Correlations Between Serum Bilirubin Levels and Diabetic Nephropathy Progression Among Japanese Type 2 Diabetic Patients: A Prospective Cohort Study (Diabetes Distress and Care Registry at Tenri [DDCRT 5])

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OBJECTIVE

To assess the correlations between serum bilirubin levels and diabetic nephropathy development and progression in type 2 diabetic patients.

RESEARCH DESIGN AND METHODS

Longitudinal data were obtained from 2,511 type 2 diabetic patients registered in a Japanese diabetes registry. To assess the independent correlations between serum bilirubin levels and either the development or progression of diabetic nephropathy, we used logistic regression analysis adjusted for potential confounders.

RESULTS

The median follow-up period was 503.4 days (range 238–777). The mean patient age, BMI, and HbA_{1c} level was 65.2 years, 24.7 kg/m^2, and 7.5% (58.5 mmol/mol), respectively. Baseline serum bilirubin levels were significantly associated with the urinary albumin-creatinine ratio at baseline ($P < 0.001$) and 1 year after registration ($P < 0.001$). Multivariable adjusted odds ratios for progression from microalbuminuria to macroalbuminuria for the second, third, and fourth quartile of serum bilirubin levels were 0.89 (95% CI 0.49–1.58), 0.93 (0.47–1.83), and 0.33 (0.13–0.84), respectively, showing a statistically significant linear trend across categories ($P = 0.032$). However, this trend disappeared after adjustment for hemoglobin levels.

CONCLUSIONS

Serum bilirubin levels were associated with diabetic nephropathy progression in type 2 diabetic patients independent of possible confounders. Serum bilirubin levels might be the link in the correlation between hemoglobin levels and nephropathy progression.

Diabetes Care 2014;37:252–258 | DOI: 10.2337/dc13-0407

Diabetic nephropathy is a common complication of type 2 diabetes and the leading cause of end-stage renal disease in developed countries (1,2). Several studies have reported that diabetic nephropathy is related to the onset of cardiovascular disease, which worsens the overall prognosis (3,4). An understanding of the clinical characteristics and risk factors associated with the development and progression of

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Received 19 February 2013 and accepted 26 August 2013.

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc13-0407/-/DC1.

Members of the Diabetes Distress and Care Registry at Tenri Study Group are listed in the APPENDIX.

diabetic nephropathy is useful to establish effective therapeutic strategies to prevent diabetic nephropathy progression and the onset of cardiovascular complications.

Previous studies have reported a negative correlation between serum bilirubin levels and cardiovascular disease (5,6). Furthermore, individuals with Gilbert syndrome exhibit mild elevations in serum bilirubin levels and tend to have a decreased incidence of vascular complications (7,8). Therefore, it is reasonable to speculate that serum bilirubin levels may be negatively correlated with diabetes complications. Moreover, several cross-sectional studies have shown that serum bilirubin levels are negatively correlated with the prevalence of diabetic microvascular complications (7,9,10); however, no study has evaluated the temporal correlations between the two. Therefore, we assessed the prospective correlation between serum bilirubin levels and the subsequent development or progression of diabetic nephropathy in a cohort of Japanese type 2 diabetic patients enrolled from a large-scale single-center registry.

RESEARCH DESIGN AND METHODS

Patients

We derived patient data from the diabetes registry maintained at Tenri Hospital, a regional tertiary-care teaching hospital in Tenri City, Japan. The details of this registry can be found elsewhere (11,12). The registry briefly recruited patients diagnosed with diabetes who visited the outpatient clinic of Tenri Hospital between October 2009 and August 2010. Patients diagnosed with prediabetes by an oral glucose tolerance test were excluded. Of a total of 5,133 diabetic patients who visited our hospital, 81 were excluded from the study because of dementia (n = 15), mental retardation (n = 6), schizophrenia (n = 3), visual disturbances (n = 35), poor general health (n = 3), or other comorbidities (n = 17). In addition, two patients who refused to complete the questionnaire survey necessary for participation were excluded. Of the 5,052 eligible patients, 3,898 (77.2%) provided consent to participate in the study. The Ethics Committee of Tenri Hospital approved the protocols used in this study.

Data Collection

At registration, patients underwent routine medical history inquiries, physical examinations, and laboratory tests. In addition, the clinical research coordinators collected demographic data, including age, sex, body weight, duration of diabetes, and treatment modalities from patient medical charts on the day of registration. Laboratory tests included random measurements (fasting was not required) of serum levels of HbA_{1c}, lipids, creatinine, total bilirubin, and uric acid and the urinary albumin-creatinine ratio (UACR) from a spot urine sample. HbA_{1c} levels were expressed in accordance with the National Glycohemoglobin Standardization Program guidelines as recommended by the Japanese Diabetes Society (13). Estimated glomerular filtration rate (eGFR) was calculated using an equation proposed by the Japanese Society of Nephrology (14). In addition to baseline data, we collected follow-up laboratory test data, which included UACR 1 year after registration.

Outcomes

One year after registration, we evaluated the transition from normoalbuminuria to microalbuminuria or macroalbuminuria, defined as diabetic nephropathy development, and the transition from microalbuminuria to macroalbuminuria, defined as diabetic nephropathy progression. According to the UACRs, we classified diabetic nephropathy stages as follows: normoalbuminuria (UACR $<$3.4 mg/mmol), microalbuminuria (UACR = 3.4–33.9 mg/mmol), and macroalbuminuria (UACR $\geq$33.9 mg/mmol) (15).

Statistical Analysis

Continuous variables were presented as the mean and standard deviation (SD) or interquartile ranges, where indicated. Differences between groups were evaluated using the unpaired Student t test for normally distributed variables and the Mann-Whitney U test for variables with skewed distributions. The trends in categorical variables were compared using the trend test. We used logistic regression analysis to estimate the odds ratio (OR) (95% CI) of the outcome of serum total bilirubin levels in patients with development and progression of nephropathy in comparison with the patients in a reference category (first quartile). Four statistical models were used. The preliminary model was crude and considered total serum bilirubin levels only, whereas model 1 was adjusted for age and sex, and model 2 was adjusted for the variables in model 1 and duration of diabetes, follow-up duration, BMI, systolic blood pressure, alcohol consumption, tobacco usage, HbA_{1c} levels, log-transformed UACR (from registration), and use of renin-angiotensin system (RAS) inhibitors (i.e., angiotensin-converting enzyme inhibitors, angiotensin type 1 receptor blockers, direct renin inhibitors, and aldosterone antagonists) and statins. Model 3 was adjusted for the variables in models 1 and 2 in addition to hemoglobin levels. All P values were two sided and those $<$0.05 were considered statistically significant. All analyses were performed using Stata/SE statistical software version 12.0 (StataCorp, College Station, TX).

RESULTS

Patient Characteristics

Between October 2009 and August 2010, 3,898 patients provided consent to participate in the study, of which 3,534 had confirmed type 2 diabetes. We excluded 1,387 as described in Fig. 1. The remaining 2,511 patients met the inclusion criteria and were included in the study. Table 1 displays the patient demographics and laboratory data according to the serum bilirubin level quartiles. There were no patients with a serum bilirubin level in the toxic range. The mean patient age, HbA_{1c} level, and BMI were 65.2 years, 7.5% (58.5 mmol/mol), and 24.7 kg/m^2, respectively. Similar to the results reported by Endler et al. (16), in our study, the proportion of males was higher than that of females in the higher category of serum bilirubin levels. Patients with elevated serum bilirubin levels tended to be younger (P = 0.012), had a shorter duration of diabetes ($P < 0.001$), and exhibited higher levels of hemoglobin, eGFR ($P < 0.001$), and diastolic pressure ($P < 0.001$). RAS inhibitors were used to a greater extent to treat patients with higher serum bilirubin levels than those with lower levels (P = 0.003). We

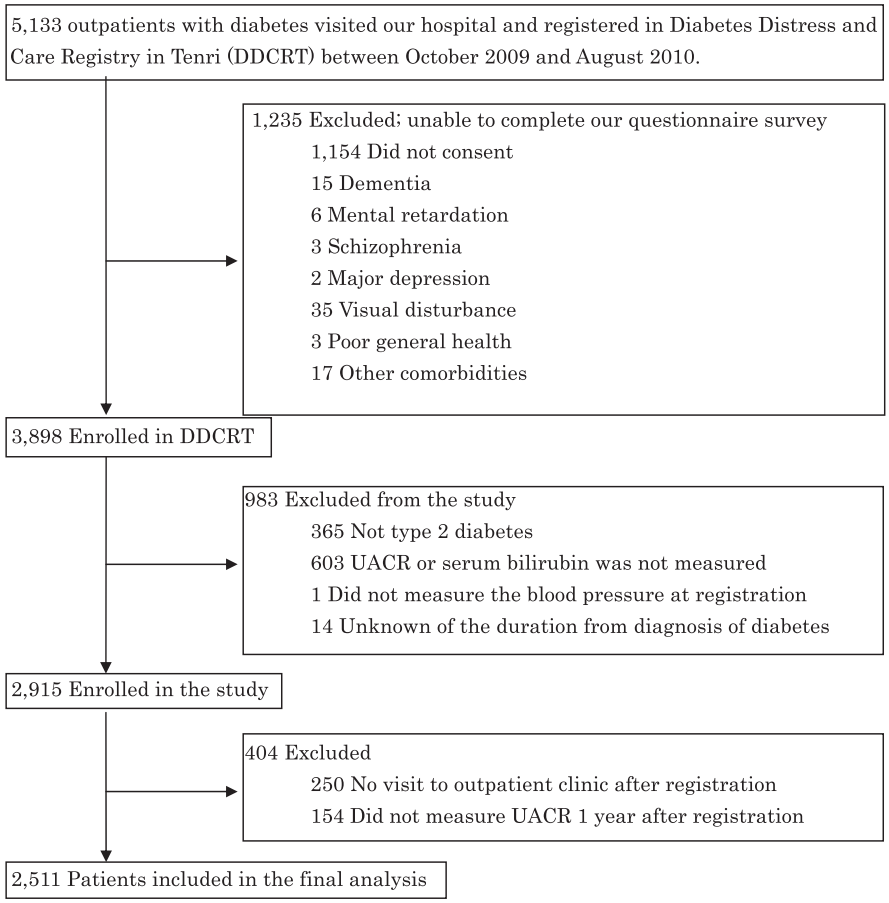

Figure 1—Flowchart of our prospective cohort study.

observed statistically significant linear trends in the UACRs across the serum bilirubin level categories for both baseline and follow-up measurements ($P < 0.001$). As shown in Supplementary Tables 1 and 2, we found similar significant differences in sex and duration of diabetes at baseline between Q1 and Q4 within the normoalbuminuria and microalbuminuria patients. However, in UACR, there was no significant linear trend between Q1 and Q4 in regards to serum bilirubin concentrations.

Next, we analyzed the development of diabetic nephropathy in 1,238 patients with normoalbuminuria and nephropathy progression in 957 patients who presented with microalbuminuria at baseline. The median follow-up period was 503.4 days (range 238–777). Table 2 displays patient characteristics and associated laboratory data on the basis of diabetic nephropathy development and progression. Of the 1,238 diabetic patients with normoalbuminuria, 243 developed microalbuminuria or macroalbuminuria, and of the 957 with microalbuminuria, 88 progressed to macroalbuminuria. Patients with newly developed diabetic nephropathy tended to be female and older, had diabetes for a relatively longer duration since diagnosis, and demonstrated higher baseline UACRs. We observed significantly higher baseline UACRs and lower baseline serum total bilirubin levels in patients with diabetic nephropathy progression. RAS inhibitors were administered more often to patients who demonstrated either diabetic nephropathy development or progression than to those who did not. Table 3 shows the correlations between serum bilirubin levels and diabetic nephropathy progression. Although the linear trend observed across the spectrum of serum bilirubin levels was not significant for diabetic nephropathy development in the crude model (data not shown), a statistically significant linear trend for diabetic nephropathy progression was observed ($P = 0.042$), and the trend remained statistically significant after adjustment for possible confounders, such as sex, age, duration of diabetes, follow-up duration, BMI, systolic blood pressure, alcohol consumption, tobacco use, HbA_{1c} levels, log-transformed UACR at baseline, and use of RAS inhibitors and statins ($P = 0.032$). However, the significance disappeared for all of these variables when hemoglobin levels were added to the multivariate analysis.

CONCLUSIONS

To the best of our knowledge, this is the first study to show that baseline serum bilirubin levels were correlated independently and negatively with diabetic nephropathy progression in a cohort of East Asian type 2 diabetic patients. However, this correlation was diminished by the addition of hemoglobin level to the multivariable statistical model. We speculated that serum bilirubin level may be a novel protective biomarker for diabetic nephropathy progression and serum

Table 1—Participant baseline characteristics on the basis of total serum bilirubin concentration quartiles

		Serum total bilirubin quartiles				
	Total	First quartile	Second quartile	Third quartile	Fourth quartile	*P* for trend
n	2,511	686	891	509	*n* = 425	
Range of serum total bilirubin (μmol/L)	3.4–61.6	3.4–6.8	8.6–10.3	12.0–13.7	15.4–61.6	
Male sex (%)	63.3	53.6	60.7	69.4	76.9	<0.001
Age (years)	65.2 (10.9)	65.7 (11.1)	65.5 (10.5)	64.9 (10.7)	64.0 (11.9)	0.012
Duration of diabetes (years)	13.3 (9.7)	14.8 (10.1)	13.3 (9.9)	12.4 (8.9)	11.8 (9.5)	<0.001
BMI (kg/m^2)	24.7 (3.9)	24.6 (4.2)	24.7 (3.9)	24.8 (3.8)	24.8 (3.7)	0.16
HbA_{1c} (mol/mmol)	58.5 (12.5)	57.4 (12.0)	59.1 (12.5)	58.5 (12.6)	58.8 (13.3)	
HbA_{1c} (%)	7.5 (1.1)	7.4 (1.1)	7.6 (1.1)	7.5 (1.1)	7.5 (1.2)	0.17
Hb (g/L)	137.1 (16.1)	128.9 (15.9)	136.9 (14.9)	142.4 (13.9)	144.6 (15.5)	<0.001
Systolic blood pressure (mmHg)	132.6 (16.2)	133.1 (16.2)	132.7 (16.5)	131.7 (16.2)	132.4 (15.5)	0.548
Diastolic blood pressure (mmHg)	743 (10.5)	73.1 (10.3)	74.2 (10.3)	74.9 (11.1)	75.4 (10.3)	<0.001
Total cholesterol (mmol/L)	5.0 (1.1)	5.0 (1.0)	5.1 (1.2)	4.9 (0.9)	5.0 (1.0)	0.525
HDL cholesterol (mmol/L)	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)	0.189
Triglyceride (mmol/L)	1.8 (1.3)	1.8 (1.4)	1.7 (1.1)	1.7 (1.0)	1.8 (1.5)	0.459
LDL cholesterol (mmol/L)	2.9 (0.8)	2.9 (0.8)	2.9 (0.8)	2.9 (0.8)	2.9 (0.8)	0.41
eGFR (mL/min/1.73 m^2)	74.7 (22.9)	71.6 (25.0)	74.6 (22.5)	75.6 (21.6)	79.0 (20.8)	<0.001
Total bilirubin (μmol/L)	10.8 (4.9)	6.2 (1.0)	9.3 (0.9)	12.7 (0.8)	19.3 (5.0)	<0.001
Uric acid (μmol/L)	319.2 (86.6)	312.9 (91.2)	319.9 (87.5)	327.3 (80.5)	318.4 (83.7)	0.011
Alcohol consumption (g/day)	6.4 (13.3)	4.4 (11.5)	6.2 (13.1)	8.5 (15.2)	7.5 (13.5)	<0.001
UACR (mg/mmol) at baseline (IQR)	24.4 (83.4) (1.7–11.7)	35.9 (101.3) (1.8–16.1)	21.6 (79.4) (1.7–10.9)	17.4 (51.4) (1.6–10.2)	20.1 (88.9) (1.4–9.6)	<0.001
UACR (mg/mmol) after 1 year (IQR)	23.3 (81.7) (1.7–12.0)	37.7 (127.1) (1.9–17.9)	19.4 (54.9) (1.7–11.8)	18.0 (57.2) (1.5–10.2)	14.7 (52.4) (1.6–9.1)	<0.001
Retinopathy (%)	42	48.8	42.5	38.5	38.5	<0.001
Statin use (%)	39.6	44.7	39.3	38.5	33.7	0.004
RAS inhibitor (%)	40.3	44.2	42.2	36.7	34.6	0.003
Smoking (%)						0.003
Never	41.5	45.5	41.8	39.9	36.7	
Past	38.6	35.1	36.4	40.9	46.4	
Current	19.8	19.4	21.9	19.3	16.9	
eGFR <60 (%)	26.3	30.2	22.8	22.6	16.2	<0.001
Progression to stage 1 or 2 to stage 3 CKD (%)	9.3	9.4	10.3	8.6	8.7	0.571

Values within parentheses represent SD if not otherwise specified. CKD, chronic kidney disease; Hb, hemoglobin; IQR, interquartile range.

bilirubin may be the link in the correlation between hemoglobin levels and diabetic nephropathy progression.

One year after registration, we observed a significant correlation between baseline serum bilirubin levels and the diabetic nephropathy progression even after adjustment for possible confounding factors. The results of several cross-sectional studies on diabetic angiopathy were in agreement with our results (7,9,10,17). Inoguchi et al. (7) first reported that diabetic patients with Gilbert syndrome, which is the most common hereditary genetic disorder causing elevated serum bilirubin levels, showed a lower incidence of vascular complications. Fukui et al. (9) discovered a significant cross-sectional correlation between serum bilirubin levels and both urinary albumin excretion and pulse wave velocity in 633 type 2 diabetic patients and concluded that lower serum bilirubin levels were associated with a higher prevalence of microalbuminuria and subclinical atherosclerosis. Yasuda et al. (10) reported a cross-sectional correlation between total serum bilirubin levels and the prevalence of diabetic retinopathy in 1,672 diabetic patients with impaired glucose metabolism. In addition, patients in the highest bilirubin level quartile exhibited a fourfold lower incidence of diabetic retinopathy than those in the lowest bilirubin level quartile. Riphagen et al. (18) found a negative correlation between baseline serum bilirubin levels and the doubling of serum creatinine levels and end-stage renal disease during a median follow-up of ~3 years in a prospective assessment in a post hoc analysis of the results of the Reduction of End Points in Non–Insulin-Dependent Diabetes Mellitus with the Angiotensin II Antagonist Losartan (RENAAL) Trial (19) and the Irbesartan Diabetic Nephropathy Trial (IDNT) (20). However, they did not adjust for hemoglobin levels.

We did not find a mechanism that could explain the correlation between serum bilirubin levels and diabetic nephropathy progression; however, the correlation between bilirubin levels and oxidative stress may be a possible explanation. Reportedly, oxidative

Table 2—Baseline characteristics of patients on the basis of the development and progression of diabetic nephropathy

	Development of diabetic nephropathy				Progression of diabetic nephropathy			
	All	No	Yes	*P* value	All	No	Yes	*P* value
n	1,238	995	243		957	869	88	
Male sex (%)	63.6	66.9	49.8	<0.0001	63.0	63.4	59.1	0.246
Age (years)	63.6 (10.8)	63.2 (10.8)	65.4 (10.5)	0.0033	67.1 (10.8)	67.2 (10.7)	66.3 (11.3)	0.442
Duration of diabetes (years)	11.8 (9.4)	11.6 (9.2)	13.0 (10.1)	0.0293	14.2 (9.7)	14.1 (9.6)	14.9 (10.1)	0.4403
BMI (kg/m^2)	24.5 (3.9)	24.4 (3.8)	24.8 (4.2)	0.1329	24.8 (3.9)	24.7 (3.9)	25.1 (3.4)	0.3562
HbA_{1c} (mmol/mol)	57.0 (11.3)	57.0 (11.1)	57.2 (11.7)	0.7584	59.7 (13.1)	59.6 (12.9)	60.9 (14.6)	0.3782
HbA_{1c} (%)	7.4 (1.0)	7.4 (1.0)	7.4 (1.1)		7.6 (1.2)	7.6 (1.2)	7.7 (1.3)	
Hb (g/L)	138.8 (14.6)	139.8 (14.1)	134.8 (16.3)	<0.0001	137.3 (16.2)	137.6 (16.1)	133.7 (17.2)	0.0305
Systolic blood pressure (mmHg)	130.0 (15.5)	129.7 (15.5)	131.3 (15.5)	0.1345	134.2 (15.5)	134.1 (16.4)	135.1 (16.9)	0.5767
Diastolic blood pressure (mmHg)	73.9 (10.5)	73.9 (10.4)	74.0 (11.1)	0.9194	74.5 (10.3)	74.4 (10.2)	75.5 (11.1)	0.3378
Total cholesterol (mmol/L)	5.0 (0.9)	5.1 (1.0)	4.9 (0.9)	0.0339	4.9 (1.2)	5.0 (1.2)	4.8 (0.8)	0.1817
HDL cholesterol (mmol/L)	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)	0.9132	1.5 (0.4)	1.5 (0.4)	1.5 (0.5)	0.9495
Triglyceride (mmol/L)	1.8 (1.3)	1.8 (1.4)	1.7 (1.3)	0.0625	1.7 (1.2)	1.7 (1.2)	1.8 (1.1)	0.6385
LDL cholesterol (mmol/L)	2.9 (0.8)	2.9 (0.8)	2.9 (0.7)	0.1303	2.9 (0.7)	2.9 (0.8)	2.8 (0.7)	0.4262
eGFR (ml/min/1.73 m^2)	79.2 (19.6)	79.1 (18.7)	79.7 (22.8)	0.6998	74.1 (23.0)	74.3 (22.6)	71.9 (26.4)	0.3494
Total bilirubin (μmol/L)	11.2 (5.0)	11.2 (5.1)	10.8 (4.9)	0.2791	10.8 (4.8)	10.9 (5.0)	9.6 (3.5)	0.0173
Uric acid (μmol/L)	304.2 (79.2)	302.8 (77.5)	310.5 (85.8)	0.1774	321.3 (84.1)	320.2 (84.5)	332.1 (79.5)	0.2052
Alcohol consumption (g/day)	6.7 (13.8)	6.8 (13.6)	6.4 (14.9)	0.7	6.7 (13.2)	6.7 (13.4)	5.5 (11.6)	0.3881
UACR at baseline (mg/mmol)	1.7 (0.8)	1.6 (0.7)	2.2 (0.7)	<0.0001	10.7 (7.6)	10.0 (7.1)	17.7 (8.1)	<0.0001
UACR after 1 year (mg/mmol)	2.8 (3.9)	1.7 (0.7)	7.5 (7.1)	<0.0001	14.3 (23.5)	9.0 (7.5)	66.0 (50.1)	<0.0001
Retinopathy (%)	30.12	28.7	35.5	0.159	42.6	46.7	66.2	0.001
RAS inhibitor (%)	31.91	30.4	37.9	0.017	48.4	41.2	56.8	0.004
Statin use (%)	37.6	37.2	39.1	0.582	40.7	40.5	42.1	0.779
Smoking (%)				0.013				0.425
Never	41.5	39.5	49.8		42.8	42.3	47.7	
Past	39.7	40.8	35		36.8	37.2	33	
Current	18.8	19.7	15.2		20.4	20.5	19.3	

Values in parentheses represent SD. Hb, hemoglobin.

stress was associated with the development of diabetic microvascular complications (21–23), and bilirubin has been regarded as the most effective endogenous antioxidant of lipid peroxidation (24). Fujii et al. (22) reported that hyperbilirubinemia and the administration of biliverdin, which is converted rapidly to bilirubin by biliverdin reductase, protected *db*/*db* mice against diabetic nephropathy via inhibition of oxidative stress. They also reported significantly lower serum bilirubin levels in diabetic hyperbilirubinemic rats than in nondiabetic hyperbilirubinemic rats. These results indicate that bilirubin acts as a radical scavenger and is consumed

Table 3—Correlation between serum total bilirubin and progression of diabetic nephropathy (shift from microalbuminuria to macroalbuminuria)

	Serum total bilirubin quartiles				
	First quartile	Second quartile	Third quartile	Fourth quartile	*P* for trend
n	255	345	188	169	
Mean serum total bilirubin (SD), μmol/L	6.1 (1.0)	9.3 (0.8)	12.7 (0.9)	18.8 (4.9)	
Range of serum total bilirubin, μmol/L	3.4–6.8	8.6–10.3	12.0–13.7	15.4–61.6	
OR for progression (crude)	Ref.	0.95 (0.56–1.62)	0.89 (0.48–1.68)	0.42 (0.19–0.95)	0.042
OR for progression (model 1*)	Ref.	0.96 (0.57–1.63)	0.90 (0.48–1.71)	0.43 (0.19–0.97)	0.054
OR for progression (model 2†)	Ref.	0.89 (0.49–1.58)	0.93 (0.47–1.83)	0.35 (0.13–0.84)	0.032
OR for progression (model 3‡)	Ref.	0.97 (0.54–1.74)	1.14 (0.55–2.39)	0.41 (0.16–1.04)	0.130

*Model 1 adjusted for sex and age. †Model 2 adjusted for the variables in model 1 and duration of diabetes, follow-up time, BMI, systolic blood pressure, drinking and smoking status, HbA_{1c}, log-transformed UACR at baseline, and use of RAS inhibitors and statins. ‡Model 3 adjusted for the variables in model 2 and hemoglobin levels.

as a means to inhibit oxidative stress. On the basis of these findings, we hypothesized that increased serum bilirubin levels can inhibit oxidative stress and inflammation, thereby preventing diabetic nephropathy progression. Although our study results showed a significant correlation between baseline serum bilirubin levels and diabetic nephropathy progression after adjustment for multiple confounders, we could not show a significant correlation after adding hemoglobin levels to the multivariable model. Reportedly, anemia may be an independent risk factor for subsequent diabetic nephropathy progression (25). However, the precise mechanisms by which lower hemoglobin levels may impact diabetic nephropathy progression remain unknown. It has been reported that anemia-induced renal hypoxia and oxidative stress accelerated the decline in renal function (26). In addition, it could be argued that bilirubin mediates the correlation between anemia and diabetic nephropathy progression, because bilirubin is a metabolic product of heme. Bilirubin has been regarded as the most effective endogenous antioxidant of lipid peroxidation (24), which might support our hypothesis that oxidative stress was not suppressed by lower serum bilirubin levels resulting from anemia caused by diabetic nephropathy progression.

In the present patient cohort, the probabilities of albuminuria development and progression were 18.9 and 9.2% per year, respectively, which were greater than those reported in the UK Prospective Diabetes Study (2%) and the Japan Diabetes Complications Study (2.8%) (27,28). These low transition rates of diabetic nephropathy may be explained by the fact that the patients in the present cohort were newly diagnosed with type 2 diabetes. On the other hand, in the Incipient to Overt: Angiotensin II Blocker, Telmisartan, Investigation on Type 2 Diabetic Nephropathy (INNOVATION) Study, transition rates to macroalbuminuria with a median follow-up period of 1.3 years (SD 0.3) were 16.7% with 80 mg of telmisartan, 22.6% with 40 mg of telmisartan, and 49.9% with placebo (29). We believe that the transition rates to macroalbuminuria in our study were comparable to those in previous reports.

Our study had several limitations. We evaluated diabetic nephropathy using single UACR measurements at baseline and during follow-up, which may have resulted in misclassification of diabetic nephropathy. Furthermore, we did not exclude patients with recent febrile illnesses or urinary tract infections or those who took nonsteroidal anti-inflammatory drugs and exercised strenuously on the day of urine sampling, which may have led to erroneous patient classification; however, this misclassification most likely occurred evenly across all bilirubin level quartile categories and resulted in nondifferential misclassification. Nondifferential exposure misclassification will inevitably lead to decreased strength of estimated exposure/disease correlations (30). Thus, we may have underestimated the correlation between bilirubin levels and nephropathy progression in our study.

In conclusion, our analysis of the results from a large longitudinal sample of diabetic patients enrolled from a Japanese registry indicated that serum bilirubin levels were negatively correlated with the progression of microalbuminuria to macroalbuminuria in type 2 diabetic patients, independent of known risk factors.

Acknowledgments. The authors would like to give special thanks to Yukari Moritsuji, Yuki Fujita, Noriko Nakamura, and Youko Sakamoto (Department of Endocrinology, Tenri Hospital) for their support of the study.

Funding. This study was partially supported by the Manpei Suzuki Diabetes Foundation and Japan Society for the Promotion of Science KAKENHI (Grant 25460641, Grant-in-Aid for Scientific Research).

The funder played no role in the performance, design, data collection, analysis, interpretation of the study, or the preparation, review, or approval of the manuscript.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. T.M. performed the literature search, conceived the study, analyzed the data, interpreted the results, and wrote the first draft of most sections of the manuscript. Y.H. obtained funding, collected data, revised the report, and participated in writing the manuscript. S.O. was the project coordinator. S.T. and H.I. organized and supervised the study, interpreted the results, and revised the manuscript. T.M. and Y.H. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Appendix

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