Original Article

Serum Levels of Bilirubin as an Independent Predictor of Coronary In-Stent Restenosis: A New Look at an Old Molecule

Takashi Kuwano¹, Shin-ichiro Miura¹, Kazuyuki Shirai², Amane Ike¹, Ken Mori¹, Tomohiko Shimizu¹, Bo Zhang¹, Atsushi Iwata¹, Hiroaki Nishikawa¹, Akira Kawamura¹, and Keijiro Saku¹

¹Department of Cardiology, Fukuoka University School of Medicine, Fukuoka, Japan

²Division of Cardiology, Hakujiji Hospital, Fukuoka, Japan

Aim: In-stent restenosis (ISR) remains the major limitation of percutaneous coronary intervention (PCI), and the mechanism of ISR is neointimal growth. Higher serum total bilirubin (TBIL) inhibits the inflammatory response and the proliferation of vascular smooth muscle cells; however, the relationship between TBIL and ISR is still unclear. This study aimed to determine whether the serum bilirubin level is associated with ISR after coronary stenting in patients with coronary artery disease.

Methods: This study investigated 1076 consecutive patients who underwent coronary stenting and follow-up angiography (average period: 236 days) using the Fukuoka University Hospital Registry. We used multivariate logistic regression analysis to identify independent predictors of ISR.

Results: The baseline TBIL level was significantly lower in patients with ISR at follow-up compared to those without ISR [0.53 ± 0.26 mg/dL vs. 0.62 ± 0.69 mg/dL, $p=0.036$]. When we divided the patients into 4 groups according to their baseline TBIL level ["low", "low-normal", "high-normal group" and "high" (0.18 ± 0.04 , 0.50 ± 0.13 , 0.93 ± 0.12 and 2.11 ± 2.68 mg/dL)], the ISR rate at follow-up was significantly correlated with the TBIL level [31.0, 32.3, 22.1, and 15.6%, respectively; $p=0.008$ for trend]. A significant negative correlation between the TBIL level and ISR was revealed by multivariate analyses [odds ratio, 0.6; 95% confidence interval, 0.39-0.89, $p=0.015$]. This risk reduction was comparable to those of other known ISR risk factors and covariates.

Conclusion: To the best of our knowledge, this is the first report to demonstrate that a higher TBIL level may be a useful independent predictor of ISR risk after PCI.

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Key words; Percutaneous coronary intervention, Stent, Restenosis, Bilirubin, Coronary artery disease

Introduction

Intracoronary stent placement is a widely accepted treatment for patients with coronary artery disease (CAD), and drug-eluting stents (DES) have been shown to significantly reduce the rate of restenosis in randomized trials^{1,2}; however, in-stent restenosis (ISR) is still a major problem even in the DES era. First, the ISR rate in patients implanted with a DES is considerable: over 10% of patients still require repeat

revascularization^{3,4}. Second, DES should be avoided in patients with an increased risk of bleeding, those who require a long-term oral anticoagulant, and those scheduled for elective surgery. A bare metal stent (BMS) remains the preferred choice of revascularization for these patients.

The pathology of ISR is an overreaction of the wound-healing response after vascular injury and is characterized by neointimal tissue formation⁵. The sequence of inflammation, granulation, extracellular matrix remodeling, and vascular smooth muscle cell (VSMC) proliferation and migration sometimes causes neointimal hyperplasia⁶⁻⁸. The bile pigment bilirubin is one of the major end-products of heme catabolism; however, until recently, bilirubin was regarded as a toxic metabolic product. Stocker *et al.* first reported

Address for correspondence: Shin-ichiro Miura, Department of Cardiology, Fukuoka University School of Medicine, 7-45-1 Nanakuma, Jonan-ku, Fukuoka, 814-0180, Japan
E-mail: miuras@cis.fukuoka-u.ac.jp

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that bilirubin acts as a potent chemical antioxidant⁹⁾, and many epidemiological studies have subsequently reported that individuals with high-normal or just-above-normal serum total bilirubin (TBIL) have a lower incidence of coronary artery disease (CAD)¹⁰⁻¹⁵⁾. Accumulated *in vitro* evidence now shows that bilirubin inhibits low-density lipoprotein oxidation, scavenges oxygen radicals, and restricts VSMC proliferation¹⁶⁻¹⁸⁾. In animal models of balloon-induced vascular injury, bilirubin can block injury-induced VSMC proliferation and prevent narrowing of the vascular lumen¹⁹⁾.

According to these reports, bilirubin may have many pleiotropic effects and has attracted attention as a possible therapeutic approach to induce the prevention of coronary stenosis. Therefore, increased serum levels of TBIL in patients undergoing percutaneous coronary intervention (PCI) may be associated with vascular remodeling and restenosis by cell migration and inflammation; however, thus far, no studies have tested the association between TBIL and the clinical outcome after PCI in patients with CAD. In this study, we determined whether the serum level of bilirubin is associated with ISR after coronary stenting in CAD patients.

Methods

Study Population and Design

We analyzed the clinical and angiographic data of consecutive PCI patients treated from March 2003 to May 2009 using the Fukuoka University Hospital (FU) Registry. Patients who were eligible for this study were those who underwent additional coronary procedures (additional PCI or follow-up angiography) after baseline PCI. We excluded patients with major cardiac events (including death, myocardial infarction, stroke, and target lesion revascularization) within 3 months after baseline PCI and patients who lacked a measurement of TBIL before PCI. Thus, 1076 patients were included in this study. The initial assessment of the patients before PCI consisted of a medical history, physical examination, blood chemistry tests for TBIL, serum aspartate transaminase (AST), alanine transaminase (ALT) and creatinine. All blood samples were drawn in the morning of PCI after an overnight fast. All laboratory measurements were carried out at the Center for Clinical Chemistry using routine standardized methods. All patients gave written informed consent and this study was approved by the ethics committee of Fukuoka University Hospital.

Coronary Angiography and PCI Procedures

All patients underwent PCI according to current guidelines. All procedure decisions, including device selection and adjunctive pharmacotherapy, were made by the individual PCI operator. All patients received more than 100 mg aspirin and either clopidogrel (75 mg/day) or ticlopidine (200 mg/day). Aspirin was continued indefinitely and clopidogrel or ticlopidine was continued for at least 6 months. Angiographic follow-up was performed at 9 months or earlier if clinical presentation or non-invasive evaluation suggested ischemia.

Coronary angiographic measurements were performed at the angiographic core laboratory of Fukuoka University Hospital using a validated edge-detection system (CMS; Medis, Leiden, The Netherlands). Standard qualitative and quantitative definitions and measurements were used. ISR at follow-up was defined as luminal narrowing of more than 50% occurring in the segment with the stent or within a 5 mm segment proximal or distal to the stent. Late luminal loss was defined as the difference between the minimal lumen diameter (MLD) at the completion of the stenting procedure and that measured during follow-up.

Statistical Analysis

Discrete variables were reported as frequencies (percentage) and were compared using chi-square analysis or Fisher's exact test, as appropriate. Continuous variables are reported as the means $\pm$ standard deviation. Continuous variables were compared with Student's *t* test if data followed a normal distribution and with Wilcoxon's rank-sum test if data were skewed. Multiple logistic regression analysis was performed to identify independent predictors of ISR. Differences were considered significant when $p < 0.05$ unless indicated otherwise. All statistical analyses were performed using the SAS software package (version 9.1, Statistical Analysis System; SAS Institute Inc., Cary, NC, USA).

Results

Patient Characteristics

A total of 1,076 patients were included: mean age, 66.5 years and mean follow-up period, 236 days. Patients were divided into 2 groups according to the occurrence of ISR at follow-up [non-ISR ($n=735$) and ISR ($n=341$) groups] (Table 1). Restenosis was observed in 31.7%. Patients with ISR were more likely to have diabetes mellitus and a lower body mass index (BMI), left ventricular ejection fraction (LVEF) and

Table 1. Baseline characteristics of the study population

	overall (<i>n</i> = 1,076)	non-ISR (<i>n</i> = 735)	ISR (<i>n</i> = 341)	<i>p</i>
Age (yrs)	66.5 ± 10.5	66.1 ± 10.8	67.3 ± 9.8	0.075
Male gender (%)	79.8	78.8	81.9	0.242
BMI (kg/m ²)	23.7 ± 3.4	23.8 ± 3.4	23.5 ± 3.3	0.091
Clinical variables				
Hypertension (%)	73.5	73.2	74.1	0.752
Diabetes mellitus (%)	50.2	46.3	58.7	<0.001
Dyslipidemia (%)	70.2	71.2	68.1	0.310
Current smoker (%)	31	29.3	34.9	0.052
Renal insufficiency* (%)	33.4	31.4	37.8	0.057
Previous MI (%)	32.1	31.1	34.4	0.283
LVEF (%)	59.3 ± 12.6	59.8 ± 12.8	58.1 ± 12.1	0.049
Laboratory values				
Total bilirubin (mg/dL)	0.57 ± 0.30	0.62 ± 0.69	0.53 ± 0.26	0.036
AST (IU/L)	31.2 ± 37.2	30.0 ± 32.4	33.8 ± 45.9	0.127
ALT (IU/L)	24.9 ± 20.1	25.1 ± 19.0	24.4 ± 22.5	0.594
Medications				
ACE inhibitor/ARB (%)	73.7	73.9	73.4	0.875
Calcium channel blocker (%)	44.9	45.2	44.1	0.728
Beta blocker (%)	12.5	11.6	14.4	0.218
Diuretics (%)	20.2	17.4	25.9	0.002
Statins (%)	71.4	73.7	66.5	0.016
Nitrate (%)	26.8	24.3	31.9	0.011
Nicorandil (%)	44.1	44.9	42.2	0.417
Angiographic variables, presurgery				
Complex lesions† (%)	61.7	61.7	65.9	0.191
Left main coronary artery (%)	4.7	4.7	7.1	0.177
Bifurcation (%)	32.1	29.5	37.6	0.036
Calcification (%)	11.2	9.2	15.8	0.002
ISR lesion (%)	20.4	17.5	26.5	<0.001
Drug-eluting stent implantation (%)	42	46	33.7	0.003
QCA analysis, presurgery				
Reference vessel diameter (mm)	2.56 ± 0.58	2.62 ± 0.59	2.44 ± 0.56	<0.001
Minimal lumen diameter (mm)	0.66 ± 0.43	0.69 ± 0.45	0.60 ± 0.40	0.021
Diameter stenosis (%)	73.0 ± 17.8	73.0 ± 16.9	72.9 ± 19.4	0.965
Lesion length (mm)	17.5 ± 10.5	16.7 ± 9.0	19.2 ± 12.9	0.005
Stent length for lesion (mm)	23.1 ± 11.9	22.6 ± 10.9	24.3 ± 13.9	0.129

Patients were divided into 2 groups according to the occurrence of in-stent restenosis (ISR) at follow-up (non-ISR and ISR groups). Values are the means ± S.D. *Renal insufficiency was defined as creatinine clearance calculated with the Cockcroft-Gault equation <60 mL per min. †Complex lesions were defined as lesion type B2 and C, according to the American College of Cardiology/American Heart Association.

ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; LVEF, left ventricular ejection fraction; MI, myocardial infarction; QCA, quantitative coronary angiography

TBIL levels than patients without restenosis. They were also less likely to be treated with statins than patients without restenosis and more likely to be treated with diuretics and nitrate. With regard to angiographic variables, the ISR group showed a signifi-

cantly higher rate of bifurcation, calcification, and ISR lesions, a shorter reference diameter and minimal lumen diameter, and a longer lesion length before PCI. Placement of a drug-eluting stent was seen more frequently in patients without restenosis. We also per-

Table 2. Classification by stent type of patient baseline characteristics

	Bare metal stent use			Drug-eluting stent use		
	no restenosis (<i>n</i> = 399)	restenosis (<i>n</i> = 226)	<i>p</i>	no restenosis (<i>n</i> = 336)	restenosis (<i>n</i> = 115)	<i>p</i>
Age (yrs)	66.2 ± 11.4	67.7 ± 9.8	0.114	65.9 ± 10.0	66.6 ± 9.8	0.510
Male gender (%)	76.6	81.7	0.138	78.8	81.9	0.242
BMI (kg/m ²)	23.9 ± 3.6	23.7 ± 3.5	0.488	23.7 ± 3.2	23.0 ± 3.0	0.025
Clinical variables						
Hypertension (%)	70.1	72.0	0.619	76.9	78.2	0.782
Diabetes mellitus (%)	42.0	53.7	0.006	51.6	68.5	0.002
Dyslipidemia (%)	66.9	66.5	0.918	76.4	71.2	0.272
Current smoker (%)	27.3	33.6	0.144	31.8	37.3	0.525
Renal insufficiency* (%)	30.3	34.0	0.359	32.9	46.5	0.028
Previous MI (%)	31.3	30.6	0.864	30.9	42.0	0.033
LVEF (%)	58.3 ± 13.1	58.1 ± 11.6	0.841	61.5 ± 12.3	58.1 ± 13.1	0.014
Laboratory values						
Total bilirubin (mg/dL)	0.61 ± 0.33	0.56 ± 0.27	0.041	0.62 ± 0.96	0.48 ± 0.23	0.017
AST (IU/L)	34.6 ± 40.1	39.1 ± 53.2	0.235	24.7 ± 18.2	23.3 ± 23.0	0.528
ALT (IU/L)	26.1 ± 20.3	26.1 ± 24.4	0.969	23.9 ± 17.3	21.2 ± 17.7	0.147
Medications						
ACE inhibitor/ARB (%)	74.8	77.0	0.555	72.7	66.4	0.204
Calcium channel blocker (%)	41.0	40.6	0.922	50.3	53.3	0.596
Beta blocker (%)	11.5	15.6	0.158	14.2	16.2	0.627
Diuretics (%)	18.3	27.8	0.008	16.9	30.5	0.003
Statins (%)	72.0	67.9	0.285	75.7	63.6	0.014
Nitrate (%)	25.7	29.7	0.293	22.6	36.1	0.006
Nicorandil (%)	47.3	43.4	0.37	42.2	39.8	0.671
Angiographic variables, presurgery						
Complex lesions [†] (%)	56.7	61.8	0.215	67.6	73.7	0.222
Left main coronary artery (%)	2.2	4.4	0.195	4.7	7.1	0.177
Bifurcation (%)	29.6	30.3	0.883	29.4	52.1	<0.001
Calcification (%)	7.0	13.2	0.010	11.8	20.9	0.018
In-stent restenosis (%)	14.0	26.6	<0.001	21.6	26.3	0.303
Drug-eluting stent implanted (%)						
QCA analysis, presurgery						
Reference vessel diameter (mm)	2.73 ± 0.61	2.49 ± 0.57	<0.001	2.49 ± 0.54	2.34 ± 0.53	0.044
Minimal lumen diameter (mm)	0.63 ± 0.46	0.60 ± 0.42	0.472	0.75 ± 0.42	0.61 ± 0.35	0.012
Diameter stenosis (%)	75.8 ± 16.8	73.5 ± 19.8	0.213	69.6 ± 16.5	71.8 ± 18.6	0.354
Lesion length (mm)	14.5 ± 6.3	17.2 ± 10.8	0.004	19.1 ± 10.9	23.3 ± 15.6	0.017
Stent length for lesion (mm)	19.3 ± 7.5	20.9 ± 11.4	0.172	25.5 ± 12.5	28.7 ± 15.6	0.079

Abbreviations as in Table 1.

formed sub-analysis in which we compared the patients according to stent type (**Table 2**). As shown in **Table 2**, the TBIL level in the restenosis group was significantly high in both the BMS and DES groups.

Relationship between Serum TBIL Levels at Baseline and ISR Rate at Follow-Up

As shown in **Table 1** and **2**, TBIL in the ISR

group was significantly lower than those in the non-ISR group, and we divided the study population into 4 groups according to their TBIL level before PCI to identify the relationship between the TBIL level and the ISR rate at follow-up (**Table 3**). The normal TBIL value at our University Hospital is 0.3 mg/dL to 1.2 mg/dL; therefore, our “low bilirubin group” was defined as TBIL <0.30 mg/dL, the “low-normal bili-

Table 3. Clinical characteristics of the study population according to serum total bilirubin levels

	Low Bilirubin (<i>n</i> =69)	Low-Normal Bilirubin (<i>n</i> =816)	High-Normal Bilirubin (<i>n</i> =156)	High Bilirubin (<i>n</i> =35)	<i>p</i> for trend
Age (yrs)	66.8 ± 11.0	66.5 ± 10.3	66.5 ± 11.2	64.1 ± 11.6	0.586
Male gender (%)	79.4	79.1	81.1	91.2	0.177
BMI (kg/m ²)	22.2 ± 3.6	23.7 ± 3.4	24.4 ± 3.2	24.1 ± 2.2	<0.001
Clinical variables					
Hypertension (%)	84.6	71.8	77.6	70.6	0.723
Diabetes mellitus (%)	62.2	50.8	45.4	35.3	0.005
Dyslipidemia (%)	68.2	69.9	70.6	79.4	0.670
Current smoker (%)	39.7	30.1	32.9	29.0	0.302
Renal insufficiency* (%)	45.5	35	24.7	18.2	0.007
Previous MI (%)	38.5	31.9	32	26.5	0.635
LVEF (%)	54.6 ± 12.8	60.1 ± 12.1	57.5 ± 14.0	57.2 ± 13.8	<0.001
Laboratory values					
Total bilirubin (mg/dL)	0.18 ± 0.04	0.50 ± 0.13	0.93 ± 0.12	2.11 ± 2.68	<0.001
AST (IU/L)	29.4 ± 37.3	29.2 ± 34.1	40.0 ± 50.8	41.6 ± 29.6	<0.001
ALT (IU/L)	19.8 ± 21.9	24.5 ± 18.9	27.0 ± 22.9	36.5 ± 26.8	<0.001
Medications					
ACE inhibitor/ARB (%)	72.3	72.1	81.5	79.4	0.039
Calcium channel blocker (%)	60.3	44.8	40.4	34.4	0.035
Beta blocker (%)	22.2	11.4	12.1	21.9	0.03
Diuretics (%)	25.4	14.7	22	21.9	0.623
Statins (%)	66.7	70.8	76.8	70.6	0.191
Nitrate (%)	25.4	28.1	22.7	15.6	0.267
Nicorandil (%)	58.7	42.8	40	62.5	0.723
Angiographic variables, presurgery					
Complex lesions [†] (%)	60.9	63.7	61.3	58.8	0.650
Left main coronary artery (%)	4	6.2	2	4.8	0.379
Bifurcation (%)	20.5	32.7	34.1	35	0.228
Calcification (%)	13.9	13.4	8.4	9.8	0.097
ISR (%)	29	21.3	14.8	5.9	0.002
Drug-eluting stent implantation (%)	39.1	35.1	28.8	37.1	0.103
QCA analysis, presurgery					
Reference vessel diameter (mm)	2.63 ± 0.54	2.56 ± 0.60	2.55 ± 0.53	2.58 ± 0.50	0.880
Minimal lumen diameter (mm)	0.62 ± 0.42	0.66 ± 0.44	0.63 ± 0.42	0.67 ± 0.40	0.884
Diameter stenosis (%)	76.2 ± 14.4	72.6 ± 18.1	72.9 ± 17.8	74.5 ± 14.8	0.605
Lesion length (mm)	18.1 ± 14.3	17.7 ± 10.6	16.4 ± 7.8	14.3 ± 4.4	0.367
Stent length for lesion (mm)	25.2 ± 15.1	23.4 ± 11.6	21.7 ± 12.6	18.3 ± 6.4	0.165

Values are the means ± SD. The *p* value are were tested for trend across TBIL levels.

Abbreviations as in Table 1.

rubin group” was defined as $0.75 \geq \text{TBIL} \geq 0.30$ mg/dL, the “high-normal bilirubin group” was defined as $1.20 \geq \text{TBIL} \geq 0.76$ mg/dL, and the “high bilirubin group” was defined as $\text{TBIL} > 1.20$ mg/dL. The clinical and angiographic characteristics were compared among the groups. There were no significant differences among the 4 groups regarding age, gender, the prevalence of hypertension and dyslipidemia. TBIL levels were significantly associated with BMI, LVEF,

the prevalence of diabetes mellitus and renal insufficiency, serum levels of AST and ALT, and the use of ACE inhibitor/ARB, calcium channel blocker and beta blocker; however, a strong association was only detected between the rate of ISR before PCI and TBIL levels at baseline.

Angiographic outcomes at follow-up are shown in **Fig. 1**. There was a significant trend for a stepwise decrease in the rate of ISR corresponding to the

Fig. 1. Rate of ISR at follow-up according to serum TBIL levels (Overall patients). Open, light gray, dark gray and closed bars indicated low bilirubin, low-normal bilirubin, high-normal bilirubin groups, respectively.

Fig. 2. Comparison of the ISR rate at follow-up for patients without liver injury (open) and those with suspected liver injury (grey). Patients with suspected liver injury were defined as those who met at least one of the following criteria: AST > 32 IU/mL, ALT > 30 IU/mL and hepatitis B or C virus-positive.

increase in TBIL (low bilirubin group=31.0, low-normal bilirubin group=32.3, high-normal bilirubin group=22.1, and high bilirubin group=5.6%, respectively; $p=0.0083$ for trend). We also performed sub-analysis that divided the patients into 2 groups according to the presence or absence of suspected liver injury (Fig. 2). Patients with suspected liver injury were

Table 4. Univariate logistic analysis of ISR at follow-up

variable	OR	95%CI	<i>p</i>
Age (yrs)	1.01	1.00-1.02	ns
Male gender	1.2	0.87-1.69	ns
BMI	0.97	0.93-1.01	ns
Hypertension	1.01	0.79-1.44	ns
Dyslipidemia	0.87	0.66-1.16	ns
Diabetes mellitus	1.66	1.27-2.16	<0.001
Current smoker	1.18	1.00-1.39	0.048
Renal insufficiency*	1.36	1.01-1.81	0.041
LVEF	0.99	0.98-1.00	0.05
Total bilirubin (per category)	0.71	0.56-0.91	0.006
AST	1	1.00-1.01	ns
ALT	1	0.99-1.00	ns
ACE inhibitor/ARB treatment	1.01	0.75-1.37	ns
Calcium channel blocker	0.95	0.72-1.24	ns
Beta blocker	1.3	0.87-1.91	ns
Diuretics	1.66	1.20-2.29	0.002
Statin	0.72	0.54-0.95	0.021
Nitrate	1.4	1.04-1.88	0.027
Bifurcation lesions	1.45	1.03-2.03	0.033
Calcification lesions	1.34	1.10-1.63	0.004
ISR lesions	1.7	1.24-2.31	<0.001
Drug-eluting stent implantation	0.95	0.92-0.98	<0.001
Reference vessel diameter	0.57	0.42-0.77	<0.001
Minimal lumen diameter	0.65	0.44-0.94	0.023
Lesion length	1.02	1.01-1.04	0.005

CI, confidence interval; OR, odds ratio. Abbreviations as in Table 1. NS, not significant.

defined as those who met at least one of the following criteria: AST > 32 IU/mL, ALT > 30 IU/mL and hepatitis B or C virus-positive. There is a correlation between TBIL levels (per category) and ISR in the absence of hepatic injury. In addition, TBIL levels (per category) tended to be associated with ISR regardless of the presence of hepatic injury.

Predictors of ISR at Follow-Up

Logistic regression analysis was used to assess independent predictors of ISR at follow-up. All factors that correlated with ISR or TBIL (Table 1 and 3) and variables that were considered to be of clinical importance (age, gender, hypertension, and dyslipidemia) were included in the univariate logistic model (Table 4). Next, we performed multiple logistic regression analysis that included all significant ($p < 0.05$) univariate predictors of ISR, as shown in Table 4, and the odds ratio and p value were adjusted for age and gender (Table 5). In the multivariate analysis model, TBIL levels (per category) [odds ratio (OR)=0.6, $p=0.015$],

Table 5. Predictors of ISR at follow-up by multivariable analysis

variable	OR	95%CI	<i>p</i>
Diabetes mellitus	1.32	0.87-2.01	ns
Current smoker	0.99	0.75-1.30	ns
Renal insufficiency*	1.39	0.88-2.16	ns
LVEF	1	0.98-1.02	ns
Total bilirubin (per category)	0.6	0.39-0.89	0.015
Diuretics treatment	2.4	1.38-4.04	0.002
Statin treatment	0.93	0.58-1.52	ns
Nitrate treatment	1.15	0.73-1.81	ns
Bifurcation lesions	1.7	1.11-2.62	0.015
Calcification lesions	1.01	0.70-1.43	ns
ISR lesions	0.9	0.52-1.54	ns
Drug-eluting stent implanted	0.9	0.85-0.95	0.002
Reference vessel diameter	0.49	0.23-0.75	0.001
Minimal lumen diameter	0.95	0.53-1.69	ns
Lesion length	1.03	1.00-1.05	0.022

Abbreviations as in Table 1.

P value and ORs were adjusted for age and gender.

diuretic treatment (OR=2.4, *p*=0.002), DES implantation (OR=0.9, *p*=0.002), and bifurcation lesions (OR=1.7, *p*=0.015), reference vessel diameter (OR=0.49, *p*=0.001) and lesion length (OR=1.03, *p*=0.022) before PCI were significant risk indicators of ISR at follow-up. The risk reduction by higher TBIL was comparable to those of other known ISR risk factors and covariates such as DES implantation, bifurcation lesions, reference vessel diameter and lesion length before PCI.

If the “low bilirubin group” is considered as a reference category, the “high-normal bilirubin group” and “high bilirubin group” had a significantly lower relative risk of restenosis, while the “low-normal bilirubin group” was not associated with superior risk reduction (Table 6).

Discussion

In the present study, we found that TBIL levels were associated with the occurrence of restenosis after PCI. Patients with high or high-normal levels of TBIL exhibited a lower rate of ISR at follow-up than patients with low TBIL. Furthermore, the risk reduction was comparable to those of other known ISR risk factors and covariates. To the best of our knowledge, this is the first report to demonstrate that serum TBIL levels before PCI serve as an independent predictor of ISR.

Several experiments have shown how bilirubin

Table 6. Relative risk of ISR according to the categorized serum bilirubin level

	OR*	95%CI	<i>p</i> *
Low bilirubin group	— [†]	— [†]	— [†]
Low-Normal bilirubin group	0.78	0.47-1.32	0.347
High-Normal bilirubin group	0.53	0.29-0.98	0.042
High bilirubin group	0.36	0.12-0.93	0.046

Abbreviations as in Table 1. “low bilirubin group” was defined as TBIL <0.30 mg/dL, “low-normal bilirubin group” was defined as 0.75 ≥ TBIL ≥ 0.30 mg/dL, “high-normal bilirubin group” was defined as 1.20 ≥ TBIL ≥ 0.76 mg/dL, and “high bilirubin group” was defined as TBIL >1.20 mg/dL.

*ORs and *p* value were adjusted for age, gender. [†]Reference category.

acts as an antioxidant. Stocker *et al.* reported that both unconjugated bilirubin and conjugated bilirubin scavenged peroxy radicals and protected against the oxidation of low-density lipoprotein^{9, 17)}. With regard to its antioxidant mechanism, it is believed that bilirubin can neutralize radicals by donating a hydrogen atom, and all forms of bilirubin have this ability, including free unconjugated bilirubin, albumin-bound unconjugated bilirubin, and conjugated bilirubin. Excess proliferation of VMSCs is considered a major pathological mechanism of ISR and bilirubin can also restrict VSMC proliferation. Ollinger *et al.* reported that bilirubin suppressed the smooth muscle cell cycle via inhibition of the mitogen-activated protein kinase signal transduction pathways and caused p53-dependent cell cycle arrest by hypo-phosphorylation of the retinoblastoma tumor suppressor protein in VSMC^{18, 19)}. The pathological mechanism of ISR with a DES has not yet been sufficiently elucidated; however, some intravascular ultrasound studies of restenosis after SES implantation have shown an increase in intimal growth in a SES restenosis group as well as in a BMS group²⁰⁾. Various factors, such as calcified lesion, bifurcation lesion and stent fracture etc., might be involved in ISR with a DES²¹⁻²³⁾. ISR also includes stent-edge restenosis. Local drug delivery may be impaired in this case and, as a result, ISR may occur; thus, neointimal hyperplasia may be the main cause of ISR with a DES. In this study, bilirubin levels were associated with ISR in both the BMS and DES groups (Table 2), and therefore a high level of TBIL may protect against ISR with a DES through the inhibition of neointimal growth, as with a BMS.

Some epidemiological studies have shown that individuals with Gilbert syndrome or uridine diphosphate-glucuronosyltransferase 1 and its polymorphism, which is related to Gilbert syndrome, were associated with a marked reduction in CAD risk^{14, 15)}. Although

these conditions are congenital, it is unknown whether higher TBIL as an acquired condition, such as due to hepatic injury or medication, is also associated with a reduction in risk. In the present study, we examined all of the subjects together regardless of the presence of hepatic injury. AST and ALT levels were significantly associated with the serum TBIL level (per category) in this study; however, AST and ALT were not associated with ISR at follow-up. It is possible that liver dysfunction could be associated with many factors, such as increased estrogen. As this result shows (**Fig. 2**), TBIL levels (per category) tended to be associated with ISR regardless of the presence of hepatic injury. This might suggest that higher TBIL might be associated with ISR reduction not only in patients with an inherent predisposition but also in patients with an acquired condition. In an animal model, TBIL increased after exogenous biliverdin administration and this prevented the formation of intimal hyperplasia²⁴. Therefore, while bilirubin levels are currently a natural factor, in the future, therapeutic intervention with regard to the bilirubin level may be an effective treatment strategy for patients in need of coronary stenting. Furthermore, our results show that slightly high TBIL is required for risk reduction in humans; thus, we believe that our findings support such a future therapeutic intervention.

However, there is little evidence that bilirubin has such an effect in humans. Heme oxygenase (HO) is a rate-limiting enzyme in the degradation of heme into biliverdin, which is rapidly converted to bilirubin. Its inducible isoform, HO-1, is a stress-responsive protein that acts as an atherogenic and inflammatory mediator via its reaction products²⁵. Previous studies have reported that the HO-1 polymorphism is associated with restenosis after stenting or balloon angioplasty^{26, 27}. In these reports, the length polymorphism of a GT repeat in the HO-1 promoter region was shown to be an independent risk factor for restenosis, and carriers of short alleles had a reduced risk for restenosis. In addition, other reports showed that carriers of short alleles in the HO-1 gene had higher TBIL levels than non-carriers^{25, 28, 29}. On the other hand, a recent study in a large population showed that the GT repeat polymorphism had no effect on restenosis³⁰. Even though TBIL levels were not described in this report, this result may contradict our findings; however, while the genotype distribution of the GT repeat polymorphism was similar in Caucasian and Chinese populations, the effect of this polymorphism on TBIL levels differed according to race^{25, 29}. Our present findings and these controversial data from previous studies indicated that a large proportion of restenosis after coronary stenting may be associated with the TBIL level itself but not the genotype of the HO-1 promoter region.

HO-1 gene promoter is also associated with increased high-density lipoprotein (HDL) cholesterol²⁵. Low serum HDL cholesterol is one of the strongest predictors of coronary risk. HDL has been reported to take up cellular cholesterol from the periphery and to mediate the transport of excess cholesterol to the liver. Although higher HDL cholesterol prevents ISR after PCI, there were no differences in serum HDL cholesterol between the non-ISR and ISR groups or among the 4 categories according to the TBIL level (data not shown).

Our study also showed that TBIL levels are associated with the prevalence of diabetes mellitus and CKD. While TBIL levels were not correlated with diabetes mellitus in middle-aged British men¹², in a Chinese population, a decrease in the TBIL concentration was significantly associated with an increase in glycated hemoglobin³¹. Regarding the relationship between TBIL and CKD, although TBIL levels were not correlated with renal insufficiency in this report, a previous report indicated that mildly elevated TBIL levels were negatively associated with the incidence of end-stage renal disease with IgA nephropathy, and bilirubin treatment was shown to improve the glomerular filtration rate (GFR) after ischemia/reperfusion injury of the kidney³². Fukui *et al.* demonstrated a positive correlation between the TBIL concentration and the estimated GFR in Japanese diabetic patients, and suggested that TBIL may play a role in diabetic nephropathy³³. Meanwhile, in a US adult population, increasing levels of TBIL were independently associated with decreasing estimated GFR and increasing albuminuria³⁴. Thus, bilirubin levels are not likely to have significant effects in all subjects as their effects may depend on the presence of diabetes mellitus or the cause and stage of CKD, and our results partly support this hypothesis.

Study Limitations

The present study presents a “real-world” evaluation of independent predictors of ISR after coronary stenting, but our study had some limitations. First, we conducted a single-center, retrospective study and patient selection bias might have occurred, since only patients who underwent the follow-up coronary angiography were enrolled. Second, the sample size was relatively small. Nonetheless, we were able to demonstrate a relationship between serum TBIL levels and the rate of ISR after coronary stenting. Third, we tried to control for baseline imbalances by a multivariate

model. However, these limitations still permit the interpretation of “real-world” data taken from a routine clinical practice. Further studies are required to evaluate this issue.

Conclusions

To the best of our knowledge, this report is the first to demonstrate that the TBIL level may independently predict the risk of ISR after PCI. Patients with higher TBIL had a lower risk of ISR than those with lower TBIL. These findings present important clinical implications for identifying risk after coronary stenting. We should pay more attention to monitoring patients with lower TBIL after stenting. Furthermore, while the TBIL level is currently a completely natural factor, our results suggest that it may become a target for future therapeutic intervention.

Conflict of Interest

The authors have no conflicts of interest to declare.

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