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Original Research Article

A study on serum bilirubin levels in coronary artery disease patients

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ABSTRACT

Background: The 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 dam-ages. Lower serum bilirubin level has been proven to be associated with endothelium and microvascular malfunction. The aim of the present study was to assess the association between serum bilirubin levels and coronary artery disease in comparison with controls without coronary artery disease.
Methods: A cross-sectional study was conducted for a period of a one year in our medical college hospital. Patients with evidence of coronary artery disease for not more than 10 years of duration confirmed by ECG, ECHO and other previous case records were taken as cases. Controls were selected matched with age, gender and other co-morbid conditions. Total of 200 subjects were included in the study with 100 cases and 100 controls. General and systemic examination was conducted on all study subjects including laboratory investigations like complete blood count, renal function test, lipid profile, viral markers such as HBsAG, HCVIgM and liver function test which includes total bilirubin, direct and indirect, liver enzymes, albumin and globulin levels. A 12 lead ECG and a transthoracic echocardiogram was performed for all patients.
Results: The various liver function test parameters were compared between the cases and controls it was found that the serum bilirubin levels which includes total bilirubin, direct bilirubin and indirect bilirubin was found to be lower among the case group compared to the control group and this difference was found to be statistically significant ($p < 0.05$). A perfect linear correlation between the ejection fraction and serum bilirubin levels, as the ejection fraction decreases the serum bilirubin levels was also decreasing.
Conclusions: This study showed a significant association between the reduced serum bilirubin levels and the occurrence of CAD; therefore, bilirubin level can serve as a predictive factor, together with other influential factors for identifying a person at risk of developing coronary artery disease.

Keywords: Coronary artery disease, Ejection fraction, Risk factor, Serum bilirubin

INTRODUCTION

Coronary artery diseases (CAD) is still the major prevail¬ing cause of mortality among advanced countries. On the other hand, the number of CAD victims is continuously increasing in developing countries.[1] 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.[2] Although various main risk factors have been identified for atherosclerosis, including hypertension (HTN), hyperlipidemia, diabetes mellitus (DM), smoking, etc.,

for many years, the bile pigment bilirubin was considered to be only a toxic waste product formed during heme catabolism.[3-8] Recent evidence, however, suggests that bilirubin acts as a potent physiologic antioxidant that may provide important protection against arteriosclerosis, coronary artery disease (CAD), and inflammation.[9-11]

The antioxidant capacity of bilirubin and its potent ability to scavenge peroxyl radicals have led to the concept that mildly increased circulatory bilirubin may have a physiologic function to protect against disease processes that involve oxygen and peroxyl radicals.[3] Indeed, inverse correlations between the presence of CAD and total bilirubin concentrations in the circulation were reported recently in several independent studies.[5]

Additionally, plasma bilirubin correlates inversely with several established risk factors for CAD, including smoking, increased LDL-cholesterol, diabetes, and obesity, but is directly proportional to the protective factor HDL-cholesterol.[12] The effect of bilirubin on the risk of cardiovascular disease is apparent in men but is less clear in women.[13]

The 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. Lower serum bilirubin level has been proven to be associated with endothelium and microvascular mal¬function.[14]

As of today, very few studies in India had been conducted to prove the association between serum bilirubin levels and coronary artery disease and so the present study was undertaken to assess the association between these two variables by comparing it with a control group.

The aim of the present study was to assess the association between serum bilirubin levels and coronary artery disease in comparison with controls without coronary artery disease.

METHODS

A cross-sectional study was conducted for a period of a one year in our medical college hospital. The study was started after getting the clearance from the institutional ethical committee.

Patients with evidence of coronary artery disease for not more than 10 years of duration confirmed by ECG, ECHO and other previous case records were taken as cases.

Patients with symptoms of congestive cardiac failure, chronic kidney disease, chronic liver disease, autoimmune diseases, COPD and malignancy were excluded from the study. Controls were selected matched with age, gender and other co-morbid conditions. Total of 200 subjects were included in the study with 100 cases and 100 controls. Informed consent was obtained from all the subjects involved in the study.

A complete socio-demographic details was obtained from all the subjects including the dietary habits and smoking/alcohol history. General and systemic examination was conducted on all study subjects including laboratory investigations like complete blood count, renal function test, lipid profile, viral markers such as HBsAG, HCVIgM and liver function test which includes total bilirubin, direct and indirect, liver enzymes, albumin and globulin levels. A 12 lead ECG and a transthoracic echocardiogram was performed for all patients.

Total serum bilirubin was measured in the laboratory by spectrophotometry method. In the Jendrassik-Grof allied methods, total bilirubin is reacted with diazotized sulfanilic acid in an acidic medium to form azobilirubin. The absorbance of the azo pigment is then measured as direct bilirubin and the total bilirubin is measured after treatment with alkaline tartrated solution, which shifts the maximum absorption of the azo pigment towards longer wavelength.

Statistical analysis

All the data were entered and analysed using SPSS version 22. Mean and standard deviation was derived for all the parametric variables and the parametric variables between the two groups (cases and controls) were compared using unpaired student T test and comparison between the frequencies was done by using chi-square test considering $p < 0.05$ as statistically significant.

RESULTS

The entire study subjects were divided into two groups of 100 cases (with CVD) and 100 controls.

Table 1 shows the age and sex wise distribution of the study subjects. It is seen from the table that majority of the subjects were in the age group between 55 and 65years. The minimum age was 42 and the maximum age was 73years.

The mean age among the cases and controls group were between 63 and 65 years. The male subjects were more than the females with a male: female ratio of 2:1 among both the cases and controls. So, it shows that the cases and controls did not show any significant difference with respect to age and gender which implies that the controls were age and sex matched.

The most common risk factors for CVD like diabetes, hypertension, smoking, obesity and family history of CVD was found to be slightly higher among the cases than the control groups but it was not found to be statistically significant and it proves that the controls

were matched for almost all the risk factors for CVD except for dyslipidemia which was found to be significantly higher among the CVD patients than the controls (Table 2).

Table 1: Age and sex wise distribution of the study subjects.

Age group	Cases		Controls		P value
	Males	Females	Males	Females	
40 - 45	4 (6%)	1 (2.9%)	5 (7%)	0	
46 - 50	7 (10.6%)	1 (2.9%)	8 (11.2%)	2 (6.8%)	
51 - 55	3 (4.5%)	2 (5.8%)	3 (4.2%)	2 (6.8%)	
56 - 60	21 (31.8%)	6 (17.6%)	23 (32.3%)	9 (31%)	
61 - 65	16 (24.2%)	15 (44.1%)	14 (19.7%)	12 (41.3%)	0.519
66 - 70	10 (15.1%)	5 (14.7%)	9 (12.6%)	3 (10.3%)	
>70	5 (7.5%)	4 (11.7%)	9 (12.6%)	2 (6.8%)	
Total	66 (100%)	34 (100%)	71 (100%)	29 (100%)	
Mean±SD	63.8±7.9	64.6±8.1	62.3±8.4	63.7±8.6	

Table 2: Prevailing risk factors for CVD among study subjects.

Risk factors	Cases (n=100)	Controls (n=100)	P value
Diabetes	33 (33%)	28 (28%)	0.318
Hypertension	55 (55%)	43 (43%)	0.154
Smoking	38 (38%)	35 (35%)	0.863
Family history of CVD	41 (41%)	32 (32%)	0.281
Obesity	27 (27%)	19 (19%)	0.182
Dyslipidemia	63 (63%)	42 (42%)	0.003

Table 3: Distribution of the cases based on their duration of CVD.

Duration of CVD	Frequency	Percentage	Mean±SD
<3 years	21	21%	4.2±2.6
3 - 5 years	48	48%	
5 - 7 years	22	22%	
>7 years	9	9%	
Total	100	100%	

The duration of CVD among the cases varied from 2 years to 9 years with majority of the subjects' duration was between 3 and 5 years and the mean duration was 4.2 years. The patients' CVD status was confirmed by history, ECG findings and ECHO reports (Table 3).

The various liver function test parameters were compared between the cases and controls it was found that the serum bilirubin levels which includes total bilirubin, direct bilirubin and indirect bilirubin was found to be lower among the case group compared to the control group and this difference was found to be statistically significant, whereas the other parameters like SGOT, SGPT and GGT levels did not show much difference between the case and control groups and the difference in values were not statistically significant (Table 4).

Table 4: Comparison of the liver function test parameters between the CVD patients and the controls.

LFT	Cases (mean±SD)	Controls (mean±SD)	P value
Total bilirubin	0.89±0.07	1.23±0.21	<0.001
Direct bilirubin	0.23±0.04	0.48±0.08	<0.001
Indirect bilirubin	0.64±0.09	0.82±0.11	<0.001
SGOT (IU/L)	25	28	0.571
SGPT (IU/L)	29	33	0.219
GGT (IU/L)	31	29	0.313

p value derived by applying student T test

For all the CVD patients an echocardiogram was performed and their ejection fraction was recorded and it was correlated with the serum bilirubin levels, authors found a perfect linear correlation between the ejection fraction and serum bilirubin levels, as the ejection fraction decreases the serum bilirubin levels was also decreasing and all the serum bilirubin parameters were found to be very low in patients with ejection fraction <50% when compared to patients with ejection fraction >60% and this association was found to be statistically significant (p <0.05) (Table 5).

DISCUSSION

Atherosclerosis is considered to be the most common underlying cause for the coronary artery disease (CAD), which is the major cause of mortality worldwide both in

developed and developing countries.[15] Whereas on the other hand antioxidants are the predominant adaptive responses by the arterial vasculature in response to the oxidative stress thereby preventing the atherosclerosis.[3] Bilirubin, being a toxic waste product formed during heme catabolism is in fact a potent physiological antioxidant that provides important protection against atherosclerosis and inflammation.[16]

A particular enzyme namely the heme oxygenase (HO) is a stress inducible enzyme in the heme catabolism which plays an important role in cell defense mechanism against oxidative injury.

The products of the catabolic reaction, i.e. bilirubin, carbon monoxide and iron have a protective role. The other important role of bilirubin, the natural antioxidants are the inhibition of vascular cell adhesion molecule VCAM-1 preventing the proliferation of the smooth muscle cells and the transendothelial migration of the leucocytes.[17]

Table 5: Association and correlation between serum bilirubin levels and the ejection fraction among the CVD patients.

Serum bilirubin	>60 % (n= 28)	50-60 % (n= 51)	<50 % (n=21)	P value	r value
Total bilirubin (mean±SD)	1.1±0.32	0.84±0.23	0.73±0.18	<0.001	0.915
Direct bilirubin (mean±SD)	0.41±0.10	0.32±0.08	0.21±0.07	<0.001	0.899
Indirect bilirubin (mean±SD)	0.72±0.24	0.66±0.09	0.60±0.05	<0.001	0.934

Plasma bilirubin inversely correlated with risk factors of CAD- smoking, diabetes and obesity, thus emphasizing the oxidative stress underlying in them, but in present study authors did not observed such correlation as authors matched most of the risk factors between the cases and controls. Inverse relationship between the presence of CAD and circulatory total bilirubin was first observed by Schwertner et al.[5]

Male gender is one of the most important risk factors for CAD. The same was found in our study. Males were predominant in cases and so we matched the controls accordingly. Authors also matched the cases and controls with regards to age and other comorbidities thereby removing the confounding factors responsible for the lowering of bilirubin as a result of the oxidative stress and other mechanisms.[18]

Present study found a significant inverse association between serum bilirubin and CAD in comparison with control, bilirubin levels found to be significantly lower in CAD patients in comparison with the controls ($p < 0.001$) and a similar type of results was also quoted by Taban SM et al, and in their study they had also found a significant association between the bilirubin levels and the severity of CAD by doing an angiogram.[19] So it seems that higher bilirubin level has a protective effect against coronary artery stenosis (CAS).

The present study among 100 CAD patients and 100 healthy controls confirmed the results of several previous epidemiological studies that low serum bilirubin levels were associated with increased risk for coronary events.[7,20,21] A recent study in patients with peripheral arterial disease (PAD) revealed similar results showing a clear association between low bilirubin concentrations and PAD.[22]

Present study showed a higher level of mean total bilirubin in males in comparison to females, but the difference was not statistically significant, however lower levels of bilirubin in females may be attributed to the influence of estrogens. This may relate to the increased secretion of bilirubin through the induction of UDP-glucuronil transferasa enzyme in liver. Estrogens also decrease LDL level, increase HDL level and reduce LDL oxidation.[23] Recently, low serum bilirubin levels have been proposed as a useful biomarker to predict cardiovascular risk and suggests that bilirubin acts as a potent physiologic antioxidant and anti-inflammatory agent. Studies have shown that elevated serum bilirubin concentrations provide important protection against atherosclerotic diseases.[3] Several authors have suggested that bilirubin plays a potential role in inhibition of lipid oxidation.[24] An inverse correlation between the presence of coronary artery disease, peripheral arterial disease, carotid intima-media thickness and bilirubin has been reported in several studies. Subnormal levels of plasma bilirubin are associated with premature coronary artery disease and cardiovascular morbidity.[25] In a previous study, the 3-year incidence of coronary artery disease was significantly lower in patients with Gilbert syndrome.[26]

This study showed a significant relation between ejection fraction with total serum bilirubin the ejection fraction showed a descending trend as serum bilirubin level decreased and a similar type of results was also quoted by Taban SM et al.[19]

One of the major limitations of this study is it was not conducted in a prospective manner to exactly identify the causal association between bilirubin levels and CAD, it was only a cross-sectional design and secondly, authors didn't associate the severity of CAD with bilirubin levels by doing an angiogram study.

CONCLUSION

This study showed a significant association between the reduced serum bilirubin levels and the occurrence of CAD; therefore, bilirubin level can serve as a predictive factor, together with other influential factors for identifying a person at risk of developing coronary artery disease. Further studies with a larger sample and a prospective design would throw more light on bilirubin being considered as an independent risk factor for coronary artery disease.

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