



Can bilirubin act as an indicator to predict coronary artery disease?

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Abstract *Introduction and Objective:* Oxidative reactions are involved in the development of atherosclerosis. The formation of oxygen and peroxy radicals and LDL-cholesterol oxidation are some examples. The aim of this study was to evaluate the relationship between serum levels of bilirubin and coronary artery disease (CAD).

Materials and Methods: We evaluated 85 patients and 92 healthy participants. Total and direct bilirubin levels were measured using the diazo method, and levels of TG and total cholesterol were measured using the enzymatic method. The poly anion-cation and the direct method were used for HDL-cholesterol and LDL-cholesterol, respectively. Data was analyzed with SPSS version 17. Chi-square and student t test was used for qualitative and quantitative variables, respectively. P values less than 0.05 were considered significant.

Results: The level of direct, indirect, and total bilirubin was 0.213, 0.375, and 0.588 in healthy controls and 0.228, 0.365, and 0.593 mg/dl in patients, respectively. There was no significant difference in the level of direct, indirect, and total bilirubin between the two groups. There was also no significant difference in the level of TG and total cholesterol between the two groups. There was a significant difference in the mean serum level of HDL-cholesterol (P=0.001), family history (P=0.006), mean blood pressure (P<0.001), and smoking (P=0.031) between the two groups.

Conclusion: The results of this study show that bilirubin can be used as an indicator to predict CAD. Our findings are in line with some previous reports and contradictory to some others.

Keywords CAD, bilirubin, antioxidants

Introduction

Bilirubin has been always considered a toxic product of heme catabolism that should be excreted. Heme oxygenase is a rate-limiting enzyme in the production of bilirubin. This microsomal enzyme is present in both central and peripheral tissues and converts heme to biliverdin and carbon monoxide [1]. Then, biliverdin is reduced to bilirubin using biliverdin reductase [2]. Recent studies suggest that bilirubin is a potential physiologic antioxidant that may have an important protective role against atherosclerosis, CAD, and inflammation. On the other hand, oxidative reactions are also involved in the pathophysiology of these diseases.

There is compelling evidence that progression of CAD is associated with lipid oxidation and oxygen radicals formation, and atherosclerosis and inflammation is associated with the formation of oxygen and peroxy radicals [3-5].

The antioxidant capacity of bilirubin and its potential ability to scavenge peroxy radicals raised points whether a



mild increase in bilirubin could have a physiologic protective role against diseases associated with oxygen and peroxy radicals [6].

Moreover, biliverdin can prevent LDL oxidation as an antioxidant [7]. Furthermore, a lower serum concentration of bilirubin is associated with defects in the endothelium function [8] and increased risk of cardiovascular diseases [9]. In pediatric and adult patients with metabolic syndrome, high levels of bilirubin have been reported to improve insulin resistance [10]. In addition, the serum bilirubin level is inversely associated with the prevalence of metabolic syndrome in adults [11-13]. The aim of this study was to investigate the relationship between bilirubin concentration, risk of CAD, oxidative stress, and lipid metabolism.

Materials and Methods

In this case-control study, the participants were selected from the patients attending Tehran Heart Center, including 85 patients (28 women (32.9%) and 57 men (67.1%) with CAD whose disease was confirmed by a specialist through angiography and 92 individuals (53 women (57.6%) and 30 men (42.4%) who were found to be CAD free through angiography. Blood samples were taken from the participants after 12 hours of fasting. After separating the serum, the bilirubin concentration was measured using the Pars Azmoon Kit. The concentration of direct and total bilirubin was measured using the diazo method (with diazotized sulfanilic acid). In this method, bilirubin reacts with diazonium salt of sulfanilic acid to produce an azo dye that is red in alkaline pH. After the complex is formed, direct bilirubin is pink in color while total bilirubin is green after adding accelerator in an alkaline medium. Indirect bilirubin is measured by subtracting direct bilirubin from total bilirubin.

The maximum absorbance of total and direct bilirubin was measured at 578 and 546 nm, respectively.

Total cholesterol and TG were measured using the enzymatic colorimetric method. The measurement of TG is based on the hydrolysis of triglycerides by enzymes and the measurement of free glycerol:

The light absorbance was read at 520 nm.

The measurement of total cholesterol is based on the hydrolysis of cholesterol esters by different enzymes and the measurement of free cholesterol

The light absorbance was read at 520 nm.

HDL-cholesterol was measured using the colorimetric method. The level of LDL-cholesterol was determined using the direct method. There is no need for preparation in this method. The sample is exposed to reagents directly in 2 steps.

The light absorbance was read at 546 nm.

SPSS 17 was used for data analysis. Chi square was used for qualitative and student t test was used for quantitative variables.

Results

The mean age of the patients and controls was 58.62 and 56.37 years, respectively. The BMI was calculated through dividing weight (kg) by the square of height (m), and its mean and standard deviation were determined in cases and controls. Moreover, the level of direct, indirect, and total bilirubin was 0.213, 0.375, and 0.588 in controls and 0.228, 0.365, and 0.593 mg/dl in cases, respectively. There was no significant difference in direct bilirubin ($p=0.320$), indirect bilirubin ($p=0.846$), and total bilirubin ($p=0.940$) between the case and control groups.

The mean \pm standard deviation of TG, total cholesterol, HDL-cholesterol, and LDL-cholesterol was determined in cases and controls. The results showed no significant difference in mean TG ($p=0.548$) and total cholesterol ($p=0.751$) between cases and controls. Although there was a significant difference in mean HDL-cholesterol between cases and controls ($P=0.001$), the difference in LDL-cholesterol was not significant ($P=0.813$).

Forty-three cases and 65 controls lacked a positive family history. The difference in family history was significant



between the case and the control group ($P=0.006$).

Three cases and 60 healthy controls lacked hypertension. There was a significant difference in the mean blood pressure between cases and controls ($P<0.001$). In addition, 3.8% of the women ($n=28$) and 96.2% of men ($n=57$) in the case group were smokers and the difference was significant ($P=0.031$).

We found a significant relationship between diabetes mellitus and CAD ($p<0.001$). Although there was an association between mean total bilirubin and diabetes in both groups, this relationship was not significant ($p=0.127$). There was a weak inverse correlation between HDL-cholesterol and bilirubin in men and women (women: $r=-0.008$, men: $r=-0.073$).

We evaluated the relationship between total bilirubin and smokers (0.597 ± 0.39) and non-smokers (0.572 ± 0.52) but found no significant relationship.

The median of total serum bilirubin was significantly lower in women (0.54 ± 0.416 mg/dl) than men (0.63 ± 0.441 mg/dl) ($p=0.001$). Table 1 presents clinical and biological parameters in case and control groups.

Table 1: Clinical and laboratory characteristics of cases and controls

	Patients (n=85)		Controls (n=92)		P
Age (year)	58.62±6.42		56.37±7.13		0.029
Sex (male, female)	male	female	male	female	
	57	28	39	53	0.001
BMI (kg/m ²)	28.54 ±4.64		28.46± 5.19		0.907
Family history	yes	no	yes	no	
(yes-no)	42	43	27	65	0.006
Hypertension	yes	no	yes	no	
(yes-no)	82	3	32	60	0/001<
Smoking	Yes	no	yes	no	
(yes-no)	26	59	20	72	0.301
Diabetes	yes	no	yes	no	
(yes-no)	43	42	20	72	0/001<
Total bilirubin (mg/dl)	0/433±0/593		0/432±0/588		0.940
Indirect bilirubin (mg/dl)	0/363±0/365		0/343±0/375		0.842
Direct bilirubin (mg/dl)	0/091±0/228		0/110±0/213		0.320
TG (mg/dl)	129/92±185/33		132/9±173/4		0.548
Total cholesterol (mg/dl)	45/51±184/78		41/7±186/85		0.751
HDL-cholesterol (mg/dl)	11/33±41/65		12/11±47/63		0.001
LDL-cholesterol (mg/dl)	38/39±105/71		36/81±104/37		0.813

All values are expressed as mean±SD

Discussion

Serum bilirubin is a potential endogenous antioxidant that is associated with many oxidative stress-related diseases, including atherosclerosis, cancer, neurodegenerative disease, etc [14]. Perlstein et al reported an inverse association between bilirubin and atherosclerosis [15]. Atherosclerosis is a chronic inflammatory disease associated with some endothelial changes. The process starts with a response to endothelial injury. Endothelial dysfunction is characterized by dysfunction and lack of endothelium. Endothelial regeneration defects prepare the grounds for atherosclerotic inflammation and wound formation, known as atherosclerotic plaque [16]. On the other hand, bilirubin, as the final product of heme catabolism, has potential antioxidant properties [7,9,17].

Heme oxygenase that catalyzes the degradation of heme is induced by oxidative stress and has antioxidant properties. This enzyme has 3 isoforms: HO-1, HO-2, and HO-3. HO-1 is the most important isoform in the vascular



system [18]. The products of this reaction (biliverdin, carbon monoxide, and ferrous iron) have a potential protective role against atherosclerosis [19]. Previous studies suggest that different forms of bilirubin in blood circulation and its precursors like biliverdin have the capacity to excrete reactive oxygen species, suppression of LDL oxidation, and induce monocyte chemotaxis [20-22]. In addition to its antioxidant capacity, bilirubin can suppress VCAM-1, delay leukocyte migration among endothelial cells [23], and stop the proliferation of smooth muscle cells [24].

Hopkins et al [25] and Troughton et al [26] found no relationship between CAD and other vascular disorders with bilirubin and its concentration. However, Erdogan et al [27] proposed that high levels of bilirubin were an independent prognostic factor for cardiovascular diseases.

High levels of bilirubin are an independent prognostic factor for the development of coronary collaterals, which play an important role in blood supply to the ischemic area in patients with coronary occlusion [28]. It has been shown that the formation of coronary collaterals depends on the severity of myocardial ischemia and there is marked association between them [29]. Studies have revealed a negative relationship between the development of collateral vessels and cardiovascular risk factors like age, diabetes mellitus, metabolic syndrome, and obesity [30,31]. All these risk factors are associated with endothelial dysfunction. However, the increased blood flow to the injured area is associated with bilirubin levels [8]. New studies suggest that bilirubin, as a potential physiologic antioxidant, can provide marked protection against atherosclerosis and inflammation. Our study was a case-control study to compare the serum bilirubin level between patients with angiography confirmed CAD and participants with no angiographic findings as healthy controls.

We evaluated the role of gender in this study and found a significant relationship between sex and the incidence of CAD ($p=0.001$). Male gender is one of the most important risk factors of CAD [32].

In our study, the serum level of total bilirubin was higher in the case group as compared with the control group but the difference was not significant.

The level of total bilirubin was higher in men (in both the case and control groups) than women but the difference was not significant ($P=0.160$). Decreased levels of total bilirubin were not detected in our study. Lower levels of serum bilirubin in women may result from the effect of estrogens [32]. It is probably related to increased bilirubin production induced by UDP-glucuronyltransferase in the liver [33]. Estrogens also decreased LDL, increase HDL, and decrease LDL oxidation [34]. Therefore, the potential effect of female sex steroids through decreasing serum bilirubin is masked by the beneficial effects of estrogens.

Another explanation for sex-related differences in total bilirubin may be different risk factors in men and women. Only 3.8% of women but 96.2% of men were smokers. The concentration of total bilirubin was lower in smokers (0.572 ± 0.520) versus non-smokers (0.597 ± 0.397) although the difference was not statistically significant ($P=0.734$). Schwertner et al reported an inverse correlation between smoking and total serum bilirubin concentration in cases and controls [9] while Endler et al [35] found that the serum concentration of total bilirubin was significantly lower in smokers versus non-smokers.

We detected a significant correlation between total bilirubin and HDL-cholesterol in men ($r=0.14$, $p=0.001$) but not in women ($r=-0.05$, $p=0.4$). Moreover, a significant difference was observed in mean HDL-cholesterol between the case and control groups ($p=0.001$). Researchers have shown that plasma bilirubin is directly associated with the HDL-cholesterol protective factor [36]. It is obvious that high levels of HDL-cholesterol are seen in healthy individuals because its increase is associated with decreased risk of CAD. Total bilirubin can be effective in CAD prevention through increasing HDL-cholesterol and decreasing inflammation [37]. Ghem et al [38] reported a negative association between total bilirubin concentration and the prevalence of CAD. Schwertner et al reported the same correlation between total bilirubin and CAD incidence (9). However, our results did not indicate any significant relationship between total bilirubin concentration and occurrence of CAD ($p=0.94$).

Total cholesterol was non-significantly higher in the control group versus the case group, probably due to the consumption of lipid lowering agents. There is no evidence that lipid lowering agents can affect bilirubin.

We found a significant difference in hypertension between cases and control ($P<0.001$). In fact, CAD is a multifactorial disease and therefore risk factors like hypertension and diabetes mellitus may be effective in its progression.



Although bilirubin alone is not a predictive molecule for CAD, it is important to conduct more studies to find new markers that can be used along with the existing markers for diagnosis and prognosis, because new markers may be effective in prevention and treatment.

Conclusion

The results of our study showed the importance of bilirubin as an index for predicting CAD. Our findings agree with some previous reports and disagree with some others.

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