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Original research article

An Investigation to Compare the Blood Bilirubin Levels of Patients with Coronary Artery Disease to Healthy Controls.

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Abstract

Aims: 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 case-control study was conducted in the Department of Geriatrics- medicine, Darbhanga Medical College and Hospital, Laheriasarai, Darbhanga,Bihar, India for 18 months. Total of 160 subjects were included in the study with 80 cases and 80 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.

Results: The mean age among the cases male and female respectively was 73.76±8.2 and 74.85±8.3 and controls group were 72.85±8.4 and 73.38±8.5 years male and female respectively. 60% were male and 40% female in case group and 63.75% patients were male and 36.25% patients were female in control. 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. The mean duration of CVD was 4.9±2.8 years. 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 .we 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 ($p < 0.05$).

Conclusion: The relationship between the decreased serum bilirubin levels and the event of CAD; in this manner, bilirubin level can fill in as a prognostic factor, together with other significant factors for recognizing an individual who is in the peril of coronary artery disease.

Keywords: bilirubin, CVD, coronary artery

Introduction

The role of inflammation in cardiovascular disease (CVD) is established. Oxidative stress plays an important role in atherosclerosis, which is a chronic inflammatory response to vascular endothelial injury caused by a variety of factors promoting inflammatory cell entry and activation.[1] The recognition of bilirubin as an important endogenous anti-inflammatory and anti- oxidant molecule has increased in recent decades. Bilirubin affects atherosclerosis by several inhibiting mechanisms, including low-density lipoprotein oxidation, vascular smooth muscle cell proliferation, and endothelial dysfunction.[1] Mildly elevated circulating bilirubin levels seems to represent a promising target for prevention and reduction of the prevalence of CVD and other oxidative-stress disorders, including type 2 diabetes mellitus (T2DM) and cancer.[2] Accordingly, the role of bilirubin as a biological predictor in the risk assessment of chronic disorders, with increasing worldwide prevalence, is of considerable medical economic importance. Indeed, CVD represent a main cause of mortality and burden of disease.[3]

Recent meta-analysis has found an inverse association between total bilirubin levels and the risk of CVD, which is independent of established risk factors.[4] Thus, serum bilirubin level may be an independent marker for environmental and genetically determined CVD risk. The reported effects of bilirubin levels on an individual basis, however, have been inconsistent in the context of CVD. Increased bilirubin levels have been associated with greater protection against CVD in some studies[5,6], whereas other research indicates that higher levels of bilirubin have increased or null associations with CVD.[7,8] Epidemiologic studies have indicated that the total bilirubin level is inversely related to diabetes mellitus, hypotension, CAD and metabolic syndrome.[9,10] Atherosclerosis and inflammation are associated with free oxygen and peroxyl radicals' formation.[11,12] Arterial protective responses and adjustment against oxidative stress have important roles in atherosclerosis prevention.[13] 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.

Material and methods

A case control study was conducted in the Department of Geriatrics-Medicine, Darbhanga Medical College and Hospital, Laheriasarai, Darbhanga, Bihar, India for 18 months after taking the approval of the protocol review committee and institutional ethics committee.

Inclusion criteria

- Patients of 60 years and above
- Patients with evidence of coronary artery disease by ECG, ECHO

Exclusion criteria

- Patients with symptoms of congestive cardiac failure
- Chronic kidney disease,
- Chronic liver disease, autoimmune diseases,
- COPD and malignancy

Controls were selected matched with age, gender and other co-morbid conditions. Total of 160 subjects were included in the study with 80 cases and 80 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. 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 25.0. 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 80 cases (with CVD) and 80 controls. Table 1 shows the mean age and sex distribution of the study subjects. Majority of the patients were above 65 years of age. The minimum age was 60 and the maximum age was 83 years. The mean age among the cases male and female respectively was 73.76±8.2 and 74.85±8.3 and controls group were 72.85±8.4 and 73.38±8.5 years male and female respectively. 60% were male and 40% female in case group and 63.75% patients were male and 36.25% patients were female in control group and 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.

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

Age group	**Cases=80**		**Controls=80**		P value
	Males=48	**Females=32**	**Males=51**	**Females=29**	>0.05
Mean±SD	74.8±7.9	75.6±8.1	73.3±8.4	74.7±8.6	

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 2: Prevailing risk factors for CVD among study subjects

Risk factors	**Cases (n=80)**	**Controls (n=80)**	**P value**
Diabetes	26 (32.5%)	22 (27.5%)	0.41
Hypertension	43 (53.75%)	33 (41.25%)	0.15
Smoking	29 (36.25%)	27 (33.75%)	0.74
Family history of CVD	33 (41.25%)	25 (31.25%)	0.32
Obesity	21 (26.25%)	15 (18.75%)	0.21
Dyslipidemia	50 (62.25%)	33 (41.25%)	0.003

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

Duration of CVD	Frequency	Percentage	Mean±SD
Below 3 years	16	20%	
3 - 5 years	40	50%	
5 - 7 years	17	21.25%	4.9±2.8
Above 7 years	7	8.75%	
Total	80	100%	

The duration of CVD among the cases varied from 1 years to 10 years with majority of the subjects' duration was between 3 and 5 years and the mean duration was 4.9±2.8 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	0.92±0.06	1.19±0.24	<0.001
Direct bilirubin	0.25±0.06	0.51±0.11	<0.001
Indirect bilirubin	0.67±0.11	0.85±0.15	<0.001
SGOT (IU/L)	16	29	0.51
SGPT (IU/L)	28	34	0.23
GGT (IU/L)	33	30	0.32

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).

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

Serum bilirubin	>60 % (n= 20)	50-60 % (n=43)	<50 % (n=17)	P value	r value
Total bilirubin (mean±SD)	1.3±0.29	0.87±0.18	0.75±0.22	<0.001	0.87
Direct bilirubin (mean±SD)	0.44±0.13	0.34±0.14	0.23±0.05	<0.001	0.77
Indirect bilirubin (mean±SD)	0.74±0.27	0.67±0.15	0.62±0.09	<0.001	0.85

Discussion

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. The remarkable prevalence of cardiovascular diseases in today's society high- lights 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 hyper- tension (HTN), hyperlipidemia, diabetes mellitus (DM), smoking, etc., it seems that there are other factors increasing the chance of CAD.[14] 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.[15] 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 is the inhibition of vascular cell adhesion molecule VCAM-1 preventing the proliferation of the smooth muscle cells and the transendothelial migration of the leucocytes.[16]
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.[17]

Male gender is one of the most important risk factors for CAD. In this study the mean age among the cases male and female respectively was 73.76±8.2 and 74.85±8.3 and controls group were 72.85±8.4 and 73.38±8.5 years male and female respectively. 60% were male and 40% female in case group and 63.75% patients were male and 36.25% patients were female in control group and 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. we also matched 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 80 CAD patients and 80 healthy controls confirmed the results of several previous epidemiological studies that low serum bilirubin levels were associated with increased risk for coronary events.[20-22] A recent study in patients with peripheral arterial disease (PAD) revealed similar results showing a clear association between low bilirubin concentrations and PAD.[23] 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 transferase enzyme in liver. Estrogens also decrease LDL level, increase HDL level and reduce LDL

oxidation.[24] 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.[14] Several authors have suggested that bilirubin plays a potential role in inhibition of lipid oxidation.[25] 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.[26] In a previous study, the 3-year incidence of coronary artery disease was significantly lower in patients with Gilbert syndrome.[27] 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]

Conclusion

This study has demonstrated a critical relationship between the decreased serum bilirubin levels and the event of CAD; in this manner, bilirubin level can fill in as a prognostic factor, together with other significant factors for recognizing an individual who is in the peril of coronary artery disease.

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