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Role of Bilirubin in Diabetic Vascular Complications: Can Bilirubin Predict More than Just Liver Disease?

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Estimating the risk of cardiovascular diseases (CVDs) is important for making clinical decisions and establishing therapeutic strategies for individual patients with type 2 diabetes. Although current guidelines do not recommend routine screening for CVD in asymptomatic patients with diabetes [1], prognostic information is still required in clinical practice. Several risk equations using data obtained from large populations have been developed. However, a recent review shows that these risk models do not reliably predict the risk of fatal CVD in patients with type 2 diabetes [2]. If a simple circulating biomarker that enabled risk prediction models was available, it could provide information in addition to that provided by equations to readily identify diabetic patients with high or intermediate risk of CVD and thus prevent cardiovascular events. A number of markers have already been suggested, including C-reactive protein, fibrinogen [3], and vitamin D [4]; however, they are not routinely measured and confirmatory trials are still required.

Bilirubin is used as a marker of cholestasis and is believed to be a toxic waste metabolite of heme catabolism, particularly in newborns. However, several studies have reported that bilirubin plays a protective role in cardiovascular and metabolic diseases. Heme is degraded by heme oxygenase (HO) producing biliverdin, carbon monoxide (CO), and ferrous iron (Fe^{2+}). There are two isoforms of HO; HO-1 is a highly inducible form responsible for oxidative stress, and HO-2 is a constitutive form with an important role in brain and testes. HO-derived CO, an important signaling gas, mediates several cytoprotective actions, including vasodilatation and reduction of inflammation [5]. Bili-

verdin is subsequently reduced to bilirubin by biliverdin reductase. Because bilirubin is a non-polar molecule, it is solubilized in vascular bed upon binding to albumin. Bilirubin is conjugated with two molecules of glucuronic acid by uridine diphosphate glucuronosyltransferase A1 (UGT1A1) to a water-soluble form for elimination. Accumulating evidence suggest a protective role of the HO-1/CO/bilirubin system in vascular dysfunction caused by diabetes and obesity.

The protective role of bilirubin has been clearly demonstrated in subjects with Gilbert's syndrome. Gilbert's syndrome is generally caused by a mutation in the promoter region of UGT1A1 gene, which reduces its ability to eliminate bilirubin. This syndrome represents benign, non-hemolytic, unconjugated hyperbilirubinemia. Diabetic patients with Gilbert's syndrome are reported to have reduced prevalence of vascular complications compared with diabetic patients with normal bilirubin levels [6] as well as reduced markers of oxidative stress and inflammation. Novotny and Vitek [7] indicated that low serum bilirubin concentration is an important risk factor for CVD in patients with diabetes receiving hemodialysis. A meta-analysis also supports the results that a modest elevation in unconjugated bilirubin, as seen in Gilbert's syndrome, is negatively correlated with the risk of developing CVD.

Numerous clinical studies have shown a negative association between circulating bilirubin levels and risk of diabetic vascular complications. Low serum bilirubin value is reported to be a significant risk factor for diabetic microangiopathy. In a Japanese cross-sectional study, patients in the lowest quartile group

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for bilirubin levels had four times higher prevalence of diabetic retinopathy than those in the highest quartile group [8]. Lower bilirubin levels have also been associated with increased urinary albumin excretion [9]. On the contrary, a large Korean study found that higher bilirubin levels reduced the risk of diabetes and diabetic nephropathy [10].

Since an inverse correlation between bilirubin and coronary artery disease (CAD) was observed [11], many studies have consistently demonstrated a low concentration of bilirubin to be a risk factor for atherosclerotic CVDs. Circulating bilirubin levels have also been found to be inversely associated with coronary artery calcification [12]. A meta-analysis has shown clear protective effects of bilirubin on atherosclerosis wherein each 1.0 μmol/L increase in bilirubin related to a 6.5% decrease in CVD risk, though only males were included in this analysis. In addition, a prospective study has indicated that bilirubin could be a protective and predictive biomarker for the development of cardiovascular events in patients with cardiac syndrome X, which is closely related to oxidative stress and vascular inflammation [13]. Turfan et al. [14] demonstrated serum bilirubin levels to be inversely related to disease severity in patients with stable CAD and suggested total bilirubin levels to be a useful marker for the severity of stable angina.

Experimental approaches have consistently shown the protective effects of bilirubin on vascular complications in diabetes. The antioxidant capacity of bilirubin potently inhibits lipid peroxidation that is closely associated with the initiation and progression of atherosclerosis [15]. In addition, bilirubin has been shown to have anti-inflammatory effects by inhibiting cytokine release (interleukin-2, interferon-γ, and Tumor necrosis factors-α) and intercepting complement proteins (C3, C1q-immunoglobulin M [IgM], and IgG complexes). Unconjugated bilirubin inhibits protein kinase C and nicotinamide adenine dinucleotide phosphate oxidase activity as well as vascular smooth muscle cell proliferation, which are well-known causes of diabetic vascular dysfunction. The mechanism underlying the antithrombotic functions of bilirubin could involve bilirubin reducing platelet activation as an antioxidant and inhibiting collagen-induced platelet aggregation and thrombus formation [16] *in vitro*. Liu et al. [17] demonstrated that serum concentrations of unconjugated bilirubin are reduced by nearly 80% in *db/db* mice, and chronic bilirubin treatment reverses endothelial dysfunction both *in vivo* and *ex vivo*. They also identified Akt as the principal target of bilirubin and showed that reduced activities of Akt and eNOS and reduced levels of nitrite in diabetes are improved by bilirubin.

In the current issue of *Diabetes & Metabolism Journal*, Leem et al. [18] demonstrated that circulating total bilirubin can be used as a biochemical marker for CAD risk in asymptomatic patients with type 2 diabetes. They defined obstructive CAD as ≥50% stenosis of the lumen using 64-channel multiple detector computed tomography. Although coronary computed tomographic angiography is not recommended for the routine screening of CAD, it may be useful to a carefully selected group for its remarkable accuracy as shown in a previous study [19].

Consistent with previous studies, higher bilirubin levels were beneficial to obstructive CAD after adjustments and this benefit persisted regardless of coronary calcium score and plaque subtype, with the exception of calcified plaque. Furthermore, total bilirubin levels provided additional risk information to the Framingham risk score (FRS) compared with FRS alone in asymptomatic patients with type 2 diabetes. These results imply that bilirubin may be clinically utilized as a predictive marker with a complementary role in established risk prediction models. However, conflicting evidence was recently reported suggesting that total bilirubin in the standard established risk factor panel does not improve CVD risk prediction [20]. Although the study included both a large prospective trial and a meta-analysis, this discrepancy may stem from a difference in the medical history of the participants, who in this case were adults without a history of diabetes or CVD.

There is a desperate need for reliable risk prediction tools for CVD that are capable of readily identifying the high-risk group from asymptomatic diabetic patients. The present study considers the possibility of bilirubin as a candidate marker. If we do not miss some messages from yellow pigment, we will be able to provide more help to diabetic patients unknowingly progressing to CVD. Future studies will help us to understand the role of bilirubin more clearly.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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