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Elevated serum bilirubin levels are inversely associated with coronary artery atherosclerosis

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ABSTRACT

Background: Inverse correlations of high serum bilirubin with metabolic and cardiovascular disease have been suggested. However, anti-atherogenic effects of bilirubin have not been well-established in terms of the presence of plaques and stenosis identified in coronary computed tomography (CT).
Methods: A cross-sectional study was conducted on 2862 men who were free of cardiovascular disease and underwent coronary CT as part of a routine medical screening examination. Coronary stenotic lesions were considered to be incidences of coronary atherosclerosis, and stenosis was classified as stenosis $<50\%$ or $\geq 50\%$, according to degree of stenosis.
Results: The prevalences of coronary atherosclerosis and stenosis $\geq 50\%$ in subjects with elevated bilirubin levels (>1.2 mg/dL) were lower than those in subjects with normal bilirubin levels (≤ 1.2 mg/dL) (19.9% vs. 27.9%, $p < 0.001$, 8.5% vs. 10.3%, $p = 0.044$). Bilirubin was inversely associated with total plaques (odds ratio [OR] 0.59, 95% confidence interval [CI] 0.48–0.73 in the 4th quartile vs. 1st quartile) and calcified plaques (OR 0.60, 95% CI 0.49–0.75) in univariate analysis. After adjusting for traditional risk factors, it was found that coronary atherosclerosis (OR 0.73, 95% CI 0.56–0.94 in the 4th quartile vs. 1st quartile) and calcified plaque (OR 0.66, 95% CI 0.53–0.84) were inversely associated with the bilirubin grade in a dose-dependent manner.
Conclusions: The serum bilirubin level was inversely associated with coronary atherosclerosis and calcified plaques in a dose-dependent manner. These results suggested that serum bilirubin could be used as a protective biomarker of coronary artery disease.

1. Introduction

Cardiovascular disease is the most common cause of mortality in developed countries and accounts for up to one-third of all deaths worldwide [1]. The overproduction of oxygen free radicals from oxidative stress is known to mediate various signaling pathways that underlie vascular inflammation in atherogenesis, from the initiation of fatty streak development to ultimate plaque rupture [2]. Although the pathogenesis of atherosclerosis has not been thoroughly investigated, oxidative stress and DNA damage induced by oxidized low-density lipoprotein (LDL) cholesterol and by diet-induced hypercholesterolemia contribute to the progression of atherosclerosis [3]. Therefore, antioxidants are thought to serve a protective role against atherosclerosis and coronary artery disease by preventing the oxidative modification of LDL cholesterol [4].

Bilirubin is a potent antioxidant under physiological conditions and suppresses the oxidation of lipids and lipoproteins. Thus, bilirubin has been demonstrated in vitro to prevent plaque formation and subsequent formation of atherosclerosis [5]. A prospective cohort study suggested that low levels of bilirubin are correlated to premature coronary artery disease [6]. Previous studies have demonstrated the inverse relationship between serum total bilirubin concentration to peripheral artery disease and carotid intima–media thickness [7,8]. A genetic association between genes that influence serum bilirubin concentration such as heme

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAC, coronary artery calcification; CT, computed tomography; CI, confidence interval; GGT, gamma-glutamyl transpeptidase; HDL, high-density lipoprotein; hs-CRP, high sensitive c-reactive protein; LDL, low-density lipoprotein; OR, odds ratio; TG, triglycerides.

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oxygenase and uridine diphosphate-glucuronosyl transferase and coronary artery disease has been addressed in several studies [9,10]. In recent studies, a high serum bilirubin level was inversely associated with the presence of coronary artery calcification (CAC) [11,12]. However, CAC is limited in its prediction of noncalcified plaques, and as a result, it fails to represent the entire spectrum of atherosclerotic plaques. CAC screening is also inadequate in evaluating the degree of stenosis caused by plaque [13]. Although individuals with low bilirubin levels have a higher prevalence of coronary artery disease, there are few studies on the effect of serum total bilirubin on coronary artery stenosis and plaques in large populations. Thus, we investigated the effect of serum bilirubin on both coronary artery stenosis and coronary plaques, as assessed by coronary computed tomography (CT), in large, apparently healthy Korean males.

2. Methods

2.1. Study population and study design

We performed a cross-sectional study in subjects who underwent a comprehensive medical check-up at Seoul National University Hospital, Healthcare System Gangnam Center from July 2006 to March 2010. Some of the subjects had voluntarily paid for a general health check-up, while others were supported through their employer. During this period, 3319 male subjects underwent a coronary CT examination for screening. All coronary CT and clinical and laboratory assessments were performed on the same day or within 6 months of each other.

Out of the 3319 subjects, we excluded 146 subjects who had a history of heart attack, coronary artery disease (including acute myocardial infarction), angina, or congestive heart failure. We also excluded 249 subjects with at least one potential cause of chronic liver disease: 182 with positive hepatitis B surface antigen, 55 with positive hepatitis C antibody, and 12 with a history of other hepatitis or liver disease (e.g., primary biliary cirrhosis, autoimmune hepatitis, Wilson's disease, etc.). An additional 34 subjects with abnormal biliary tracts (as observed in abdominal ultrasonography) and 28 subjects (mean ± standard deviation of creatinine: 1.61 ± 0.26 mg/dL) with abnormal renal function (creatinine level > 1.4 mg/dL) were also excluded. Finally a total of 2862 subjects were enrolled in this study.

The Institutional Review Board of Seoul National University Hospital approved the study protocol, and the study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

2.2. Clinical and laboratory evaluation

In addition to a laboratory examination, each subject underwent a questionnaire assessment and an anthropometric assessment. Systolic and diastolic blood pressures were measured twice on the same day, and the mean of the two values was used in the analysis. Height and body weight were measured using a digital scale. Body mass index was calculated as weight divided by height (in meters) squared, and waist circumference was measured at the midpoint between the lower costal margin and the iliac crest. These values were measured by a well-trained nurse. Subjects were classified as current smokers if they had been smoking for at least 1 year.

The laboratory evaluation included measurements for aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), total bilirubin, total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, fasting glucose, high sensitive c-reactive protein (hs-CRP), hepatitis B surface antigen, and an antibody to the hepatitis C virus. Venous blood samples were taken from all subjects before 10 AM, following a 12-h overnight fast.

Hypertension was defined as having a systolic blood pressure ≥140 mmHg, a diastolic blood pressure ≥90 mmHg and/or current use of antihypertensive medications. Subjects with a fasting plasma glucose level of ≥126 mg/dL or currently on anti-diabetic treatments were defined as having diabetes mellitus [14].

2.3. Measurement of coronary artery stenosis and plaque by coronary artery CT

Coronary CT was performed using a 16-row multi-slice CT (Sensation 16, Siemens Medical Systems, Erlangen, Germany) as previously described [15]. Briefly, after obtaining a topogram of the chest, a calcium score scan and angiography were performed using the retrospective method with a tube voltage of 120 kV and a 110 effective mAs tube current with a 200 mm field of view and ECG-gated dose modulation. Prior to the CT scan, the patient's heart rate was measured. Patients with a heart rate higher than 60 beats per minute were given a 50–100 mg dose of metoprolol.

All of the images were analyzed by a radiologist who was blinded to the clinical and laboratory results of the subjects. Each lesion was identified using a multi-planar reconstruction technique and maximum intensity projection of the short-axis, two- and four-chamber views.

The degree of stenosis and the plaque characterization were measured from the coronary CT images with a dedicated computer 3D workstation (Rapidia; Infinitt, Seoul, Korea). Coronary artery stenosis was estimated from the contrast enhanced portion of the coronary lumen. The stenosis was semi-automatically traced at the maximal stenotic site and then compared with the mean value for the proximal and distal reference sites [16]. Subjects with any coronary stenotic lesions were classified as having coronary atherosclerosis. Stenosis was classified as <50% or ≥50% based on the degree of stenosis. Number of stenosis was assessed using the 15-segment model of American Heart Association [17].

Plaques were identified as structures that were larger than 1 mm^2 within or adjacent to the vessel lumen. The plaques were clearly distinguished from the lumen and surrounding epicardial fat. The plaque type was classified as follows: (a) Plaques containing calcified tissue that comprised more than 50% of the plaque area (attenuation >130 HU on native images) were classified as calcified. (b) Plaques with less than 50% calcium in the plaque area were classified as mixed. (c) Plaques without any calcium were classified as noncalcified plaques [16].

2.4. Statistical analysis

The data were expressed as the mean ± standard deviation or as a median with an interquartile range for continuous variables. Comparisons between groups were performed using the Chi-squared test for categorical variables, and continuous data were analyzed using Student's *t*-test or the Mann–Whitney *U* test and the analysis of variance or the Kruskal–Wallis test, as was appropriate. To explore the association between bilirubin and coronary stenosis in a dose-dependent manner, serum bilirubin was stratified into quartiles (1st quartile: ≤0.8 mg/dL, 2nd quartile: 0.8–1.0 mg/dL, 3rd quartile: 1.0–1.2 mg/dL, 4th quartile: >1.2 mg/dL). Logistic regression analysis was used to analyze the association between serum bilirubin and both coronary stenosis and each type of plaque, while also controlling for potential confounders. Covariates in the multivariable model, which were chosen for clinical importance as well as statistical significance, included age, sex, body mass index, waist circumference, diabetes, hypertension, total cholesterol, triglycerides, HDL cholesterol, daily alcohol