low. We do not consider that these drugs lowered bilirubin concentration.

The patients included in the present study had a long duration of diabetes with poor glycemic control. With regard to known risk factors, hypertension, dyslipidemia, and smoking were present in our patients with prevalence of 62.6, 44.7, and 48.1%, respectively. Collectively, the patients in this study were at an extremely high risk for micro- and macrovascular diseases, and the prevalence of diabetic retinopathy and nephropathy was high in our patients. In addition, many patients were complicated by CBVD (15.0%) and CAD (19.0%). The prevalence of PAD was low (6.4%) compared to that of CBVD and CAD, which suggests the possibility that PAD was underdiagnosed in our subjects. It has been previously reported that two-thirds of PAD patients are asymptomatic and that PAD is often underdiagnosed [35]. According to an analysis of REACH (Reduction of Atherothrombosis for Continued Health) Registry data, patients with PAD at baseline had twice the probability of progression to polyvascular disease within the next 3 years than those with only CBVD or CAD [6]. The timely diagnosis and appropriate treatment of patients with PAD are crucial.

Patients with polyvascular disease have been shown to have higher cardiovascular disease risks than those with single arterial bed disease [2, 6, 11]. The identification of these high-risk patients is important for the prevention of cardiovascular events. Among several screening methods, ABI, which is convenient, inexpensive, and noninvasive, has been shown to be a particularly useful screening method for polyvascular disease [11, 36]. In addition, the reactive hyperemia peripheral arterial tonometry index (RHI), which noninvasively evaluates the peripheral microvascular endothelial function in the microvasculature of the finger, is reported to be useful in identifying patients with polyvascular disease [37]. However, measuring RHI requires specialized equipment, and it is not routinely performed. In this regard, measuring the total bilirubin level is an inexpensive and commonly performed test and is available in most hospital laboratories. Measurement of the serum total bilirubin concentration can provide a simple and clinically useful biomarker of polyvascular disease.

Although the precise mechanisms and pathophysiological conditions that link hypobilirubinemia and ASCVD are not clear, one possible mechanism is vascular endothelial dysfunction. Endothelial dysfunction is involved in all stages of atherosclerosis [38–40], and the bilirubin-

increasing drug atazanavir improves endothelial function [41]. However, based on our logistic regression analyses, the relationship of bilirubin with CBVD was stronger than that seen with PAD or CAD. There may be heterogeneity in the way the different vascular beds interact with serum total bilirubin concentrations. With regard to the relationship between bilirubin and the locations of the arterial bed, heterogeneity might exist. Further studies are warranted to determine whether unique mechanisms are involved between the CBVD and total serum bilirubin concentration specifically.

Our study has several limitations. First, we used only a single blood sample for measurement of the total bilirubin concentration. Plasma bilirubin concentrations exhibit substantial variability within individuals [42]. Second, the cross-sectional study design, with a relatively small number of subjects, cannot establish causality between bilirubin concentration and ASCVD. The possibility of reverse causation cannot be excluded. In addition, the presence of various biases and confounding factors cannot be excluded. Large-scale prospective studies are needed to address these points. Third, our subjects were treated at a university hospital, and our results may not be applicable to the general population of patients with type 2 diabetes treated in a primary care setting. Finally, the patient history of ASCVD (CBVD, CAD, and PAD) was not confirmed objectively. Incorrect information regarding the patients' ASCVD history may have hindered a precise evaluation of the relationship between bilirubin and ASCVD. More reliable criteria to document the presence of ASCVD are needed.

In conclusion, our study demonstrated that a decreased serum total bilirubin concentration is associated with an increased likelihood for the presence of polyvascular disease in patients with type 2 diabetes mellitus. The total bilirubin concentration can provide a useful clinical biomarker that identifies type 2 diabetes patients with a high risk of polyvascular disease. Further studies, including prospective epidemiological studies, are needed to better understand the role of bilirubin in determining a patient's susceptibility to polyvascular disease.

Compliance with ethical standards

Conflict of interest This research did not receive any special grant from funding agencies in the public, commercial, or not-for-profit sectors. All authors declare that they have no conflict of interest related to the content of this article.

Human rights statement All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (Keio University School of Medicine, Ethics Committee, date of approval: 24 October 2011, approval no. 20110195) and with the Helsinki Declaration of 1964 and later versions.

Informed consent Informed consent or a substitute for it was obtained from all patients for being included in the study.

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