INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Increased Serum Bilirubin Level is Related to Good Collateral Development in Patients with Chronic Total Coronary Occlusion

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Abstract

Objective Bilirubin is a bile pigment with potent anti-oxidant properties; in previous studies it has been reported to be negatively associated with coronary artery disease (CAD). Although elevated serum bilirubin concentrations may protect against endothelial dysfunction, it is not clear whether higher serum bilirubin levels (SBLs) in physiological ranges may work in favor of good collateral development in patients with chronic total coronary occlusion (CTO).

Methods The study population consisted of patients who underwent coronary angiography with a suspicion of CAD. Patients who had CTO in at least one major coronary artery were included. Coronary angiograms of 179 eligible patients from our database were reanalyzed and 110 of them had good collateral development and 69 had poor collateral development according to the Cohen-Rentrop method.

Results Patients with good collateral development had a lower fasting plasma glucose level (FPG) ($p=0.024$) and higher SBLs in comparison to patients with poor collateral development ($p<0.001$). The number of CTO vessels ($p=0.013$) and left ventricular ejection fraction (EF%) were higher in good collateral group than poor collateral group ($p=0.017$). In multivariate analysis, FPG negatively (odds ratio [OR]: 0.981, $p=0.003$) and SBL (OR for per 0.1-mg/dL increase: 1.832, $p<0.001$) and the number of CTO vessels (OR: 5.642, $p=0.007$) were positively related to coronary collateral development.

Conclusion This study suggests that higher bilirubin levels within relatively normal ranges were related with favorable coronary collateral growth in patients with CTO. SBL may be responsible for the difference in coronary collateral vessel development among different patients with coronary artery disease. The anti-inflammatory and anti-oxidant properties of bilirubin may mediate this effect.

Key words: coronary artery disease, collateral development, bilirubin, chronic total occlusion, glucose, anti-oxidant

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Introduction

Atherosclerosis is a chronic inflammatory disease, which develops as a process occurring in a vessel wall, beginning with a response to endothelial injury. Endothelial dysfunction is characterized by dysfunction and loss of monolayer cells covering the inside of the vessels, which is endothelium. Endothelial dysfunction is the first stage in atherosclerosis. The regenerative capacity of endothelium provides protection against atherosclerosis. Failure of the endothelial repair initiates atherosclerotic inflammation and lesion for-

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mation, so-called plaque, especially in non-laminar flow stress points in the vascular bed (1).

Coronary angiogenesis and collateral growth are chronic adaptations to myocardial ischemia to restore coronary blood flow and salvage myocardium in the ischemic region. Good coronary collateral development has potential protective roles such as limited infarct size, less aneurysm formation in the ventricle wall, improved ventricular function, fewer future cardiovascular events and improved survival in patients with occlusive coronary lesions (2, 3).

Bilirubin is the end product of heme catabolism by heme oxygenases. Bilirubin is a bile pigment with potent antioxidant properties in the blood (4-7); in previous studies it has been reported to be negatively associated with coronary artery disease (CAD) and other atherosclerotic vascular involvement (8, 9). Although elevated SBL may protect against atherosclerotic diseases, it is not clear whether higher serum bilirubin level (SBL) within physiological ranges work in favor of good collateral development in patients with chronic total coronary occlusion (CTO). We tested the hypothesis that higher bilirubin may be associated with good coronary collateral development.

Materials and Methods

The study had a cross-sectional observational design. The study population consisted of patients who underwent coronary angiography between May 2009 and December 2010 with a suspicion of CAD at our institute's outpatient clinic. Clinical characteristics, which consisted of multiple descriptors from each patient's history and physical examination, were collected by physicians from the cardiology clinic for each patient at the time of cardiac catheterization and were stored in the database of coronary angiography laboratory at our institution. Standard selective coronary angiography with at least 4 views of the left coronary system and 2 views of the right coronary artery was performed for all patients using the Judkins technique.

Patients who had CTO in at least one major coronary artery were included in the current study. The relation of SBL with collateral vessel growth was investigated. These patient's coronary angiograms were reevaluated for collateral development by two experienced interventional cardiologists who were totally blinded to the study. Collateral grading was performed for the vessel with CTO; if a patient had more than one vessel with CTO and collateral development, collateral grading was defined according to the vessel that had better collateral development. The grade of coronary collateral development was determined according to the Cohen-Rentrop (10) method: grade 0, no filling of any collateral vessels; grade 1, filling of side branches of the artery to be perfused by collateral vessels without visualization of epicardial segment; grade 2, partial filling of the epicardial artery by collateral vessels; and grade 3, as complete filling of epicardial artery by collateral vessel.

Patients with grade 0-1 collateral development were regarded as poor collateral group and patients with grade 2-3 collateral development were regarded as good collateral group. Coronary angiograms of 179 eligible patients from our database were reanalyzed and 110 of them had good collateral development and 69 had poor collateral development according to Cohen-Rentrop method.

Blood samples were drawn by venipuncture to perform routine blood chemistry analyses after fasting for at least 8 hours before coronary angiography. Fasting blood glucose, serum creatinine, SBL, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglyceride levels were recorded. Glucose, creatinine, and lipid profile were determined by standard methods.

Patients with symptomatic peripheral vascular disease (transient ischemic attack, stroke, intermittent claudication, peripheral revascularization, or amputation), prior PCI and/or CABG, non-ischemic dilated cardiomyopathy, evidence of ongoing infection or inflammation, hepatic or cholestatic disease, recent acute coronary syndrome either with or without ST-segment elevation (at most one month before enrollment), hematological disorders and known malignancy were excluded from the study.

Statistical analysis

Continuous variables were given as mean ± standard deviation; categorical variables were defined as percentages. Continuous variables were compared by Student's *t* test and the $\chi 2$ test was used for the categorical variables between two groups. Logistic regression analyses were used for the multivariate analysis of all independent variables. All tests of significance were two-tailed. Statistical significance was defined as $p<0.05$. The SPSS statistical software (SPSS 15.0 for windows, SPSS Inc., Chicago, IL, USA) was used for all statistical calculations.

Results

Patients with good collateral development had a lower fasting plasma glucose level (FPG)(115±33 vs 131±54 mg/dL, p=0.024) and higher SBL in comparison with patients with poor collateral development (total bilirubin, 0.80±0.27 vs 0.53±0.19, p<0.001; indirect bilirubin, 0.50±0.20 vs 0.33±0.16, p<0.001 and direct bilirubin, 0.30±0.15 vs 0.20±0.08, p<0.001)(Fig. 1). The number of CTO vessels (1.3±0.85 vs 1.1±0.3, p=0.013) and left ventricular ejection fraction (EF%) were higher in good collateral group than poor collateral group (51±13 vs 42±12, p=0.017)(Table 1). In multivariate analysis, FPG negatively (odds ratio [OR]: 0.981; 95% confidence interval [CI], 0.968-0.993, p=0.003) and SBL (for per 0.1-mg/dL increase in total bilirubin, OR: 1.832; 95% CI, 1.460-2.299, p<0.001) and the number of CTO vessels (OR: 5.642; 95% CI, 1.613-19.7, p=0.007) were positively related to collateral development (Table 2).

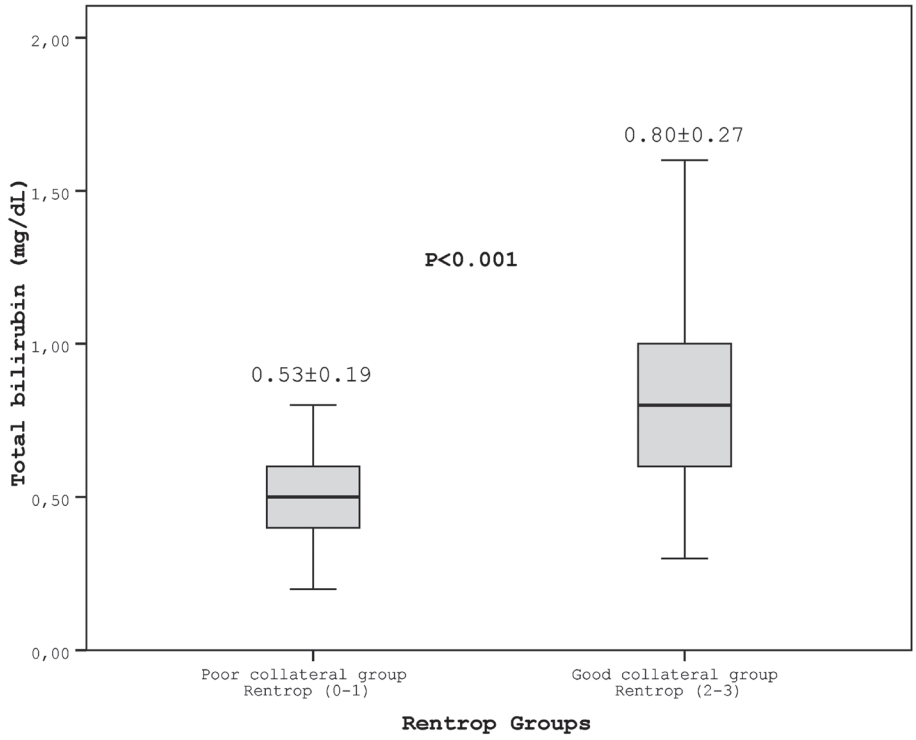

Figure 1. **Total bilirubin levels between patients with good and poor collateral groups.**

Discussion

In this study, we aimed to evaluate the effect of SBL on coronary collateral growth. Our findings suggested that higher level of bilirubin is an independent predictor for good coronary collateral formation independent of cardiovascular risk factors. Additionally, FPG was negatively related to collateral growth.

The collateral vessels can fulfill an important function in patients with significant obstruction of a coronary artery by providing an extra blood supply to the ischemic area (11). It is shown that collateral vessels are induced by the severity of myocardial ischemia and there is a good correlation between them (12). But in clinical practice we do not observe the same degree of collateral vessel development in every patient with significant ischemia. Thus there may be modifiers other than ischemia for collateral development. In a previous study related to collateral development, various cytokines were studied but significant relations were not obtained (13). Identification of the mechanisms affecting collateral formation may help to facilitate the development of new strategies to enhance collateral formation.

Previous studies showed that there is a negative relationship between collateral development and cardiovascular risk factors such as age, diabetes mellitus, metabolic syndrome and obesity (14-17). All of these risk factors are related with endothelial dysfunction. The endothelium is the most important structure for maintenance of normal vascular functions like control of vascular tonus, homeostasis and inflammation. Endothelial dysfunction causes problems in vasoactive, anticoagulant and anti-inflammatory effects of healthy endothelium, which leads to atherosclerosis in the vascular system.

Previous studies have shown that the plasma bilirubin concentration is inversely correlated with several risk factors for CAD such as smoking, diabetes, and obesity and also it is inversely correlated with endothelial dysfunction (6, 7, 17, 18). Flow-mediated dilatation which represents endothelial functions was shown to be related with bilirubin levels (19). Endothelial dysfunction is an early pivotal event in the development, progression, and manifestation of atherosclerosis (20, 21). Recent evidence suggests that bilirubin acts as a potent physiologic antioxidant that may provide important protection against atherosclerosis and inflammation. The inverse correlation between the presence of CAD and SBL was reported in several studies (6, 8). In a previous study, the 3-year incidence of coronary artery disease (CAD) was significantly low in patients with Gilbert syndrome in a follow-up of study (22). Furthermore, previous studies have also demonstrated an reverse relationship between SBL and peripheral arterial disease (PAD) and carotid intima-media thickness (CIMT). Subnormal levels of plasma bilirubin are associated with premature CAD and cardiovascular morbidity (19, 23, 24).

In previous studies, it was found that different forms of bilirubin are potent antioxidants and serum bilirubin plays a potent role as antioxidant substance in the defense against oxidative stress (4). In an *in vitro* study, bilirubin was demonstrated to be >20 times more effective than alpha-tocopherol (a vitamin E analogue) in the prevention of LDL oxidation (25). Similarly, all forms of bilirubin were shown

Table 1. Baseline Characteristics of the Study Population

N (179)	Good Collateral (110)	Poor Collateral (69)	p value
Age (yrs)	62 ± 10	64 ± 10	NS
Gender (male)	85%	78%	NS
Height (cm)	1.68±0.07	1.67±0.08	NS
Weight (kg)	80±13	80±14	NS
BMI (kg/m^2)	28.7±4.7	28.5±4.5	NS
Hypertension	58%	63%	NS
Diabetes Mellitus	33%	45%	NS
Smoking	46%	58%	NS
Family history of CAD	20%	22%	NS
Hyperlipidemia	73%	75%	NS
Total cholesterol (mg/dL)	190 ± 47	190 ± 52	NS
LDL (mg/dL)	125 ± 35	123 ± 37	NS
HDL (mg/dL)	36 ± 7	39 ± 13	NS
Triglycerides (mg/dL)	152 ± 79	143 ± 72	NS
Previous MI	20%	10%	NS
Number of severely narrowed coronary arteries (≥50%)	2.6 ± 0.6	2.6 ± 0.6	NS
Number of CTO vessels	1.3 ± 0.85	1.1 ± 0.3	0.013
LAD	50%	38%	
CX	9%	14%	NS
RCA	41%	48%	
Glucose (mg/dL)	115 ± 33	131 ± 54	0.024
Creatinine (mg/dL)	1.0 ± 0.4	1.0 ± 0.3	NS
Total bilirubin(mg/dL)	0.80 ± 0.27	0.53 ± 0.19	<0.001
Indirect bilirubin (mg/dL)	0.50 ± 0.20	0.33 ± 0.16	<0.001
Direct bilirubin (mg/dL)	0.30 ± 0.15	0.20 ± 0.08	<0.001
Leukocytes (/mm^3)	7802± 2256	7793 ± 2217	NS
Platelets (10^3/mm^3)	276 ± 74	277 ± 83	NS
Hemoglobin (mg/dL)	13.6 ± 1.4	13.5 ± 1.5	NS
Ejection fraction (EF %)	51 ± 13	42 ± 12	0.017
Medications			
Aspirin	55%	49%	NS
Clopidogrel	8%	8%	NS
ACEI	35%	42%	NS
ARB	16%	9%	NS
Beta blockers	61%	66%	NS
CCB	9%	11%	NS
Diuretics	17%	9%	NS
Statin	57%	53%	NS
OAD	10%	12%	NS
Insulin	16%	16%	NS

CAD: Coronary artery disease, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, CTO: chronic total coronary occlusion, ACEI: Angiotensin-converting enzyme inhibitor, ARB: Angiotensin II receptor blocker, CCB: calcium channel blocker, OAD: oral anti-diabetic, LAD: left anterior descending, CX: circumflex, RCA: right coronary artery

to be effective in the protection against the peroxidation of LDL (4, 26-28). The physiological importance of bilirubin as an antioxidant beyond LDL oxidation was also suggested in prior various studies. It was demonstrated that bilirubin, at as low as 10nM, may protect neuronal cultures from a 10,000-fold excess of H_2O_2 (29). Furthermore, bilirubin may also have some cytoprotective properties for erythrocytes and ventricular myocytes against oxyradicals (28, 30, 31). Elevated concentrations of plasma bilirubin were suggested to be able to prevent atherogenesis (25, 32). Strong ability to scavenge peroxyl radicals and the antioxidant capacity of bilirubin functioning even in slightly increased concentration in circulation have led to the concept that it may have a physiological function to protect against disease processes involving oxygen and peroxyl radicals (33).

The development of atherosclerosis in vasculature includes some oxidative reactions such as the formation of oxygen and peroxyl radicals, and especially oxidation of low-density lipoprotein cholesterol (LDL) (33-35). The uptake of oxidized LDL by intimal macrophages leads to the accumulation of lipid-rich foam cells in vascular intima. Inflammatory cells further potentiate the formation of oxygen and peroxyl radicals. Those radicals play an important role in the inflammatory atherosclerotic process, especially due to disruption of the function of various cells including endothelial cells (22). Therefore, antioxidants may play a protective role from atherosclerotic vascular involvement preventing oxidative modification of LDL (36, 37).

Although SBLs are down-modulated by a wide range of cardiovascular risk factors known to suppress angiogenesis, an independent relation between SBLs and good collateral vessel formation has not been established. To date, a pathophysiologic mechanism for angiogenic/arteriogenic effects of bilirubin is not provided. Anti-oxidant/anti-inflammatory effects of bilirubin would not be expected to promote angiogenesis, as collateral vessel formation is dependent on in-

Table 2. Multivariate Analysis Using the Logistic Regression Method‡ for Good Collateral Development

Model 1		
Independent Variables	**p value**	**Odds Ratio (95% Confidence Interval)**
Age (yrs)	0.584	0.983 (0.926-1.044)
Gender, *male*	0.171	0.264 (0.039-1.780)
BMI (kg/m^2)	0.439	0.943 (0.814-1.094)
Hypertension, +	0.249	0.483 (0.140-1.663)
Diabetes Mellitus, +	0.792	0.780 (0.123-4.957)
Smoking, +	0.876	1.105 (0.315-3.874)
Hyperlipidemia, +	0.139	2.929 (0.706-12.147)
Glucose (mg/dL)	0.061	0.972 (0.944-1.001)
Number of CTO vessels	0.250	2.630 (0.505-13.7)
Total bilirubin levels†	<0.001	1.673 (1.273-2.197)
Indirect bilirubin†*	0.001	1.894 (1.317-2.723)
Direct bilirubin†*	0.008	1.901 (1.187-3.046)
Ejection fraction	0.115	1.043 (0.990-1.100)
Constant	0.533	8.542
R^2 (*Cox & Snell/ Nagelkerke*)		0.361 / 0.491
Model 2 *(After exclusion of irrelevant variables from model with pre-elimination)*		
Glucose (mg/dL)	0.003	0.981 (0.968-0.993)
Number of CTO vessels	0.007	5.642 (1.613-19.7)
Total bilirubin†	<0.001	1.832 (1.460-2.299)
Indirect bilirubin†*	<0.001	1.964 (1.484-2.600)
Direct bilirubin†*	<0.001	2.085 (1.464-2.969)
Constant	0.007	0.043
R^2 (*Cox & Snell/ Nagelkerke*)		0.361 / 0.493

‡*Logistic regression analyses were used for the multivariate analysis of all independent variables in* ***Model 1****. Afterward, logistic regression with backward LR method was repeated for multivariate analysis of all independent variables. After exclusion of irrelevant variables from the model, logistic regression with Enter method were performed with the remaining significant variables and then the obtained results were presented in* ***Model 2****.*

**when included in the analysis.* † *for per 0.1-mg/dL increase in bilirubin level.*

flammatory mediator signaling. On the other hand, if the importance of healthy endothelium for coronary collateral vessel development is considered, SBL may affect collateral development. Heterogeneity in collateral formation despite similar degrees of coronary obstruction can be related to variable effects of endothelial cells and endothelial cell function on development of collaterals. Nitric oxide (NO) which represents the status of endothelial health plays a major role in collateral vessel development. Asymmetric dimethylarginine (ADMA) which is an endogenous inhibitor of NO synthesis impairs effective coronary collateral vessel development. Increased plasma ADMA levels are related to poor coronary collateral development (38, 39). In a previous study, Gullu et al showed that elevated concentrations of bilirubin may serve as a protective factor in the development of coronary flow reserve (CFR) impairment, coronary microvascular dysfunction, and possibly in the development of coronary atherosclerosis. They concluded that bilirubin shows the beneficial effects independent of the known coronary risk factors. They speculated that serum bilirubin concentrations in the upper portion of the reference interval for young adult population provide protection against coronary microvascular dysfunction and CFR impairment by improving endothelium-dependent coronary vasodilator function (40).

In a recent small study, Dekker et al induced hyperbilirubinemia by Atazanavir treatment which is an antiretroviral agent and then they tested the hypothesis whether treatment with Atazanavir would ameliorate oxidative stress and vascular inflammation and improve endothelial function in Type 2 DM. Induced hyperbilirubinemia was associated with a significant improvement of endothelial function in Type 2 DM (41). Another means to achieve therapeutic purposes may be induction of Heme oxygenase-1 (HO-1) which catalyzes the first and rate-limiting step in the metabolism of free heme into equimolar amounts of ferrous iron, carbon monoxide (CO), and biliverdin. Biliverdin is subsequently converted to bilirubin by biliverdin reductase. HO-1 has recently been identified as a promising therapeutic target in the treatment of vascular inflammatory disease, including atherosclerosis (42, 43). If this relation is confirmed, synthetic or recombinant conformational analogs of bilirubin may enhance collaterals or protect against atherosclerosis.

Additionally in our study, we also found that FPG was related to poor collateral growth. This finding supports that the patients with DM have poor collateral growth which had been found in a previous study (14).

To our knowledge, this is the first study to investigate

possible effects of bilirubin on coronary collateral development. SBL may be an important mediator for the mechanisms which augment collateral formation.

Study Limitations

The relatively low number of study population may be a limitation for the interpretation of the results. Larger clinical studies will be necessary for confirmation of this relationship, but this study may give an idea about the difference in the coronary collateral development in patients with coronary artery disease. The other limitation of our study is that we cannot examine coronary microcirculation by coronary angiography. This may render an underestimation of the degree of coronary collateral development. Since we applied the same method to determine the degree of coronary collateral vessel development in both groups, this problem should not change the interpretation of the results. Another point is that, the duration of CTO is important for collateral growth as well as number of CTO which was included in multivariate analyses. But duration of CTO is not clear in our study and we added it as a limitation of this study. Lastly, this study was cross-sectional in nature and this may partially limit the precise conclusions on cause and effect.

Conclusion

This study suggests that higher SBL in a relatively normal range were related with favorable coronary collateral growth in patients with CTO. Bilirubin may be responsible for the difference in coronary collateral vessel development among different patients with coronary artery disease. The anti-inflammatory and anti-oxidant properties of bilirubin may mediate this effect. SBL may be one of the factors that determine the degree of collateral development and may provide a new target for the treatment strategies to enhance coronary collateral vessel development. Studies with larger study populations are required to clarify this relationship between SBL and coronary collateral development.

The authors state that they have no Conflict of Interest (COI).

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