Serum Bilirubin and Coronary Artery Disease: Intricate Relationship, Pathophysiology, and Recent Evidence

Vardhmaan Jain, MD, Raktim K. Ghosh, MD, Dhrubajyoti Bandyopadhyay, MD, Meera Kondapaneni, MD, Samhati Mondal, MD, Adrija Hajra, MD, Wilbert S. Aronow, MD, and Carl J. Lavie, MD

Abstract: Coronary artery disease (CAD) is a major cause of morbidity, mortality, and healthcare expenditure. A number of environmental and genetic risk factors have been known to contribute to CAD. More recently, a number of studies have supported as well as opposed a possible protective benefit of bilirubin in CAD, since it has anti-inflammatory, antioxidant, and antiaggregatory properties that may reduce atherogenesis. It also shares associations with different forms of CAD, namely stable CAD, unstable angina pectoris, stable angina pectoris, and acute myocardial infarction. Lack of sufficient evidence, however, has failed to elucidate a causal relationship between serum bilirubin level and risk of CAD. Therefore, in this update, we attempted to simplify this intricate relationship between bilirubin and CAD, revisit the pathophysiology of disease, how bilirubin may be protective, and to summarize the findings of the current literature. (Curr Probl Cardiol 2021;46:100431.)

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Introduction

Atherosclerosis is a progressive disorder characterized by accumulation of lipids and fibrous elements in the blood vessels. Lipid engorged macrophages, known as foam cells, deposit in the subendothelium resulting in a reduction in blood supply.[1] When such lesions occur in the coronary arteries, they give rise to coronary artery disease (CAD), which affects 16.8 million people in the United States and is a major cause of healthcare expenditure.[2] The traditional risk factors associated with increased risk of CAD are classified as those with a strong genetic component and environmental factors. The former includes elevated low density lipoprotein (LDL)/very low density lipoprotein,[3] reduced high-density lipoprotein,[4] elevated lipoprotein(a),[5] elevated blood pressure,[6] raised homocysteine levels,[7] family history of CAD,[3] and male gender.[8] Additional environmental factors include ongoing systemic inflammation,[9] depression and other adverse behavioral factors,[10] diets high in fat and carbohydrates, smoking, lack of exercise,[3] infectious agents,[11] and low antioxidant levels.

The interaction of the liver and heart is multifold and has long been a topic of interest. Long-standing heart failure leads to hepatic congestion resulting in cardiac cirrhosis, and advanced liver disease is associated with cirrhotic cardiomyopathy, pulmonary hypertension, and various electrophysiological abnormalities. Nonalcoholic fatty liver disease is associated with a higher prevalence of CAD due to increased insulin resistance, accelerated atherosclerosis, and coronary artery calcification (CAC).[12] However, there is an interesting paradox between serum bilirubin level with development and progression of CAD. It was first studied by Shwertner et al who found a decreased risk of CAD in patients with high-normal bilirubin.[13] Since then, a number of studies have shown a possible protective role of bilirubin, as well as the contrary. Therefore, in this update, we attempt to simplify this intricate relationship between bilirubin and CAD, revisit the pathophysiology of disease and how bilirubin may be protective, and to summarize the findings of the current literature on this controversial topic as a guide for physicians and other healthcare providers.

Bilirubin and Atherogenesis

To better understand how bilirubin may be protective in CAD, it is important to recall the pathogenesis of atherosclerosis (Fig 1). The process begins with deposition of LDL and other apolipoprotein-B containing lipids in the subendothelial space which then get oxidized. Minimally

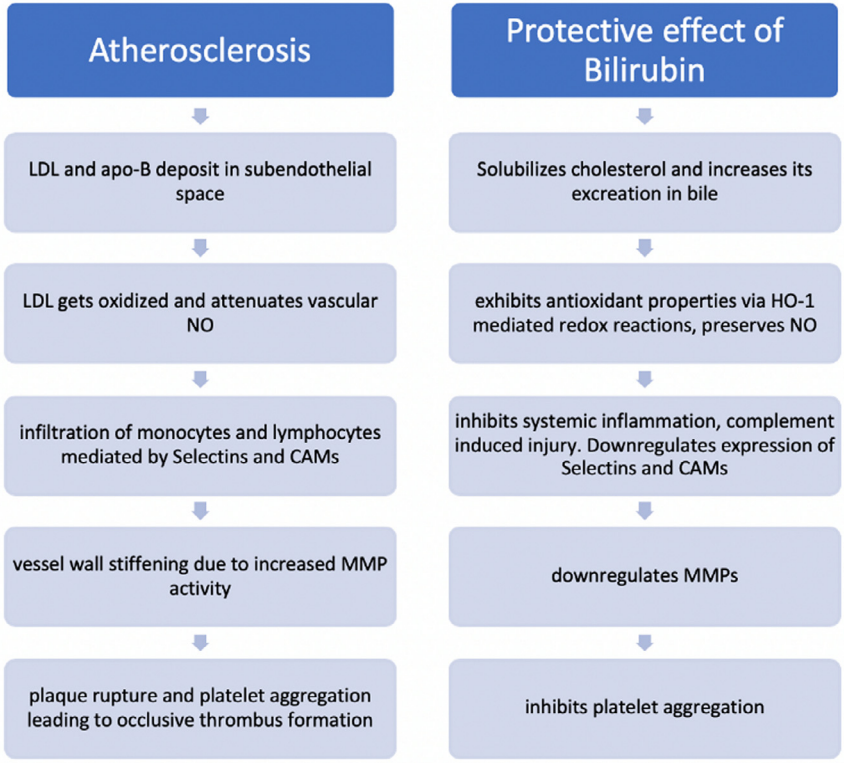

FIG 1. Pathophysiology of atherosclerosis and role of bilirubin. (LDL, low density lipoprotein; NO, Nitric Oxide; CAMs, Cell Adhesion Molecules; MMP, Metallic Metallproteinases; HO-1, Heme Oxygenase-1).

oxidized LDL recruits monocytes and lymphocytes, and downregulates the production of vasoprotective nitric oxide. Infiltration of inflammatory cells involves a complex interplay of adhesion molecules including P and E selectins, platelet and vascular cell adhesion molecules, and integrins. Highly oxidized LDL then gets engulfed by resident macrophages forming foam cells. Infiltration of smooth muscle cells and deposits of extracellular matrix results in the formation of fibrous plaques. Advanced and vulnerable plaques, such as those with thin fibrous caps, can eventually undergo rupture and thrombus formation due to platelet adhesion and aggregation on the exposed necrotic plaque core.[1] The resulting limitation in blood flow leads to the clinical manifestations of CAD.

Bilirubin may play a number of protective roles in the pathogenesis of CAD. Beginning from the initiation stage, bilirubin has been shown to play a role in solubilizing and excreting cholesterol. Genetic disorders associated with raised bilirubin levels have been shown to be

associated with higher high-density lipoprotein/LDL ratio, and lower apolipoprotein-B/apolipoprotein A-1 and total cholesterol levels.[14] Bilirubin subsegments have been shown to exhibit antioxidant properties that may prevent lipid peroxidation, which is predominantly due to the heme-oxygenase-1 mediated redox reactions involved in its catabolism.[15] Vascular nitric oxide plays a protective role in vessel wall stiffness and platelet aggregation; Heme oxygenase has been shown to preserve vascular nitric oxide (which otherwise gets downregulated in atherosclerosis).[16,17] In that sense serum bilirubin levels can be considered as a relative measure of vascular nitric oxide content. Next, bilirubin and biliverdin have also been shown to downregulate endotoxin induced P and E selectin expression which account for their antiplatelet aggregatory effect.[18] Higher concentrations of unconjugated bilirubin, such as those seen in Gilbert syndrome, have been shown to inhibit collagen and adenosine-diphosphate-induced platelet aggregation.[14] Finally, bilirubin may also be inhibitory to the inflammatory process intrinsic to atherosclerosis and has been shown to have an inverse relationship with markers of inflammation, including C-reactive protein, neutrophil-leucocyte ratio, and red cell distribution width.[19] It exhibits anticomplement properties in mice models that help slow down inflammation-mediated endothelitis.[20] Stable CAD is associated with increased vessel wall stiffness due to digestion of elastic fibers by increased activity of matrix metalloproteinases, most commonly matrix metalloproteinases-2 and matrix metalloproteinases-9.[21,22] Bilirubin and Heme oxygenase-1 products have been shown to downregulate matrix metalloproteinases and improve vessel wall elasticity, which manifests as lower pulse wave velocity, attenuation index, and CAC score.[23-26] The protective benefits of serum bilirubin have been summarized in Table 1.

UGT1A1 Polymorphism and CAD

After hepatic uptake of unconjugated bilirubin, it is conjugated by the action of action of uridine diphosphoglucuronate-glucuronosyltransferase (UDP-GT); UGT1A1 (bilirubin UDP-glucoronosyltransferase-1 family, polypeptide-A) is the major gene regulating hepatic bilirubin glucuronosylation, and codes for the enzyme UDP-GT. Mutations in its coding region have been associated with decreased enzyme activity and increased levels of unconjugated bilirubin, such as in Criggler-Najjar syndrome and Gilbert syndrome.[27] In theory, genetic polymorphisms of UGT1A1 (most common one being UGT1A1*28) should be related with increased bilirubin levels and decreased CAD risk, making them an important piece of the puzzle. A number of studies have been carried out to evaluate this association, all finding a significant association between UGT1A1 polymorphisms and bilirubin levels, and

TABLE 1. Possible protective effects of bilirubin

• Solubilizes and excretes cholesterol leading to lower LDL levels, apolipoprotein B/apolipoprotein-A1 ratio, and higher HDL levels.
• Has antioxidant properties that prevent LDL oxidation and foam cell formation
• Preserves vascular nitric oxide that maintains vessel wall elasticity
• Has anti-inflammatory and antiaggregatory benefits that prevent thrombus formation
• Downregulates matrix metalloproteinases preventing vessel wall stiffness

between bilirubin levels and incidence of CAD. However, most of them, including the Rotterdam,[28] the Etude Cas Temoins de l'Infarctus du Myocarde,[29] and the Cardiovascular Disease in Patients with Intermittent Claudication[30] studies failed to find an association between UGT1A1 polymorphisms and lower risk of CAD. On the other hand, the Framingham Offspring Study was the first to find a significant inverse relationship between UGT1A1*28 homozygosity and reduced risk of CAD[31]; similar results were seen in a subgroup of Chinese men.[32] Although there are a number of studies providing evidence of serum bilirubin and reduced incidence of CAD, the same cannot be said for UGT1A1*28 and CAD risk. This has been one of the major fallacies in the argument in favor of bilirubin's protective effects, especially when it comes to establishing a causal relationship.

Bilirubin and CAD Risk Factors

As mentioned above, the established risk factors for CAD include hypertension, obesity, smoking, metabolic syndrome, and systemic inflammation among others. The relationship between serum bilirubin levels and CAD risk factors, including obesity, metabolic syndrome, and hypertension, and how they predispose to CAD, are thus an important consideration. Nishimura et al evaluated the association between serum bilirubin and polyvascular disease (cerebrovascular disease, CAD, and peripheral vascular disease) in 674 patients with type 2 diabetes mellitus, finding that serum bilirubin levels did not differ in patients of type 2 diabetes mellitus with and without CAD. However, bilirubin levels were lower in the type 2 diabetes mellitus patients who had cerebrovascular disease and peripheral artery disease.[33] On the other hand, a Taiwanese study of 190 adolescents with metabolic syndrome found that the prevalence of the metabolic syndrome decreased from 6.6 ± 1.2% among the participants with concentrations of total bilirubin <8.6 μm/L to 2.1 ± 1.9% among those with concentrations >13.7 μm/L.[34] The results from the nationwide National Health and Nutrition Examination Surveys 1999-2012 (N = 31,069) showed that systolic

blood pressure decreased progressively up to −2.5 mmHg ($P< 0.001$) and the prevalence of hypertension was up to 25% lower ($P < 0.001$) in those with bilirubin ≥1.0 mg/dL.[35] A very interesting study by Tang et al assessed 691 subjects of prehypertension for carotid intima media thickness, serum bilirubin, systolic blood pressure, and C-reactive protein levels, finding that carotid intima media thickness was positively associated with systolic blood pressure (r = 0.257, $P < 0.001$), C-reactive protein (CRP) levels (r = 0.327, $P < 0.001$), total cholesterol (r = 0.218, $P = 0.002$), but significantly inversely associated with serum total bilirubin (r = −0.489, $P < 0.001$). However, multivariable stepwise logistic regression analysis demonstrated that total bilirubin (odds ratio [OR] = 0.476; 95% confidence interval [CI]: 0.253, 0.764; $P < 0.001$), systolic blood pressure (OR = 1.142; 95% CI: 1.003, 1.202; $P = 0.012$), CRP (OR = 1.233; 95% CI: 1.015, 1.563; $P < 0.001$), and total cholesterol (OR = 1.167; 95% CI: 1.028, 1.518; $P = 0.019$) were significantly correlated with carotid atherosclerosis.[36] Thus, given the conflicting results of various studies, it is hard to conclude whether serum bilirubin levels may be causal or protective for CAD risk factors, including hypertension and metabolic syndrome.

Bilirubin and Stable CAD

Schwertner et al first evaluated the association between serum bilirubin and risk of having CAD in a group of 619 US air force pilots and found that a 50% decrease in serum total bilirubin was associated with 48% higher odds of having greater severity of CAD.[13] Since then a number of studies have been conducted to verify these findings. A recent study of 2862 Korean men undergoing coronary computed tomography as a part of routine screening showed that serum bilirubin level was inversely associated with degree of coronary atherosclerosis and calcified plaques in a dose dependent manner.[37] Zhu et al recently published their work on bilirubin levels and coronary plaque characteristics on intravascular ultrasound in patients of CAD and showed that serum bilirubin levels were positively associated with fibrous plaques and negatively associated with plaque burden and remodeling index.[38] A Japanese study conducted in 637 participants to assess the relationship between CAC and serum bilirubin levels found that an increment of 1 μm/L in serum bilirubin concentration was associated with 14% decrease in the odds for CAC score ≥400 after adjustment for several risk factors.[24]

While many studies have provided evidence regarding the protective benefits of bilirubin against CAD, there are a number of studies providing

evidence for the contrary. The prospective British Regional Health Study followed 7685 patients over 11.5 years and demonstrated that there was a "U"-shaped correlation between bilirubin level and risk of CAD, with higher risk at bilirubin levels <0.4 mg/dL and>0.7 mg/dL.[39] The large sample size of the British Regional Health Study study as compared to other similar studies[40] gave it much more credibility.

Bilirubin and Acute Coronary Syndrome

Although serum bilirubin levels have been shown to have an inverse relationship with incidence of stable CAD, they have shown to be associated with higher in-hospital mortality and troponin levels in patients of acute myocardial infarction (MI; AMI).[41,42] A recent study conducted by Ozturk et al in 782 patients of non-ST-elevation acute coronary syndrome (ACS) with and without troponin elevation found a significant and positive correlation between admission total bilirubin levels and troponin levels.[42] They postulated that Heme Oxygenase-1 is a stress-inducible enzyme, and increased activity in AMI corresponds to raised bilirubin levels. Okuhara et. al investigated the levels of Heme Oxygenase-1 enzyme and bilirubin levels in patients admitted for AMI, and found a significant positive correlation, providing further evidence for this argument.[43] The role of bilirubin in predicting adverse outcomes in patients of CAD undergoing percutaneous coronary intervention (PCI) is also interesting. A recent study by Zhang et al in 450 hospitalized patients of stable CAD undergoing PCI found that low fasting serum bilirubin levels were independent predictors of reduced major adverse cardiovascular event-free survival.[44] However, in studies involving patients with AMI undergoing PCI, serum bilirubin levels were positively associated with higher in-hospital mortality, in-hospital adverse cardiovascular events, and higher degree of impaired vascular flow (Pre-PCI TIMI score <= 2).[41] Huang et al recently conducted a large-scale retrospective study of 3013 patients with angiographically obstructive CAD. They divided these into 3 groups – stable CAD, unstable angina, and AMI, and evaluated the relationship of total bilirubin with 30-day and long-term mortality. Serum bilirubin levels were associated with high short-term mortality in AMI group (OR 2.35, 95% CI 1.15-4.77) and were also predictive of long-term mortality in stable CAD (hazard ratio 0.34, 95% CI 0.16-0.70) and unstable angina (hazard ratio 0.49, 95% CI 0.31-0.78) groups. However, there was no statistically significant relation between total bilirubin and long-term mortality in AMI groups.[45] Thus, there is a contradictory relationship between serum bilirubin levels with CAD and ACS. Given the findings of present studies, a stress-induced

increased activity of Heme Oxygenase-1 may be a possible explanation. However, a large number of ACS patients have a prior history of long-standing stable CAD. Whether an acute change can lead to lower bilirubin levels at admission still remains controversial, and further studies may offer an alternate explanation.

Bilirubin and Peripheral Artery Disease

A number of important studies have been published recently to evaluate the relationship between serum bilirubin and incidence of peripheral artery disease. A Japanese study of 935 patients evaluated the relationship between serum bilirubin levels and ankle-brachial index in patients without prior history of angioplasty or bypass graft of lower limb vessels and found a significant and negative correlation between serum bilirubin levels and prevalence of peripheral artery disease.[46] A Spanish study recently investigated total bilirubin levels and peripheral arterial atherosclerosis in patients of familial hypercholesterolemia. They evaluated 464 individuals with familial dyslipidemia, 332 with familial hypercholesterolemia, and 142 people with familial combined hypercholesterolemia and found a significant and negative correlation between serum bilirubin levels and carotid intima media thickness, plaque incidence, as well as plaque burden. Only patients with familial combined hypercholesterolemia were found to have an inverse relationship between bilirubin and femoral plaque height.[47] Perlstein et al found similar results in the National Health and Nutrition Examination Survey. In their study, they found that each 0.1 mg/dL increase in serum bilirubin led to a 6% reduction in the odds of having peripheral artery disease.[48] Key points of our findings have been summarized in Table 2.

TABLE 2. Key points

1. Serum bilirubin has been proposed to have a protective benefit in CAD.
2. Bilirubin has been shown to have anti-inflammatory, antiproliferative, and antiaggregatory benefits in atherosclerosis. It may help preserve vascular nitric oxide levels and vessel wall elasticity.
3. UGT1A1 polymorphisms have not shown an inverse relationship with the incidence of coronary artery disease, implying that the synthesis of bilirubin rather than its excretion may be responsible for the beneficial effect.
4. Various studies have shown a direct as well as an inverse relationship between bilirubin levels and risk of coronary artery disease.
5. Individuals with lower serum bilirubin levels may be at increased risk of CAD. This has significant clinical importance for internists and other healthcare providers. Further studies are required to establish a possible causal relationship.

TABLE 3. Summarizing key clinical studies related to serum bilirubin and CAD

No.	Title, author	Journal, year	Location	Sample size	Males	Mean age in years	Selection criteria	Observations	Comments
1	Association of low serum concentration of bilirubin with increased risk of coronary artery disease. Schwertner et al.	Clinical Chemistry 1994	USA	619 (group A), 258 (group B)	100%	41.8 (21-61) 43.7 (23-65)	Asymptomatic male United States Air Force (USAF) pilots and navigators who underwent coronary angiography to determine their fitness for flying duty.	Total bilirubin was inversely and statistically significantly r elated to the presence of CAD, both univariately and multivariately after adjustment for age, total cholesterol, high-density lipoprotein cholesterol, smoking history, and systolic blood pressure	Serum bilirubin has a statistical and inverse relationship with CAD risk.
2.	The relationship between serum levels of total bilirubin and coronary plaque vulnerability Zhu et al.	Coronary artery disease 2016	USA	85	67%	63 (54-72)	85 Consecutive patients [45 with ACS and 40 with stable angina pectoris (SAP)] from Hangzhou First People's Hospital (Zhejiang, China) were enrolled.	1. Bilirubin levels lower in ACS group than SAP and control group ($P < 0.01$). 2. Total bilirubin positively associated with fibrous plaques, negatively associated with plaque burden, lipid plaque, and remodeling index .	Serum bilirubin levels are negatively associated with plaque vulnerability. Low bilirubin levels may a contributory factor in plaque formation.
3.	Association between the UGT1A1*28 allele, bilirubin levels, and coronary heart disease in the Framingham heart study Lin et al.	Circulation 2006	USA	1780	49%	36 (26-46)	In 1971, the Framingham Offspring began with 5124 subjects aged 5 to 70 years at entry, including the children of the original	Homozygote UGT1A1*28 allele carriers with higher serum bilirubin concentrations exhibited a strong association with lower risk of CVD	UGT1A1*28 polymorphisms as well as serum bilirubin are inversely associated with risk of CAD

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TABLE 3. (*continued*)

No.	Title, author	Journal, year	Location	Sample size	Males	Mean age in years	Selection criteria	Observations	Comments
							cohort and their spouses. 1780 randomly selected patients from this cohort formed the study population.		
4.	*UGT1A1*28*allele and coronary heart disease: the Rotterdam study Bosma et al.	Clinical Chemistry 2003	USA	185	61.6%	70	Population based	After adjustment for age, gender, smoking habits and pack-years of smoking, body mass index, diabetes mellitus, systolic blood pressure, total cholesterol, and HDL cholesterol, there was no significant difference in risk of CAD in among different polymorphisms of UGT1A1 gene	No clear relation between UGT1A1 polymorphism and CAD risk
5.	Serum total bilirubin concentration in patients with type 2 diabetes as a possible biomarker of polyvascular disease Nishimura et al.	Diabetology international 2018	Japan	674	61%	65 (50-80	674 Patients with type 2 DM admitted from 2008-2013	1. Patients with CBVD and PAD showed significantly lower serum total bilirubin concentrations than did those patients without those diseases. 2. The bilirubin concentration did not differ between patients	Serum bilirubin independent predictor of CBVD, not of PAD and CAD in type 2 DM

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TABLE 3. (*continued*)

No.	Title, author	Journal, year	Location	Sample size	Males	Mean age in years	Selection criteria	Observations	Comments
								with and without CAD 3. Total bilirubin concentration was an independent predictor of CBVD, but not of CAD or PAD	
6.	Serum bilirubin and risk of ischemic heart disease in middle-aged British men Breimer et al.	Clinical Chemistry 1995	Britain	7685	100	50	Prospective, population based	Compared with men in the lowest fifth of the distribution (bilirubin < 7 μm/L), those in the middle range(8-9 μm/L) showed a 30% reduction in relative risk of CAD, whereas those in the top fifth (>12 μm/L) showed similar risk to the lowest fifth.	U-shaped relationship between serum bilirubin levels and risk of CAD
7.	The correlation between serum total bilirubin and outcomes in patients with different subtypes of coronary artery disease. Huang et al.	Clinica chimica acta 2017	China	3013	80%	64	Retrospective analysis of patients admitted with angiographically obstructive CAD	Higher bilirubin levels associated with increased risk of short-term mortality in AMI group, not in UAP and SCAD group. Serum TB was able to independently predict the long-term mortality in SCAD and UAP groups. There was no significant relation between TB and long-term mortality in AMI groups	Outcomes of different subtypes of CAD correlate differently with serum TB.

Conclusion

There have been a number of studies supporting as well as contradicting a possible protective role of bilirubin in CAD (summarized in Table 3). UGT1A1 gene polymorphisms have not correlated well with lower rates of CAD. These findings would imply that the synthesis of bilirubin via Heme Oxygenase-1 and other reaction intermediates (like carbon monoxide and iron) would be responsible for a protective effect, if any, and that raised bilirubin levels might be a marker rather than a mediator of CAD.[49] Finally, low bilirubin may be indicative of increased oxidative stress (and hence increased utilization of antioxidants), or decreased Heme Oxygenase-1 activity. In that regard, low serum bilirubin may not be a cause, but rather reflective of individuals at increased risk of developing CAD. Nonetheless, bilirubin may be a prospective, noninvasive marker of CAD and further studies are required to investigate a possible causal relationship.

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