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Association of serum bilirubin levels with development and progression of albuminuria, and decline in estimated glomerular filtration rate in patients with type 2 diabetes mellitus

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ABSTRACT

Aims/Introduction: Recent observational studies suggest elevated levels of bilirubin, an endogenous anti-oxidant, might protect against kidney disease. We carried out an observational cohort study to assess whether higher baseline levels of bilirubin, within normal range, could predict the rate of development and progression of diabetic nephropathy in patients with type 2 diabetes.

Materials and Methods: Japanese type 2 diabetic patients with normo- or microalbuminuria and normal serum bilirubin (<1.2 mg/dL) were recruited from a single center, and categorized according to baseline serum bilirubin levels. Two independent end-points were specified: development or progression of diabetic nephropathy, based on transition to a more advanced stage of albuminuria (albuminuria cohort), and the rate of change in estimated glomerular filtration rate (eGFR cohort).

Results: Albuminuria and eGFR cohorts were constructed consisting of 1,915 patients and 1,898 patients, respectively, with 1,738 patients overlapping. Mean follow up was 4.4 and 5.4 years for the two cohorts, respectively. Within the albuminuria cohort, 132 (9%) of 1,418 patients with normoalbuminuria developed microalbuminuria, and 56 (11%) of 497 patients with microalbuminuria developed macroalbuminuria. Higher baseline bilirubin levels were associated with significantly lower risk of progression from microalbuminuria to macroalbuminuria in both the univariate and multivariate analyses. In normoalbuminuric patients, an inverse association was found when restricted to a subgroup with elevated hemoglobin A1c levels. There was no relationship between bilirubin levels and the rate of change in eGFR.

Conclusions: Higher serum bilirubin levels, within normal range, might be predictive of a lower risk of progression of nephropathy in type 2 diabetic patients.

INTRODUCTION

Diabetic kidney disease (DKD) is the most common cause of chronic kidney disease and end-stage kidney disease[1]; therefore, an improved understanding of the factors involved in the development and progression of DKD is urgently required. Oxidative stress is a potential factor in the pathogenesis of diabetic vascular complications including DKD[2,3]. In non-clinical studies, reactive oxygen species (ROS) are cytotoxic to kidney cells, and promote inflammatory and fibrogenic reactions in the kidneys of diabetic rats[4]. Clinical studies also show a significant association between oxidative stress and DKD[5,6]. Although hyperglycemia is thought to be a contributor to oxidative stress, uncertainty remains regarding the potential role of anti-oxidants in slowing the progression of DKD[7,8].

Bilirubin, an endogenous product of heme catabolism, is a potent anti-oxidant that effectively scavenges peroxyl radicals, and suppresses the oxidation of lipids and lipoproteins[9]. Several non-clinical studies have shown a protective effect of bilirubin in preventing kidney damage[10,11]. In diabetic patients with Gilbert

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syndrome, a common hereditary disorder (incidence of 5–10% of the population) characterized by high levels of unconjugated bilirubin, vascular complications including DKD were reported to be infrequent[12]. Furthermore, serum bilirubin concentrations were shown to be negatively correlated with urinary albumin levels, and positively correlated with glomerular filtration rate (GFR) in cross-sectional studies in patients with type 2 diabetes mellitus[12–15]. In contrast, there were no associations between serum bilirubin levels with estimated GFR (eGFR) or albuminuria in the USA diabetic population[16]. We, therefore, carried out the present longitudinal study to further clarify the association between serum bilirubin levels, and the development and progression of DKD in patients with type 2 diabetes.

MATERIALS AND METHODS

Participants

This was a single-center longitudinal observational cohort study involving adult Japanese patients with type 2 diabetes. Participants were recruited from ambulatory and hospitalized patients presenting at the Diabetes Center, Tokyo Women's Medical University Hospital in Tokyo, Japan, during the period from July 2003 to December 2004. Type 2 diabetes was diagnosed according to the Japan Diabetes Society (JDS) criteria[17].

At a regular ambulatory visit or at the time of hospitalization, participants underwent baseline anthropometric and physical examinations. Laboratory measurements included serum bilirubin, lipids, creatinine and hemoglobin A1c (A1c) in random spot blood samples, and urinary albumin excretion measured in the first morning urine specimen. In the present study, patients confirmed to have normoalbuminuria or microalbuminuria and an eGFR ≥15 mL/min/1.73 m^2 were enrolled. Definition of normo-/microalbuminuria and estimation of GFR are described later. Patients were excluded if their serum bilirubin levels were >1.2 mg/dL because of the potential for confounding hepatobiliary or hemolytic diseases. Patients were also excluded if they had gallstones, cirrhosis, hepatitis B or C, alcoholic liver disease or malignant diseases, if they had undergone renal replacement therapy, or if they were pregnant.

The study protocol was designed and carried out in adherence with the Declaration of Helsinki, and was approved by the Ethics Committee of Tokyo Women's Medical University School of Medicine.

Outcome Measurements

In the present study, two independent renal outcomes were specified (Figure 1). The first was onset or progression of albuminuria, defined as the transition from normo- to micro- or macroalbuminuria (onset of albuminuria), or from micro- to macroalbuminuria (progression of albuminuria). Both transitions required confirmation from at least two consecutive urinary albumin-to-creatinine ratio (ACR) measurements. Patients were followed for at least 6 months.

The second outcome measurement was the annual rate of decline in eGFR, as described in detail previously[18]. For the analysis of this outcome, patients were excluded if their follow-up period was <2 years from study entry (Figure 1). This minimum observation period was selected based on a previous recommendation for an observation period of at least 2 years to assure valid determination of the rate of decline in eGFR[19]. Patients were excluded from the analysis if the rate of change in eGFR was equal to or >5 mL/min/1.73 m^2/year (Figure 1), as such values are clinically implausible, likely because of imprecision in the eGFR calculation, and could artificially skew the analyses.

Measurements

Serum bilirubin concentrations were measured by an enzymatic method involving bilirubin oxidase using an automatic analyzer (Hitachi Labospect 008; Hitachi, Japan; normal range 0.2–1.2 mg/dL). Serum creatinine and total cholesterol were

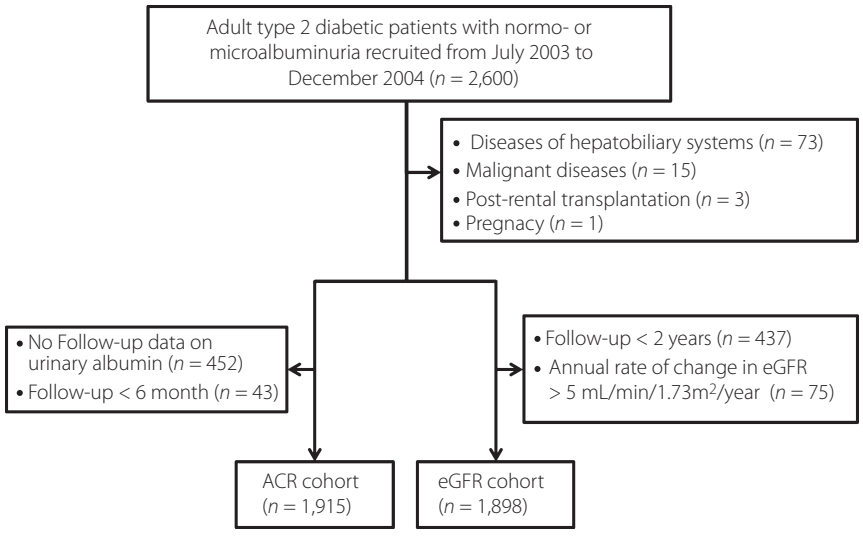

Figure 1 | The composition of the study population.

determined by enzymatic methods. A1c was measured by high-performance liquid chromatography (HPLC), using a set of calibrators assigned by the JDS (normal range 4.3–5.8%). To internationally standardize A1c values to the National Glycohemoglobin Standardized Program (NGSP) units, the following formula was used: A1c (NGSP) (%) = 1.02 × measured A1c (JDS) (%) + 0.25[20].

Urinary albumin levels were measured using the latex agglutination method, and normalized by urinary creatinine determined by an enzymatic method. The stage of albuminuria was defined as normoalbuminuria if urinary ACR was <30 mg/g, microalbuminuria if ACR was 30–299 mg/g and macroalbuminuria if ACR was equal to or higher than 300 mg/g. GFR was estimated using the following three-variable equation, as proposed by the Japanese Society of Nephrology: eGFR (mL/min per 1.73 m^2) = 194 × age (years)$^{-0.287}$ × serum creatinine (mg/dL)$^{-1.094}$ × 0.739 (if female)[21].

Statistical Analysis

Separate tertiles were obtained for normoalbuminuric and microalbuminuric patients according to baseline bilirubin levels. Continuous variables were expressed as arithmetic mean ± SD or geometric mean with 95% confidence interval (CI), as appropriate according to the data distribution. Categorical data were expressed by actual frequencies and percentages. For statistical analyses, Student's *t*-test, analysis of variance (ANOVA), Spearman's correlational analysis, multiple regression analysis and analysis of covariance (ANCOVA) were carried out.

Cumulative incidence of transition of albuminuria stage was estimated by the Kaplan–Meier method, and the statistical differences among groups were examined by the log–rank test. Hazard ratios and the corresponding 95% CIs for reaching each outcome were calculated using univariate and multivariate Cox proportional hazard model analyses. In the multivariate Cox model, all of the following parameters were considered as potential covariates: age, sex, use of renin–angiotensin–aldosterone system blockers, systolic blood pressure, diastolic blood pressure, body mass index, A1c, high-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, eGFR, logarithmically transformed urinary ACR values, hemoglobin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) at baseline. Then, variables were selected using the stepwise variable-selecting procedure specifying the significant levels for entering another explanatory variable into the model as 0.25, and that for removing an explanatory variable from the model as 0.15, respectively. *P*-values <0.05 were considered significant. All statistical analyses were carried out using the SAS version 9.2 (SAS Institute, Cary, NC, USA).

RESULTS

Baseline Demographic and Clinical Characteristics

During the entry period between July 2003 and December 2004, 2,600 adult Japanese patients with type 2 diabetes were assessed for eligibility. A total of 1,915 patients had sufficient baseline and follow-up data to qualify for inclusion in albuminuria cohort, and 1,898 patients qualified for inclusion in the eGFR cohort, with 1,738 patients overlapping (Figure 1). Table 1 shows the clinical and laboratory data for patients in the albuminuria and eGFR cohorts, with the albuminuria cohort divided into subgroups of patients with baseline normoalbuminuria or microalbuminuria. As 1,738 patients (90.8% of albuminuria cohort and 91.6% of GFR cohort) overlapped, demographic and clinical characteristics of the two cohorts were almost identical. Within the albuminuria cohort, patients with microalbuminuria were more likely than those with normoalbuminuria to be men, to be older and to have higher body mass index, systolic blood pressure, and levels of A1c and creatinine, and lower levels of eGFR. Serum levels of total bilirubin in the normoalbuminuria or microalbuminuria subgroups were identical. Clinical and laboratory data for patients with normoalbuminuria and microalbuminuria, classified according to serum bilirubin levels, are listed in the Table 2.

Associations Between Bilirubin Levels and the Progression of Albuminuria

In the 1,418 normoalbuminuric patients from the albuminuria cohort, 132 patients (9.3%) progressed to microalbuminuria during a mean follow-up period of 4.5 ± 1.7 years (range 0.5–6.9 years). As shown in Figure 2a, there were no significant differences in the cumulative incidence of microalbuminuria among the tertiles of baseline serum bilirubin levels (log–rank test, $P = 0.37$). In the multivariate Cox regression hazard analysis, hazard ratios for patients in the second and third bilirubin tertiles were not statistically significant compared with those in the first tertile (Table 3). Even when bilirubin level was modeled as a continuous variable, there was no significant association between serum bilirubin levels and the progression from normoalbuminuria to microalbuminuria using either univariate or multivariate analyses (Table 3).

Among the 497 microalbuminuric patients, 56 patients (11.3%) progressed to macroalbuminuria during the mean follow-up period of 4.3 ± 2.0 years (range 0.5–7.1 years). The cumulative incidence of macroalbuminuria significantly decreased across increasing levels of serum bilirubin (log–rank test, $P < 0.001$; Figure 2a). The adjusted hazard ratio for patients in the third versus first tertile of bilirubin was 0.24 (95% CI 0.08–0.71, $P = 0.009$; Table 3). When bilirubin level was treated as a continuous variable, higher bilirubin levels were associated with a significantly lower risk of progression of albuminuria in both the univariate and multivariate analyses (Table 3).

As hyperglycemia is a major contributor to oxidative stress in diabetic patients, glycemic control per se might modify the relationship between bilirubin levels and progression of albuminuria. Therefore, we carried out the following subanalyses, by further dividing normo- and microalbuminuric patients into subgroups based on the median level of A1c. In normoalbuminuric patients, the interaction between bilirubin and A1c levels (low or high) on

Table 1 | Demographic and laboratory data at baseline in albumin-to-creatinine ratio cohort and estimated glomerular filtration rate cohort

	ACR cohort			eGFR cohort (n = 1,898)
	Normoalbuminuria (n = 1,418)	Microalbuminuria (n = 497)	Overall (n = 1,915)	
Men (%)	58.2	60.2	58.5	59.6
Age (years)	59 ± 12	61 ± 12	59 ± 12	60 ± 12
BMI (kg/m^2)	23.7 ± 3.4	25.0 ± 4.2	24.0 ± 3.7	24.0 ± 3.7
Duration of diabetes (years)	14 ± 9	15 ± 9	14 ± 9	15 ± 9
Diabetic retinopathy (%)	31.7	34.3	40.4	40.9
Medication for diabetes (none/oral/insulin)	13.0/51.2/35.8	8.2/44.4/47.4	12.2/49.3/38.5	9.8/49.3/39.9
SBP (mmHg)	132 ± 19	139 ± 21	134 ± 19	134 ± 19
DBP (mmHg)	76 ± 11	76 ± 12	76 ± 12	76 ± 12
Use of RAS blockers (%)	34.7	63.9	42.1	43.0
Use of other antihypertensive drugs (%)	42.7	72.0	50.2	52.3
Laboratory data				
A1c (%)	8.0 ± 1.7	8.3 ± 1.8	8.1 ± 1.5	8.2 ± 1.5
HDL cholesterol (mg/dL)	55 ± 15	51 ± 15	54 ± 15	54 ± 15
Non-HDL cholesterol (mg/dL)	142 ± 33	134 ± 50	140 ± 39	138 ± 40
Creatinine (mg/dL)	0.75 ± 0.19	0.80 ± 0.20	0.76 ± 0.20	0.77 ± 0.21
Total bilirubin (mg/dL)	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2
AST (U/L)	23 ± 11	24 ± 13	23 ± 12	23 ± 12
ALT (U/L)	26 ± 19	28 ± 20	26 ± 19	27 ± 19
γ-GTP (U/L)	30 (29–32)	33 (31–36)	31 (30–32)	31 (30–32)
eGFR (mL/min/1.73 m^2)	77.6 ± 17.7	74.2 ± 20.4	76.8 ± 18.5	75.9 ± 18.5
Urinary ACR (mg/g)	10.0 (9.7–10.3)	74.0 (70.0–78.2)		16.6 (15.9–17.3)

γ-GTP, γ-glutamyl transferase; A1c, hemoglobin 1c; ACR, albumin-to-creatinine ratio; ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; RAS, renin–angiotensin system.

the progression of albuminuria was significant (P = 0.04). For patients with A1c ≥7.7% (n = 668), higher bilirubin levels, treated as either categorical or continuous variables, were significantly associated with a lower risk of progression of albuminuria in the multivariate analysis (Table 3). For patients with A1C <7.7% (n = 735), there was no association between bilirubin levels and the progression of albuminuria. As there was no significant interaction between bilirubin and A1C levels in microalbuminuric patients (P = 0.69), a subanalysis based on A1c levels was not carried out.

Associations Between Bilirubin Levels and Decline in eGFR

For the eGFR cohort, mean follow up was 5.4 ± 1.1 years (range 2.0–7.1 years), and the mean number of follow-up serum creatinine measurements, used in determining the rate of change in eGFR, was 13 ± 9 per patient. The overall mean rate of change in eGFR was −0.97 ± 2.07 mL/min/1.73 m^2/year. Neither crude nor adjusted rate of decline in eGFR was significantly different among patients classified according to baseline serum bilirubin levels (Figure 3). There was also no significant relationship between baseline bilirubin levels and the rate of change in eGFR using simple correlational analysis (Spearman's correlation coefficient = 0.029, P = 0.21) or in a multivariate regression analysis adjusted for other clinical factors (P = 0.95).

DISCUSSION

In the present single-center longitudinal observational cohort study of patients with type 2 diabetes, we found that higher serum bilirubin levels, within the normal range, were associated with a lower risk of the progression from microalbuminuria to macroalbuminuria. An association between bilirubin and progression of albuminuria was not observed in the subgroup of patients with normoalbuminuria; however, when a subanalysis was carried out, a lower risk of progression was observed for patients with elevated A1c levels. These relationships were confirmed by treating bilirubin levels as either a continuous or categorical variable. Furthermore, these associations were independent of other variables that are well-known risk factors for development of DKD. In contrast, we did not find a relationship between baseline bilirubin level and the rate of GFR decline. Previous cross-sectional studies in diabetic patients yielded conflicting results regarding the association between serum bilirubin levels and prevalence of albuminuria[13,16,22]. In addition, cross-sectional studies do not provide definite information about causal relationships. This is the first longitudinal study to assess the relationship between serum bilirubin levels and progression of albuminuria in diabetic patients, and thus provides support for the hypothesis of a cause-and-effect relationship.

Table 2 | Demographic and laboratory data at baseline in albumin-to-creatinine ratio cohort and estimated glomerular filtration rate cohort classified by baseline bilirubin levels

	Normoalbuminuria			*P*-value	Microalbuminuria			*P*-value
	First tertile (n = 381)	Second tertile (n = 553)	Third tertile (n = 484)		First tertile (n = 147)	Second tertile (n = 207)	Third tertile (n = 143)	
Range of total bilirubin (mg/dL)	0.1–0.4	0.5–0.6	0.7–1.1		0.1–0.4	0.5–0.6	0.7–1.1	
Men (%)	61.4	52.1	62.8	<0.001	66.0	58.5	57.3	0.25
Age (years)	58 ± 13	60 ± 12	58 ± 12	0.04	61 ± 11	63 ± 13	57 ± 13	<0.001
BMI (kg/m^2)	23.5 ± 3.3	23.8 ± 3.6	23.8 ± 3.5	0.37	24.0 ± 3.6	25.1 ± 4.0	26.0 ± 4.6	<0.001
Duration of diabetes (years)	14 ± 9	14 ± 9	14 ± 9	0.34	16 ± 10	16 ± 9	12 ± 8	<0.001
Diabetic retinopathy (%)	35.2	30.6	30.0	0.14	66.7	66.2	63.6	0.84
Medication for diabetes (none/oral/insulin)	13.6/46.5/39.9	12.3/52.8/34.9	10.5/53.3/32.6	0.10	6.8/39.5/53.7	11.6/38.2/50.2	7.7/57.3/35.0	0.002
SBP (mmHg)	131 ± 18	133 ± 19	131 ± 19	0.24	138 ± 22	139 ± 22	141 ± 191	0.50
DBP (mmHg)	74 ± 11	76 ± 12	77 ± 11	0.005	75 ± 12	76 ± 12	78 ± 12	0.03
Use of RAS blockers (%)	33.3	35.3	34.9	0.83	65.3	64.7	60.8	0.68
Use of other antihypertensive drugs (%)	42.3	43.8	41.9	0.82	73.5	72.5	69.9	0.76
Laboratory data								
A1c (%)	8.0 ± 1.5	8.0 ± 1.5	8.1 ± 1.5	0.42	8.4 ± 1.6	8.5 ± 1.6	8.4 ± 1.6	0.88
HDL cholesterol (mg/dL)	54 ± 15	55 ± 15	56 ± 16	0.48	52 ± 16	52 ± 15	50 ± 13	0.29
Non-HDL cholesterol (mg/dL)	142 ± 37	143 ± 32	141 ± 33	0.83	135 ± 48	131 ± 54	135 ± 47	0.71
Creatinine (mg/dL)	0.75 ± 0.19	0.74 ± 0.20	0.76 ± 0.20	0.17	0.85 ± 0.26	0.80 ± 0.22	0.73 ± 0.18	<0.001
Total bilirubin (mg/dL)	0.3 ± 0.1	0.5 ± 0.1	0.8 ± 0.1	<0.001	0.3 ± 0.1	0.5 ± 0.1	0.8 ± 0.1	<0.001
AST (U/L)	22 ± 10	23 ± 9	24 ± 13	0.03	23 ± 10	25 ± 12	25 ± 12	0.16
ALT (U/L)	25 ± 16	25 ± 15	29 ± 24	0.002	25 ± 16	28 ± 23	31 ± 20	0.06
γ-GTP (U/L)	30 (28–33)	30 (29–32)	30 (29–32)	0.19	34 (30–38)	33 (30–37)	33 (30–38)	0.75
eGFR (mL/min/1.73 m^2)	79.0 ± 19.6	76.9 ± 16.5	77.5 ± 17.6	0.20	71.3 ± 22.3	72.0 ± 19.5	80.5 ± 18.5	<0.001
Urinary ACR (mg/g)	9.5 (9.1–10.0)	10.0 (9.5–10.4)	10.2 (9.7–10.6)	0.46	81.3 (77.7–85.0)	73.3 (70.0–76.7)	72.9 (69.7–76.3)	0.09

Data are expressed as percentage, mean ± SD or geometric mean (95% confidence interval). Categorical data were compared using Fisher's exact probability test or the Cochran–Armitage trend test, and continuous data were compared by ANOVA. γ-GTP, γ-glutamyl transpeptidase; ACR, albumin-to-creatinine ratio; ALT, alanine aminotransferase; AST, asparatate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; OHA, oral hypoglycemic agents; RAS, renin–angiotensin system; SBP, systolic blood pressure.

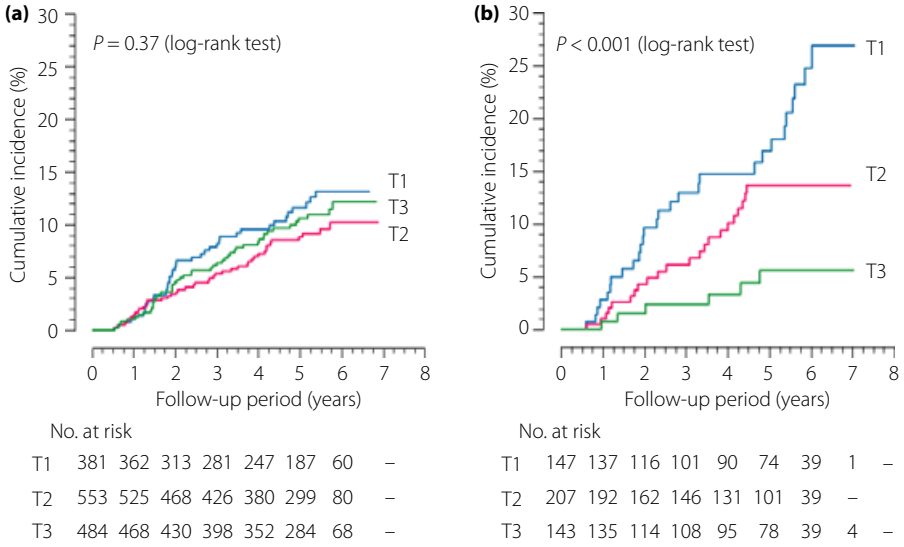

Figure 2 | Comparison of cumulative incidence of (a) development of microalbuminuria in normoalbuminuric patients and (b) progression to macroalbuminuria in microalbuminuric patients among three groups classified into tertiles by baseline bilirubin levels. Blue line: lowest bilirubin tertile (T1; 0.1–0.4 mg/dL); red line: middle bilirubin tertile (T2; 0.5–0.6 mg/dL); green line: highest bilirubin tertile (T3; 0.7–1.1 mg/dL). Numbers below the curves indicate the number of patients at risk. The difference among three groups was significant as determined by the log–rank test in (b) microalbuminuric patients ($P < 0.001$), but not in (a) normoalbuminuric patients ($P = 0.37$).

Table 3 | Univariate and multivariate hazard ratio of progression of albuminuria in diabetic patients with normoalbuminuria and microalbuminuria treating serum bilirubin levels as continuous and categorical variables

	Hazard ratio (95% CI) by tertiles of serum bilirubin levels compared with first tertile as a reference group		Hazard ratio (95% CI) for an increment of 0.1 mg/dL serum bilirubin
	Second tertile	Third tertile	
Normoalbuminuria			
Univariate analysis	0.73 (0.48–1.13)	0.87 (0.57–1.33)	0.98 (0.91–1.07)
Multivariate analysis			
Overall (n = 1,403)	0.70 (0.44–1.12)	0.77 (0.49–1.22)	0.94 (0.85–1.03)
A1c <7.7% (n = 668)	0.95 (0.39–2.29)	1.49 (0.64–3.47)	1.11 (0.95–1.28)
A1c ≥7.7% (n = 735)	0.53 (0.31–0.91)*	0.51 (0.29–0.88)*	0.87 (0.70–0.99)*
Microalbuminuria			
Univariate analysis	0.56 (0.32–0.98)*	0.21 (0.09–0.50)*	0.73 (0.62–0.86)*
Multivariate analysis	0.63 (0.34–1.17)	0.24 (0.08–0.71)*	0.77 (0.64–0.92)*

*$P < 0.05$. A1c, hemoglobin 1c, CI, confidence interval.

Hyperglycemia causes mitochondrial superoxide overproduction in vascular endothelial cells. Of the many enzymatic systems implicated in ROS generation in the kidney, nicotinamide adenine dinucleotide phosphate oxidase (NOX) is considered to be particularly important[23–25]. Among the renal NOXs, NOX-4 is most abundantly expressed in the kidney[23,24,26]. A recent animal study has shown that in diabetic rats with hereditary hyperbilirubinemia, expression of NOX-4 in the kidney was suppressed, resulting in protection against progression of DKD, specifically by suppressing renal mesangial expansion and preventing albuminuria[11]. Furthermore, bilirubin is known to have anticomplement properties[27], and to inhibit protein kinase C activity[28]. These biological properties of bilirubin might contribute to the findings in diabetic kidney disease observed in the present study, as well as in the apparent protective effect of bilirubin in cardiovascular diseases[29,30].

In normoalbuminuric patients, higher serum bilirubin levels were associated with a lower risk of progression of albuminuria only for the subgroup of patients with poorer glycemic control. Patients with poor glycemic control are likely to have more oxidative stress, partly through increased expression of NOX-4 compared with those with good glycemic control[31,32]. Diabetic patients with microalbuminuria are also more likely to have increased oxidative stress compared with those with normoalbuminuria[33,34]. Taken together, the present results suggest that bilirubin might have a protective role in progression of diabetic

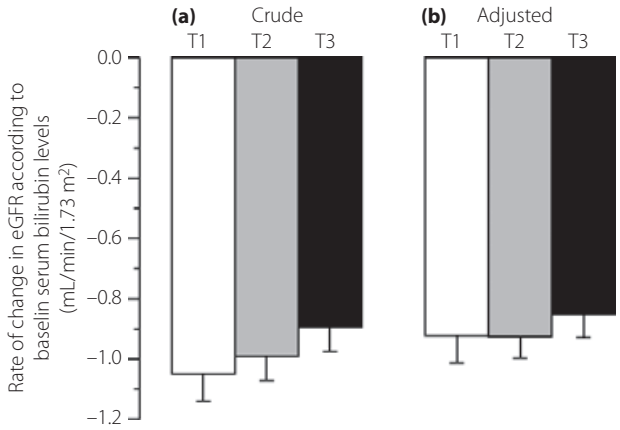

Figure 3 | Comparison of rate of change in estimated glomerular filtration rate (eGFR) among groups classified according to baseline serum bilirubin levels: lowest bilirubin tertile (T1; $n = 526$), middle bilirubin tertile (T2; $n = 745$) and highest bilirubin tertile (T3; $n = 627$), in (a) the crude model using ANOVA and in (b) the adjusted model (ANCOVA). There was no significant difference in the rate in the either crude or adjusted model.

nephropathy, particularly in diabetic patients with greater oxidative stress. Further studies will be required to address the potential mechanisms by which bilirubin might protect against progression of diabetic kidney disease.

In contrast to the progression of albuminuria, changes in eGFR were not associated with bilirubin levels, which was inconsistent with previous cross-sectional studies[13–15]. Although the reasons for this are not clear, several explanations might be postulated. Participants in this diabetic cohort had no or only mild nephropathy (normo-or microalbuminuria), whereas in the typical natural history of DKD, GFR has been considered to decline subsequent to development of macroalbuminuria[35–37]. Therefore, the follow-up period in the present study might be inadequate to evaluate GFR decline. The UK Prospective Diabetes Study found different risk factors for the progression of albuminuria and decline in GFR[38]. Therefore, the present results might reflect differences of the pathogenesis of the two renal manifestations, albuminuria and decline in GFR, in diabetes.

The present study had several limitations. First, we were unable to completely exclude patients with hepatobiliary or hemolytic disease. Second, we did not differentiate direct and indirect bilirubin from total serum bilirubin. Third, we did not evaluate the effect of time-dependent changes in serum bilirubin levels. Fourth, we did not investigate alcohol use, smoking and socioeconomic status or physical exercise. Fifth, information on the use of antihypertensive drugs, including renin–angiotensin–aldosterone system blockers, was obtained only at baseline and not during the follow-up period. Finally, the present study was carried out in an urban university hospital in an ethnically homogenous population in Japan, which might not be representative of other type 2 diabetic patient populations. Conversely, the large sample size, prospective study design and consistent use of first-morning specimens for measurement of albuminuria are strengths of the study.

In conclusion, the present observational cohort study provided evidence that higher serum bilirubin levels are associated with lower risk of the progression of albuminuria in patients with microalbuminuria and in normoalbuminuric patients with poor glycemic control. In light of the present findings, serum bilirubin levels, easily measured in conjunction with traditional risk factors, could help identify diabetic patients at higher or lower risk of DKD progression. These findings require confirmation in prospective, multicenter studies, as well as in non-diabetic kidney diseases. The relationship between serum bilirubin levels and diabetic macrovascular diseases, in which oxidative stress has also been implicated, should be assessed in future studies.

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