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The Relation of Serum Bilirubin Level With Coronary Artery Disease Based on Angiographic Findings

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Abstract

Objective: Lipid oxidation and generation of free radicals are important factors contributing to the formation of atherosclerotic plaque. Bilirubin is supposed to play a protective role against atherosclerosis, coronary artery diseases (CAD) and inflammation for its strong antioxidant property. Thus, this study aims at investigating the relationship of bilirubin level with the severity and type of coronary artery stenosis (CAS) in different patient groups.

Materials and Methods: In this cross-sectional study 200 consecutive patients, who underwent elective angiography in Madani Heart hospital, Tabriz, Iran, were selected and their blood samples were measured for total, direct, and indirect bilirubin level, with Diazo method using colorimetric technique. Following angiography, comparisons were made between the severity and location of CAS and therapeutic follow-up plan with total, direct, and indirect bilirubin level.

Results: Of 200 studied patients, 129 (64.5%) and 71 (35.5%) subjects were male and female, respectively. The cases were classified into 5 subgroups based on angiography results as follows: 59 (29.5%) cases with normal angiography, 11 cases (5.5%) with minimal CAD, 56 cases (28%) with single vessel involvement, 35 (17.5%) cases with two vessel involvement and 39 cases (19.5%) with three vessel involvement. The mean total bilirubin level was 1.47 ± 0.8 mg/dl, 1.27 ± 0.12 mg/dl, 1.27 ± 0.06 mg/dl, 1.6 ± 0.04 mg/dl and 0.98 ± 0.05 mg/dl, respectively for the cases with above order. The mean difference in serum total bilirubin between normal angiography group and three-vessel involvement group was 0.49 mg/dl ($P < .0001$). There was a significant inverse relation between bilirubin level (total, direct and indirect) and number of involved vessels and involvement intensity increased as serum bilirubin level decreased. Severity of coronary arteries stenosis as well as the number of involved vessels increased as serum bilirubin level decreased.

Conclusion: According to results, there was a significant inverse relation between serum bilirubin level and coronary involvement (type and intensity). Higher bilirubin serum levels played a protective role against CAD, even in the presence of other risk factors. Therefore, bilirubin level can be used as a predictor of CAD in the future.

Keywords: Angiography, Atherosclerosis, Bilirubin, Coronary vessels

Introduction

Coronary artery diseases (CAD) is still the major prevailing cause of mortality among advanced countries. On the other hand, the number of CAD victims is continuously increasing in developing countries. The remarkable prevalence of cardiovascular diseases in today's society highlights the necessity of the identification of risk factors and screening of vulnerable individuals in using preventive and treatment methods. Although various main risk factors have been identified for atherosclerosis, including hypertension (HTN), hyperlipidemia, diabetes mellitus (DM), smoking, etc., it seems that there are other factors increasing the chance of CAD (1). However, there are other factors, like plasma bilirubin level, with protective and preventive properties against coronary atherosclerosis (2-8).

The development of coronary atherosclerosis is associated with lipid oxidation and generation of free radicals. Eventually, atherosclerosis and inflammation are associated with the formation of oxygen and peroxyl radicals (1,9).

Several mechanisms have been attributed to antiatherogenic property of bilirubin. The first protective effect of bilirubin relates to the antioxidant property of bilirubin, which prevents lipid oxidation, especially Low-density lipoprotein (LDL), and inhibits free radical-induced damages. The second protective effect relates to the anti-complement and anti-inflammation properties of bilirubin. This effect is introduced through different forms of bilirubin including direct bilirubin (10-15).

Higher concentration of bilirubin, however, may play a protective role through varying other effective compounds in the generation of such bilirubin as hemebiliverdin and iron (16). Lower serum bilirubin level has been proven to

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be associated with endothelium and microvascular malfunction (16,17).

Serum bilirubin level has an inverse relation with the prevalence of metabolic syndrome in adults; in that, the higher levels of bilirubin in adults and children with metabolic syndrome are associated with the improvement of insulin resistance (18-20).

A study on patients with Gilbert syndrome, who normally have a higher serum bilirubin level, showed that they were less prone to CAD (2). Another study found that higher serum bilirubin is associated with decreased risk for early familial coronary artery disease (11).

The aim of this study is to evaluate the difference of bilirubin level between different groups of patients based on the number of involved vessels and the intensity of CAD, employing angiography. This substance may be used as a factor to predict the intensity of CAD and to determine the type of therapy and cardiovascular risks.

Materials and Methods

This is a cross-sectional study, where 200 patients (aged 40-60 years) visiting Tabriz Madani Heart hospital for elective coronary angiography were included. Patients with hepatocellular disorders, acute coronary syndrome in past three months, liver problem (Liver Function Tests [LFT] >2 fold normal level), kidney problem (creatinine >2 mg/dl), malignant disease, erythrocyte diseases, connective tissue diseases and history of alcohol consumption were excluded from the study.

Prior to angiography, total, direct and indirect bilirubin levels in the blood samples of all cases were measured by diazo method (diazotized sulfanilic acid) and with colorimetric technique. Moreover, the level of total cholesterol and triglyceride were measured using enzymatic colorimetric method.

Angiography films were studied separately by two interventional cardiologists of the center. The intensity of CAD was expressed in percentage and the number of involved vessels was recorded as follows:

1. Less than 50%
2. 50%-69%
3. 70%-89%
4. Equal or greater than 90%
5. Chronic total occlusion

First of all, the subject and purpose of the study were explained to the patients and their questions concerning the study were replied. The study had no additional cost to patients and angiography was a routine procedure of treatment. The written consent of all patients was obtained and the study was confirmed by the ethics committee of the university.

Categorical variables, expressed as percentages, were compared by Fisher or χ^2 exact tests. Continues data, shown as mean±SD, were compared by Mann-Whitney U test and independent sample *t* test. Multivariate regression analysis was performed to determine the independent variables for predicting adverse events. *P* values less than 0.05 were considered to indicate statistical significance. Data recording and analysis were performed using SPSS version 17.0 software (SPSS Inc., Chicago, IL).

Results

Of 200 patients, 129 (64.5%) and 71 (35.5%) subjects were male and female, respectively. The average age of subjects was 58.23 ± 0.24 years (min = 40 and max = 60). The mean age of male and female cases was 57.91 ± 0.34 and 58.82 ± 0.32 years, respectively (min = 40, max = 60). Table 1 shows the history of previous risk factors of cardiovascular disease.

Following angiography, the cases were classified into following five groups based on
the obtained results:

1. 59 patients (29.5%) had normal angiography
2. 11 cases (5.5%) with minimal CAD problem
3. 56 cases (28%) with single vessel involvement
4. 35 cases (17.5%) with two coronary arteries stenosis
5. 39 cases (19.5%) with three coronary vessels stenosis

Table 2 illustrates total, direct and indirect bilirubin levels in the patients by angiography results. Table 3 illustrates the same items by involvement intensity.

However, the cases were analyzed in terms of normal, single vessel disease and multi vessel disease groups. According to results, the single vessel disease and multi vessel disease groups were significantly different in total, direct and indirect bilirubin levels (*P* values were .048, .044 and .025, respectively) (Figure 1).

Regarding the role of confounding factors in the developing of CAD, regression analysis was conducted in the presence of such risk factors such as DM, HTN, smoking, family history, gender, and total, direct and indirect bilirubin levels. Results indicate that bilirubin independently influences the number of involved vessel and intensity of CAD.

Table 1. History of Previous Risk Factors of Cardiovascular Disease

Risk Factors	Normal	Minimal CAD	Single VD	2VD	3VD	P value
Smoking	11 (18.6)	1 (9.1)	15 (26.8)	14 (40)	18 (46.2)	.01
Hypertension	12 (20.3)	2 (18.2)	19 (33.9)	13 (37.1)	26 (66.7)	<.0001
Diabetes	15 (25.4)	2 (18.2)	7 (12.5)	12 (34.3)	16 (41)	.02
Family History	3 (51)	0 (0)	3 (5.4)	5 (14.3)	0 (0)	.08
History of Previous MI	0 (0)	0 (0)	11 (19.6)	19 (54.3)	9 (23.1)	<.0001

Abbreviation: CAD, coronary artery disease; MI, myocardial infarction.
Data are presented in frequency (percentage).

Table 2. Laboratory Findings About the Amount of Serum Bilirubin (Total, Direct, Indirect) in Patients Based on the Results of Angiography

Bilirubin	Normal	Minimal CAD	Single VD	2VD	3VD	*P* Value
Total	1.47 ± 0.08	1.2 ± 0.12	1.27 ± 0.06	1.16 ± 0.04	0.98 ± 0.05	< .0001*
Direct	0.35 ± 0.02	0.23 ± 0.02	0.27 ± 0.01	0.19 ± 0.02	0.22 ± 0.02	< .0001*
Indirect	1.1 ± 0.05	0.97 ± 0.09	0.98 ± 0.04	0.94 ± 0.02	0.75 ± 0.04	< .0001*

Data are presented as Mean ± SD.

Table 3. Levels of Bilirubin (Total, Direct, Indirect) in Patients According to the Severity of Coronary Artery Disease

Bilirubin	Severity of coronary Artery					*P* Value
	<50	50%– 69%	70%-89%	90%	10±0 %	
Total	1.47±0.08	1.18 ± 0.11	1.33 ± 0.13	1.14 ± 0.06	1.13 ± 0.04	.002*
Direct	0.35±0.02	0.22 ± 0.02	0.33 ± 0.03	0.22 ± 0.02	0.24 ± 0.01	.001*
Indirect	1.11± 0.05	0.95 ± 0.09	0.93 ± 0.13	0.92 ± 0.05	0.88 ± 0.03	.007*

Data are presented as Mean ± SD.

Discussion

This was a cross-sectional study aiming at comparing serum bilirubin level with the number and intensity of CAD in patients who underwent angiography.

According to Table 2, there is a significant inverse relation between total, direct and indirect bilirubin levels with the intensity of CAD; in that, the intensity of involvement and the number of involved vessels increase as serum bilirubin level decreases. The difference in the mean total bilirubin between normal angiography group and three-vessel involvement group was 0.49 mg/dl ($P < .0001$). A number of previous studies have reported an inverse relation between the total bilirubin concentration and CAD prevalence (5,7,11,21).

Our study, however, found a significant inverse relation between serum bilirubin level and the intensity of CAD; in that, lower serum bilirubin levels were associated with more extensive CAD ($P = .002$). So it seems that higher bilirubin level has a protective effect against coronary artery stenosis (CAS).

Our study showed a higher level of mean total bilirubin in males (1.3 ± 0.04) in comparison to females (1.15 ± 0.05), but the difference was not significant ($P = .4$). Lower levels of bilirubin in females may be attributed to the influence of estrogens (22). This may relate to the increased secretion of bilirubin through the induction of UDP-glucuronil transferasa enzyme in liver (23). Estrogens also decrease LDL level, increase HDL level and reduce LDL oxidation (24). Therefore, the potential effect of sex steroid hormones in females is obscured via reduced serum bilirubin induced by the beneficial effect of estrogens. However, different risk factors in males and females are another gender-related cause of difference in bilirubin concentrations of men and women.

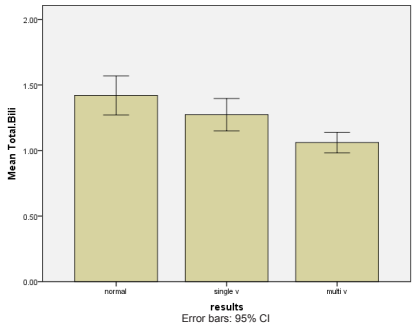

Figure 1. Regression analysis of total Bill with other risk factors based on severity of coronary artery disease (CAD).

This study investigated the CAD incidence in terms of sex and found a significant relation between sex and CAD ($P = .005$, correlation factor = 0.19), which is consistent with previous studies. Maleness is an important risk factor for CAD (22).

In our study, only 14.6% of females were smoker; whereas, this rate was 85.4% in males. Total bilirubin concentration was slightly higher in smokers (1.24 ± 0.08) compared with non-smokers (1.24 ± 0.03), but the difference was not significant ($P = .31$). Schwertner et al reported an inverse relation between smoking and total bilirubin concentration in normal people and patients (5). Endler et al (25), however, reported that total bilirubin concentration was significantly lower in smokers as compared to non-smokers, contradicting our findings (25).

This study also showed a significant relation between total bilirubin, total cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride in that 10 mg/dl increase in total cholesterol may increase the number of involved vessels by 1.32. Researchers have suggested that plasma bilirubin is directly correlated with the protective factor HDL-cholesterol (26). Total bilirubin can play an effective role in the prevention of CAD through increasing HDL-cholesterol and decreasing inflammation (27). In addition, it has been shown that total serum bilirubin level was independently and inversely associated with the severity of coronary atherosclerosis in patients with stable CAD. Total bilirubin level was inversely correlated with CRP. These results suggest that higher serum total bilirubin level may exhibit an anti-inflammatory effect in the atherosclerotic process (28).

This study showed a significant relation between ejection fraction with total serum bilirubin ($P < .0001$) and CAS intensity ($P < .0001$), in that the ejection fraction showed a descending trend as serum bilirubin level decreased.

Coronary arteries atherosclerosis is a multifactorial problem. Although bilirubin is not considered as a predictive

marker for CAD by itself, studies on introducing new markers that can be used together with current risk factors for diagnosis and prognosis purposes is very importance. This is because new markers may be effective in the prevention and treatment of such problems.

Previous studies suggest that different forms of bilirubin in blood circulation and its precursors, like biliverdin, are capable of eliminating various reactive oxygen species (ROS), and controlling LDL oxidation and chemotactic monocytes (10,13,29).

In addition to its antioxidant property, bilirubin is able to inhibit vascular cell adhesion molecule (VCAM-1), delay the migration of leucocytes from endothelial cells, and terminate the reproduction of smooth muscle tissue cells (8). Several study proved an inverse relation between CAD and other vascular diseases with bilirubin and its concentration (7,11,30,31). Erdogan et al suggested that high levels of bilirubin can serve as an independent predictive factor for cardiovascular diseases (32). In one study it has been shown that total serum bilirubin levels have a prognostic association with in-hospital major adverse cardiac events (MACEs) in male patients with myocardial infarction (33).

Collateral arteries have a vital role in patients with occlusive coronary arteries through perfusion to the ischemic areas (32,34,35). According to studies, there is a negative relation between collateral arteries growth and cardiovascular risk factors like age, diabetes mellitus, metabolic syndrome and obesity (36,37). These risk factors result in related endothelium malfunction but there is a relation between increased perfusion of the damaged area and bilirubin level (17).

Conclusion

This study showed that there is a significant inverse relation between serum bilirubin level and the number of involved vessels and involvement intensity; in that higher serum bilirubin levels have a protective role against CAD, even in the presence of other risk factors. Therefore, bilirubin level can serve as a predictive factor, together with other influential factors, of the number of involved coronary arteries and involvement intensity.

Ethical issues

Ethical considerations was considered in this study and all participants are not be identifiable in any way by reader of the final report or dissertation.

Conflict of interests

We declare that we have no conflict of interests.

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