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High Serum Bilirubin Is Associated with the Reduced Risk of Diabetes Mellitus and Diabetic Nephropathy

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Several studies have suggested a potential effect of serum bilirubin as an antioxidant and cytoprotectant factor. For the results presented here, we evaluated the correlation between serum bilirubin and diabetes mellitus (DM) or chronic kidney disease originated from DM (DMCKD) in a Korean population. We used a cross-sectional, population-based design to examine 93,909 subjects (aged 18-96 years, 53.0% male). The trend of *P* values in the odds ratios for being DM and DMCKD was calculated using patients separated into five groups based on individual serum bilirubin concentrations. The prevalence of DM and DMCKD was 6.7% and 0.8%, respectively. Higher serum bilirubin levels were significantly associated with decreased prevalence of DM in both men (*P* trend < 0.001) and women (*P* trend = 0.014). The risk of DMCKD also decreased as bilirubin levels increased in women (*P* trend = 0.011), but not in men (*P* trend = 0.467). Serum bilirubin level was inversely related to insulin resistance using the homeostasis model assessment (HOMA-IR), serum insulin, and C-reactive protein (CRP) levels in multiple linear regression analyses. The regression coefficients (*B*) of log-HOMA-IR, log-insulin, and log-CRP were as follows: −0.09, −0.13, and −0.60 in men; −0.07, −0.09, and −0.50 in women, respectively. All the regressions were statistically significant (*P* < 0.001). These results indicate that serum bilirubin might have some protective function against DM and DMCKD, although the association between high serum bilirubin and decreased prevalence of DMCKD is observed only in women.

Keywords: bilirubin; chronic kidney disease; diabetes mellitus; diabetic nephropathy; metabolic syndrome
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Diabetes mellitus (DM) is one of the most prevalent disorders worldwide and its incidence continues to rise (Cowie et al. 2006). Complications due to DM are prevalent, and DM is the most common cause of end-stage renal disease (Hostetter 2001). Improvements in prevention and treatment of DM are surely needed. Despite considerable effort, the societal burden of DM remains substantial.

Bilirubin is a natural product of heme catabolism by heme oxygenase (HO) and is excreted by the liver cell (Arias 1966). Traditionally, bilirubin was considered a toxic waste product or the secondary product of physiological conditions. However, in 1937, Najib-Farah first postulated possible protective actions of bilirubin against bacterial infection. Since Bernhard's research in the 1950's (Bernhard et al. 1954), bilirubin has been known to have a physiologic role as an antioxidant (Stocker et al. 1987) and cytoprotectant (Baranano et al. 2002). Several studies have been conducted to examine potential applications in atherosclerosis and cardiovascular disease (Ollinger et al. 2005; Chen et al. 2008; Perlstein et al. 2008). Furthermore, recent studies have explored both molecular mechanisms for protection by bilirubin and the effects of genetics on serum bilirubin levels (Johnson et al. 2009; Lin et al. 2009a). The basis for these studies is the fact that the basic mechanism of atherosclerosis and cardiovascular disease involves oxidative stress.

Recently, the induction of HO activity was shown to reduce the metabolic and cardiovascular complications in the laboratory and clinical settings (Lin et al. 2009b). In addition, some nephrologists have focused on the role of bilirubin in the development of chronic kidney disease (Fukui et al. 2008; Chin et al. 2009; Lee et al. 2009). Specifically, chronic inflammatory and oxidative stress could lead to metabolic syndrome and chronic kidney disease. However, previous studies have explored this in the laboratory setting or in limited cohorts. Therefore, the relationship between serum bilirubin and DM or chronic kidney disease originated from DM (DMCKD) remains to be determined in a large number of subjects. Herein, we evaluate the association between serum bilirubin level and the preva-

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lence of DM and DMCKD in a Korean population.

Methods

Study design and population

This was a cross-sectional and population-based study. We examined 106,765 subjects who had undergone health examinations between May 2003 and April 2009 at the Seoul National University Bundang Hospital and the Seoul National University Healthcare System Gangnam Center in Seoul, Korea. Medical examinations were carried out because of individuals' requests for a general health check. Of the candidates, 103,964 (97.4%) were selected because bilirubin data were available. We limited inclusion for analysis to the 103,626 participants who were 18 years old and over. We excluded 3,187 subjects with serum bilirubin of more than 2.0 mg/dL and 145 subjects in whom it was difficult to identify the presence of DM. In addition, 6,385 subjects with positive hepatitis viral markers (hepatitis B surface antigen, antibody to hepatitis C virus) were excluded. In total, the number of study subjects was 93,909 (88.0%). The institutional review boards at all participating sites approved the study (No. B-1003/095-104).

Measurements and Definitions

The clinical parameters investigated were age, sex, weight (kg), height (m), waist circumference (cm) and history of hypertension and diabetes mellitus, ever-smoking, and ever-consuming alcohol. History of ever-smoking and ever-drinking was obtained in 67.8% and 73.4% of subjects, respectively. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured after participants had rested for at least 3 minutes. BMI was calculated as weight (kg) divided by the square of height (m). All biochemical determinations were carried out using the same standard laboratory methods. Study visits were conducted in the fasting state. Serum measurements included hepatitis B surface antigen, antibody to hepatitis C virus, hemoglobin, glucose, bilirubin, hemoglobin A1c (HbA1c), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyltransferase (GGT), total protein, albumin, total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, uric acid, C-reactive protein (CRP), insulin, and creatinine. Serum levels of glucose, bilirubin, creatinine, and CRP were assessed using an automatic chemistry analyzer (Toshiba 200FR; Toshiba Lab. Medical, Tokyo, Japan). Plasma insulin was determined by immunoradiometric assay. Insulin resistance was estimated by the homeostasis model assessment (HOMA-IR) (Haffner et al. 1997). Serum insulin and HOMA-IR were available for 17,945 subjects (19.1%). Serum CRP was assessed in 44,434 participants (47.3%). The estimated glomerular filtration rate (eGFR) was calculated for all participants using the Modification of Diet in Renal Disease (MDRD) study equation; eGFR = 186 × (serum creatinine)$^{-1.154}$ × (age)$^{-0.203}$ × (0.742, if patient is female) (Levey et al. 1999). Urinary albumin excretion was evaluated by a dipstick urine test.

DM was defined as a fasting glucose level of 126 mg/dL or greater, or the use of hypoglycemic agents. Hypertension was defined as one of the following conditions: SBP of 140 mmHg or greater, DBP of 90 mmHg or greater, or the use of antihypertensive medication irrespective of BP. Proteinuria was defined as albumin 1+ or greater by a dipstick urine test. CKD was defined as an eGFR of less than 60 ml/min/1.73 m^2, consistent with the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI). The components of metabolic syndrome as defined by the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (NCEP/ATP III) criteria were used (Cook et al. 2003).

Statistical analysis

All analyses were performed using SPSS software (SPSS version 16.0, Chicago, IL, USA). Data were presented as mean/standard deviation (SD) for continuous variables and as proportions for categorical variables. Differences between men and women were analyzed using the Student's *t* test for continuous variables and the chi-square test for categorical variables. Participants were divided into five groups according to the quintile of serum bilirubin levels. Logistic and linear regression analyses were used to examine the correlation between serum bilirubin and DM/DMCKD and calculate odds ratio (OR). Adjustments were made with multiple variables, including traditional risk factors of outcome (DM and DMCKD) and hepatic markers. Associations among serum bilirubin and various metabolic and inflammatory components were obtained using univariate analysis of variance (UNIANOVA) and multiple linear regression analysis. Log transformation was performed for variables showing significant skew from a normal distribution. A *P* value of less than 0.05 was considered significant.

Results

Baseline characteristics of study population

Of the 93,909 subjects, 53.0% were men and 47.0% were women (Table 1). The median age was 48 years (range, 18-96 years). The prevalence of DM was 6.7%. The range of serum bilirubin was 0.1 mg/dL to 2.0 mg/dL. Quintile values of serum bilirubin were different between the sexes (in men: Q1, < 0.9 mg/dL; Q2, 0.9-1.0 mg/dL; Q3, 1.1 mg/dL; Q4, 1.2-1.3 mg/dL; and Q5, > 1.3 mg/dL; in women: Q1, < 0.7 mg/dL; Q2, 0.7 mg/dL; Q3, 0.8-0.9 mg/dL; Q4, 1.0-1.1 mg/dL; and Q5, > 1.1 mg/dL). The prevalence of CKD and DMCKD among all subjects was 7.0% and 0.8%, respectively; 12.3% of DM subjects showed DMCKD.

Correlation between serum bilirubin and the prevalence of diabetes mellitus

Table 2 shows the association between serum bilirubin and DM. In men, increased serum bilirubin was associated with decreased prevalence of DM from 12.0% to 6.8%. In women, the trend of DM prevalence (from 5.8% to 3.4%) was similar to that in men. In univariate analysis, the ORs of DM increased with the quintiles of serum bilirubin, irrespective of sex. The prevalence of DM in men was 46% (44% in women) lower in the fifth quintile group than in the first quintile group. Although adjustment of multiple variables was made, the trend between serum bilirubin and DM in men did not change. However, in women, the ORs from the second to the fourth quintile groups versus the first quintile group were not statistically significant. Only the fifth quintile group had a significant OR compared to the first quintile group. When excluding the subjects (20.0%) with abnormal hepatic function, defined as serum ALT/AST > 40 U/L, ALP > 120 U/L, or GGT > 60 U/L, the adjusted ORs of DM were 0.76 (95% CI, 0.68-0.86; $P < 0.001$), 0.69 (95%

Table 1. Baseline characteristics of study subjects according to sex.

	Men (n = 49,742)	Women (n = 44,167)	Total (n = 93,909)
Age †	48.8/12.00	48.5/12.25	48.7/12.12
BMI (kg/m^2) ‡	24.5/2.84	22.4/3.02	23.5/3.11
Waist circumference (cm) ‡	87.3/7.67	80.8/8.20	84.2/8.55
SBP (mmHg) ‡	120.2/14.33	113.7/16.00	117.2/15.47
DBP (mmHg) ‡	78.3/11.15	70.5/11.27	74.6/11.86
Hypertension (%) ‡	24.0	15.5	20.0
Diabetes mellitus (%) ‡	8.8	4.3	6.7
Ever-smoking (%) ‡	55.8	9.2	34.3
Ever-drinking (%) ‡	55.5	40.6	48.6
Serum findings			
Hemoglobin (g/dL) ‡	15.5/1.07	13.1/1.08	14.4/1.58
Glucose (mg/dL) ‡	100.6/22.44	93.2/16.11	97.1/20.07
Bilirubin (mg/dL) ‡	1.12/0.33	0.91/0.30	1.02/0.33
AST (U/L) ‡	26.1/14.01	21.8/18.75	24.1/16.56
ALT (U/L) ‡	31.6/22.74	19.6/17.71	26.0/21.38
ALP (U/L) ‡	66.7/18.84	61.5/20.70	64.2/19.91
GGT (U/L) ‡	45.5/47.01	20.0/20.84	33.5/39.21
Total protein (g/dL) ‡	7.29/0.42	7.25/0.42	7.27/0.42
Albumin (g/dL) ‡	4.44/0.27	4.33/0.24	4.39/0.26
Total cholesterol (mg/dL) ‡	197.6/34.43	195.3/35.81	196.5/35.10
Triglyceride (mg/dL) ‡	140.4/86.27	95.2/55.66	119.2/76.87
HDL cholesterol (mg/dL) ‡	50.9/12.13	60.7/14.11	55.5/13.98
Uric acid (mg/dL) ‡	6.26/1.27	4.46/0.96	5.4/1.44
CRP (mg/dL) ‡	0.16/0.50	0.10/0.33	0.13/0.43
HbA1c (%) ‡	5.77/0.77	5.66/0.63	5.72/0.71
Insulin (μU/mL) ‡	10.0/5.13	8.9/5.09	9.4/5.14
Creatinine (mg/dL) ‡	1.13/0.16	0.88/0.14	1.01/0.19
Proteinuria (%) ‡	5.6	4.5	5.1
eGFR (ml/min/1.73 m^2) ‡	75.6/11.91	75.0/13.11	75.3/12.49
eGFR < 60 ml/min/1.73 m^2 (%) ‡	6.2	7.9	7.0

Data were presented as mean/standard deviation (S.D.) for continuous variables and as proportions for categorical variables.

Statistical differences between sexes were calculated: *P < 0.05, †P < 0.01, ‡P < 0.001.

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyltransferase; HDL, high-density lipoprotein; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate.

CI, 0.59-0.80; P < 0.001), 0.66 (95% CI, 0.58-0.74; P < 0.001), and 0.60 (95% CI, 0.52-0.70; P < 0.001) in men and they were 0.86 (95% CI, 0.72-1.02; P = 0.089), 0.87 (95% CI, 0.75-1.01; P = 0.086), 0.78 (95% CI, 0.65-0.94; P = 0.012), and 0.74 (95% CI, 0.61-0.89; P = 0.003) in women. Each of these sets of adjusted ORs compares the second to fifth quintile groups to the first group. P trends of DM according to the bilirubin groups were < 0.001 in men and 0.003 in women.

Bilirubin and components of the metabolic syndrome

Table 3 shows the components of metabolic syndrome according to the quintile groups. Increases in serum bilirubin were correlated with decreased waist circumference and decreased levels of triglyceride, glucose, and HbA1c. The correlation between serum bilirubin and HDL cholesterol was also positively significant. Although our study showed significant differences in BP among the five bilirubin groups, the tendency of BPs to decrease as serum bilirubin increased was not seen in men, but was seen in women. The prevalence of hypertension was negatively correlated with serum bilirubin in women. In women, the prevalence of hypertension was 17.3%, 16.1%, 15.0%, 14.9%, and 14.7% from the first to the fifth groups (P < 0.001 by the chi-square test), respectively, whereas in men, the prevalence was 24.8%, 23.9%, 23.2%, 24.4%, and 23.5% from the first to the fifth groups (P = 0.095 by the chi-square test), respectively.

Table 2. Prevalence and odds ratio of diabetes mellitus by the quintiles of serum concentrations of bilirubin.

	Bilirubin quintile	Prevalence (%)	Unadjusted		Adjusted [a]	
			OR (95% CI)	*P* value	OR (95% CI)	*P* value
Men	Q1 (*n* = 10,660)	12.0	1 (Referent)		1 (Referent)	
	Q2 (*n* = 12,354)	9.2	0.74 (0.68 - 0.81)	< 0.001	0.82 (0.75 - 0.90)	< 0.001
	Q3 (*n* = 5,843)	8.2	0.66 (0.59 - 0.73)	< 0.001	0.76 (0.68 - 0.86)	< 0.001
	Q4 (*n* = 9,305)	7.6	0.60 (0.55 - 0.66)	< 0.001	0.71 (0.64 - 0.79)	< 0.001
	Q5 (*n* = 11,580)	6.8	0.54 (0.49 - 0.59)	< 0.001	0.67 (0.61 - 0.74)	< 0.001
	P trend for ORs			< 0.001		< 0.001
Women	Q1 (*n* = 7,875)	5.8	1 (Referent)		1 (Referent)	
	Q2 (*n* = 6,853)	4.7	0.80 (0.69 - 0.93)	0.004	0.88 (0.75 - 1.03)	0.111
	Q3 (*n* = 13,300)	4.1	0.71 (0.62 - 0.80)	< 0.001	0.89 (0.77 - 1.01)	0.077
	Q4 (*n* = 8,165)	3.9	0.67 (0.58 - 0.77)	< 0.001	0.88 (0.75 - 1.02)	0.097
	Q5 (*n* = 7,974)	3.3	0.56 (0.48 - 0.65)	< 0.001	0.77 (0.66 - 0.91)	0.003
	P trend for ORs			< 0.001		0.014

[a]Adjusted for age, body mass index, hypertension, and the serum levels of total cholesterol, triglyceride, high-density lipoprotein cholesterol, and hepatic markers including aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and gamma glutamyltransferase.

Bilirubin quintiles in men: Q1, < 0.9 mg/dL; Q2, 0.9-1.0 mg/dL; Q3, 1.1 mg/dL; Q4, 1.2-1.3 mg/dL; Q5, > 1.3 mg/dL.

Bilirubin quintiles in women: Q1, < 0.7 mg/dL; Q2, 0.7 mg/dL; Q3, 0.8-0.9 mg/dL; Q4, 1.0-1.1 mg/dL; Q5, > 1.1 mg/dL.

OR, odds ratio; CI, confidence interval.

Table 3. Metabolic abnormality according to level of serum bilirubin.

		Q1	Q2	Q3	Q4	Q5	*B*	*P* value
Waist (cm)	M (*n* = 31,455)	87.9/7.98	87.6/7.53	87.3/7.56	87.0/7.48	86.6/7.68	–0.52	< 0.001
	F (*n* = 28,170)	81.8/8.31	81.5/8.42	80.8/7.99	80.4/8.12	79.8/8.17	–0.05	0.560
Log-TG	M (*n* = 49,702)	4.89/0.53	4.83/0.51	4.80/0.52	4.77/0.51	4.72/0.50	–0.18	< 0.001
	F (*n* = 44,142)	4.56/0.51	4.49/0.47	4.43/0.45	4.37/0.44	4.34/0.43	–0.19	< 0.001
HDL chol (mg/dL)	M (*n* = 49,720)	48.6/11.66	50.3/11.69	51.2/12.26	51.8/12.18	52.6/12.55	3.98	< 0.001
	F (v = 44,143)	57.7/13.75	56.0/13.94	60.9/14.00	62.0/14.18	62.6/14.24	3.97	< 0.001
SBP (mmHg)[a]	M (*n* = 37,683)	119.2/14.28	118.9/13.84	118.9/13.69	119.0/13.78	119.2/13.85	0.74	< 0.001
	F (*n* = 33,120)	112.8/15.34	112.4/14.98	111.8/14.73	111.6/14.65	111.3/14.61	0.57	0.020
DBP (mmHg)[a]	M (*n* = 37,683)	77.0/11.09	77.3/10.96	77.6/10.86	77.7/10.99	77.8/10.96	1.51	< 0.001
	F (*n* = 33,120)	69.3/10.90	69.4/10.66	69.3/10.80	69.2/10.86	69.0/10.75	0.67	< 0.001
Glucose (mg/dL)[b]	M (*n* = 35,395)	100.0/20.78	98.6/19.01	98.5/20.44	97.5/17.55	96.8/17.38	–2.10	< 0.001
	F (*n* = 31,206)	92.3/12.47	91.8/11.59	91.3/12.49	90.7/13.04	90.1/12.81	–1.04	< 0.001
HbA1c (%)[b]	M (*n* = 33,818)	5.86/0.72	5.78/0.65	5.74/0.67	5.69/0.57	5.64/0.57	–0.188	< 0.001
	F (*n* = 29,957)	5.73/0.47	5.70/0.47	5.66/0.44	5.61/0.47	5.58/0.45	–0.126	< 0.001

Data was presented as mean/S.D. according to the quintile group of serum bilirubin.

[a]Excluded for subjects taking anti-hypertensive medication.

[b]Excluded for subjects taking hypoglycemic medication.

Analyses were made using linear regression analysis and regression coefficients (*B*) were presented.

Adjusted for age, body mass index, the serum levels of uric acid, and hepatic markers including aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and gamma glutamyltransferase.

TG, triglyceride; HDL chol, high-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c.

Bilirubin and chronic kidney disease in patients with diabetes mellitus

The prevalence of DMCKD in each quintile of serum bilirubin was illustrated in Table 4. In men, the first quintile group had the highest prevalence of DMCKD (12.7%) among the five quintile groups. In men, from the second to the fifth quintile groups, the prevalence of DMCKD was similar, around 9%. In women, the prevalence of DMCKD

Table 4. Association between bilirubin and chronic kidney disease in patients with diabetes mellitus.

	Bilirubin quintile	Prevalence (%)	Unadjusted		Adjusted [a]	
			OR (95% CI)	*P* value	OR (95% CI)	*P* value
Men	Q1 (*n* = 1,347)	12.7	1 (Referent)		1 (Referent)	
	Q2 (*n* = 1,223)	9.0	0.68 (0.52 - 0.88)	0.003	0.72 (0.54 - 0.94)	0.018
	Q3 (*n* = 516)	9.8	0.74 (0.53 - 1.05)	0.088	0.88 (0.62 - 1.27)	0.498
	Q4 (*n* = 753)	9.3	0.71 (0.52 - 0.96)	0.024	0.82 (0.60 - 1.13)	0.219
	Q5 (*n* = 857)	9.5	0.72 (0.54 - 0.96)	0.025	0.88 (0.64 - 1.19)	0.399
	P trend for ORs			0.068		0.467
Women	Q1 (*n* = 485)	21.1	1 (Referent)		1 (Referent)	
	Q2 (*n* = 337)	18.3	0.84 (0.59 - 1.20)	0.340	0.77 (0.52 - 1.14)	0.191
	Q3 (*n* = 586)	16.0	0.71 (0.52 - 0.98)	0.037	0.79 (0.56 - 1.12)	0.181
	Q4 (*n* = 338)	14.3	0.63 (0.43 - 0.92)	0.017	0.71 (0.47 - 1.08)	0.107
	Q5 (*n* = 280)	12.5	0.53 (0.35 - 0.82)	0.004	0.68 (0.43 - 1.08)	0.104
	P trend for ORs			0.001		0.011

[a]Adjusted for age, body mass index, hypertension, and the serum levels of total cholesterol, triglyceride, high-density lipoprotein cholesterol, uric acid, proteinuia, and hepatic markers including aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and gamma glutamyltransferase.

OR, odds ratio; CI, confidence interval.

decreased from 21.1% to 12.5% as serum bilirubin increased. Univariate logistic regression analysis showed that the prevalence of DMCKD decreased as the serum bilirubin increased. However, multivariate analysis in men did not show a significant inverse correlation. In women, the ORs of being DMCKD decreased significantly with the quintiles of total bilirubin (*P* trend = 0.011).

Factors related to insulin resistance and inflammatory status

Fig. 1 shows the levels of HOMA-IR, serum insulin, and CRP according to the quintile groups of serum bilirubin. Irrespective of sex, all the values decreased, as serum bilirubin increased. Using linear regression analysis, the inverse correlation between factors and serum bilirubin did not change. After adjustments for multiple variables (as shown in Table 3), regression coefficients (*B*) of log-HOMA-IR, log-insulin, and log-CRP were as follows: –0.09, –0.13, and –0.60 in men; –0.07, –0.09, and –0.50 in women, respectively. All the regressions were statistically significant ($P < 0.001$).

Discussions

The present study evaluated the correlation between serum bilirubin level and the prevalence of DM and DMCKD among a large Korean population. When the correlations were evaluated by sex stratification, the bilirubin level was inversely correlated with the prevalence of DM in both men and women. The correlation with DM seemed to be stronger in men than in women after adjustment of multiple variables. Furthermore, all of the components of metabolic syndrome were inversely associated with serum bilirubin, except for BP. In univariate analysis, the prevalence of DMCKD changed according to the serum bilirubin levels and was greater in women than in men. However, after adjustments for multiple variables, an inverse correlation was seen only in women. To determine the reason for this inverse correlation between serum bilirubin and DM, we also studied the relationship with insulin resistance and inflammatory component. Increases in serum bilirubin were associated with decreased levels of insulin resistance and the inflammatory markers, including HOMA-IR, serum insulin, and CRP. Therefore, we concluded that high serum bilirubin level was correlated with decreased prevalence of DM in both of sexes and that of DMCKD in women. This correlation may be attributable to the protective nature of serum bilirubin against insulin resistance and inflammation.

Serum bilirubin level is known to be sex-dependent. Serum bilirubin level is greater in men than in women (Rosenthal et al. 1984). The basis for this difference is unclear. One possible mechanism is the gender difference of HO expression (Toth et al. 2003). Another possible mechanism is the effect of sex hormones on bilirubin excretion. Muraca et al. (1983) showed that bilirubin excretion decreased in women and increased in men after gonadectomy. We therefore created sex-stratified groups for this study.

The present study does not explain why serum bilirubin level was inversely correlated with the prevalence of DM, although the protective role of serum bilirubin against insulin resistance and inflammation was suggested. One possible reason is the antioxidant nature of serum bilirubin. Results from several experimental and clinical studies suggest that oxidative stress plays a major role in the pathogenesis of both types of DM (Maritim et al. 2003). First, HO plays an important role in cytoprotection. HO-1 is an

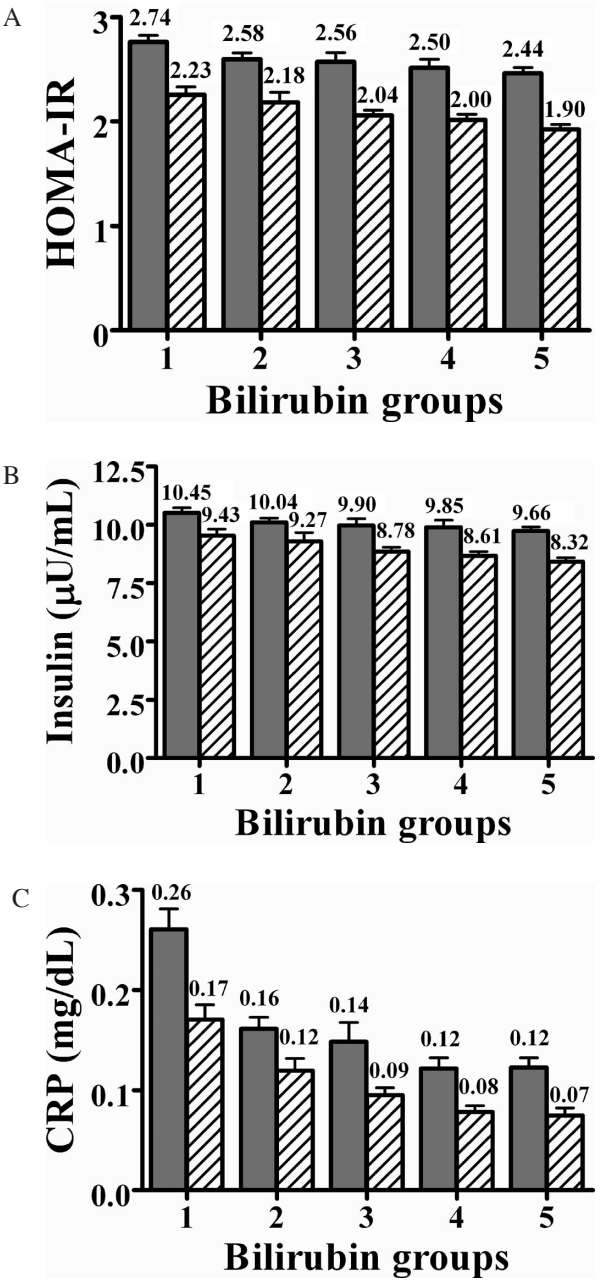

Fig. 1. Relationship between serum bilirubin levels and insulin resistance or inflammatory status. Shown are mean levels (95% CI) of HOMA-IR (A), serum insulin (B), and CRP (C) with respect to the quintile of serum bilirubin. Irrespective of sex, all the differences in the mean level between bilirubin groups are significant (*P* values by UNIANOVA methods are less than 0.001). Solid bar: men; slashed bar: women.

enzyme that catalyzes the degradation of heme and produces biliverdin/bilirubin, iron, and carbon monoxide. Lack of HO-1 induction has been shown to lead to an increase in oxygen radical (O_2^-) formation, abnormalities in cell cycling, and apoptosis of endothelial cells (Abraham et al. 2003). Recent study reveals that expression level and polymorphism in HO-1 gene promoter is associated with the prevalence of type 2 DM (Song et al. 2009). In addition to HO-1, HO-2 is known to have a cytoprotective role in the diabetic state (Goodman et al. 2006). Second, this role is interpreted to be one component of the biliverdin reductase (BVR) cycle. BVR is an enzyme that converts biliverdin to bilirubin, a step relying on nicotinamide adenine dinucleotide phosphate (NADPH). When the BVR transcription was blocked by RNA interference, the level of reactive oxygen species was markedly augmented, and this caused apoptotic cell death (Baranano et al. 2002). Small amounts endogenous BVR, which is abundant with a high turnover rate, can sufficiently convert biliverdin into bilirubin. Therefore, as little as 10 nanomolar bilirubin can protect an individual from oxidative stress of up to 10,000 times higher concentrations of hydrogen peroxide (Dore et al. 1999). Therefore, the manipulation of HO-1/BVR/biliverdin/bilirubin represents a new target of therapy for DM.

Insulin resistance itself is a risk factor for DM. Insulin resistance results from impaired signaling; insulin receptor substrate is rapidly phosphorylated and activates the two primary downstream signals: phosphatidylinositol 3-kinase (PI3K) and ras-mitogen-activated protein kinase (MAPK) (Muoio and Newgard 2008). Some evidence suggests that HO-1 is correlated with an anti-inflammatory mechanism (Idriss et al. 2008). BVR is identified as both a substrate for insulin receptor tyrosine kinase activity and a kinase for serine phosphorylation of insulin receptor substrate-1 (Lerner-Marmarosh et al. 2005). Therefore, BVR plays an important role in modulating insulin signaling. Pachori et al. showed that BVR could enhance PI3K activity by binding to p85, a regulatory subunit of PI3K (Pachori et al. 2007). The inverse correlation between serum bilirubin and serum insulin as well as HOMA-IR and CRP in our data could be explained by the results obtained in these studies.

Recently, Fukui et al. (2008) researched the relationship between serum bilirubin and DMCKD in Japanese patients with DM (aged 64.4 years). This cross-sectional study evaluated albuminuria according to the ratio of albumin to creatinine from random urine samples, and eGFR with the MDRD study equation. The results showed that serum bilirubin had an inverse correlation with albuminuria and a positive correlation with eGFR. However, the sample size was modest ($n = 633$) and the correlation coefficient (r) was relatively low (0.202 and 0.106, respectively). The present study showed a negative correlation with DMCKD in the women, but not in the men, using eGFR. When evaluating the correlation with DMCKD according to CKD stages (e.g., < 45 ml/min/1.73 m^2), the results were insignificant in both of sexes (data not shown). It may be due to extremely low proportion of DM patients with eGFR less than 45 ml/min/1.73 m^2 (0.4%). We did not collect the daily urinary albumin excretion. However, the correlation between bilirubin and proteinuria using dipstick test was not significant (data not shown). It may be because the dipstick test is much less sensitive compared to proteinuria diagnosed from 24-hour urine collection sample or random urine albumin to creatinine ratio. These results cannot be

explained entirely by our data. Further research is needed to delineate the correlation between serum bilirubin and DMCKD.

Although our results are informative, this study has some limitations. First, the study design was cross-sectional, which prevented conclusions regarding the temporal nature of the observed association between serum bilirubin and DM/DMCKD. Second, there were missing data, especially on history of ever-smoking and ever-drinking, HOMA-IR and CRP. Third, we did not collect urine to quantify daily urinary albumin excretion. This may mean patients with stage 1 and 2 CKD are diagnosed as false negatives. Fourth, we did not differentiate direct and indirect bilirubin from total serum bilirubin.

To date, no report has been published on the correlation between serum bilirubin and DM/DMCKD in a large population. Our data revealed that serum bilirubin was inversely correlated with the prevalence of DM, but sex-dependent correlation was seen between serum bilirubin and DMCKD. These results could be explained by an antioxidant or insulin-sensitizing role of serum bilirubin and HO-1/BVR. The present study will be helpful for determining the correlation between serum bilirubin and DM/DMCKD, as well as routine clinical practice.

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