

## ASSOCIATION BETWEEN SERUM BILIRUBIN LEVELS AND CORONARY ARTERY DISEASE: A CASE-CONTROL STUDY

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### Abstract

**Background:** Bilirubin is a strong antioxidant under physiological conditions and suppresses the oxidation of lipids and low density lipoproteins and prevents free radical injury. Lesser serum bilirubin levels are associated with Protein Kinase C activation, inflammation, and oxidative stress, which are known mediators of endothelial and microvascular dysfunction. **Aim:** 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 Medicine, Nalanda Medical College and Hospital Patna, Bihar, India from Jan 2019 to Jan 2020. 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 62.76±8.2 and 63.85±8.3 and controls group were 61.85±8.4 and 62.38±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 4.4±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:** 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.

### Introduction

Coronary artery disease (CAD) is one of the most common causes of death worldwide. In 2010, about 7 out of total 53 million deaths were due to ischemic heart disease.<sup>1</sup> Heart muscle as an oxidative tissue and with constant activity is one of the tissues susceptible to oxidative damage caused by reactive oxygen species such as superoxide, hydrogen peroxide and hydroxyl.<sup>2</sup> Oxidative stress plays an important role in the pathogenesis of degenerative diseases or chronic such as atherosclerosis, diabetes and cancer.<sup>3,4</sup> Oxidative stress as a result of an imbalance between the production of free radicals and reactive oxygen species on the one hand

and the antioxidant defence system on the other hand. In other words, aerobic biological systems in order to cope with free radicals and reactive oxygen species, antioxidant defence mechanisms designed to neutralize the deleterious effects of these factors attacker, or to a minimum.<sup>4</sup> New scientific studies have suggested that bilirubin is a potential physiological antioxidant that can be an important protective role against atherosclerosis, CAD and inflammation take over.<sup>5</sup> Many factors increase the risk of CAD, including low serum bilirubin level, which is associated with increased risk of CAD.<sup>6</sup> Several studies have shown that bilirubin, the final product of hem metabolism has potent antioxidant capacity.<sup>7</sup> A study

performed on healthy individuals. Dividing them according to serum bilirubin levels into 3 groups of low, intermediate and high, has shown that high bilirubin level prevents coronary flow reserve impairment, microvascular dysfunction and probably coronary atherosclerosis.<sup>8</sup> Epidemiologic studies have indicated that the total bilirubin level is inversely related to diabetes mellitus, hypotension, CAD and metabolic syndrome.<sup>9,10</sup> Atherosclerosis and inflammation are associated with free oxygen and peroxy radicals' formation.<sup>11,12</sup> Arterial protective responses and adjustment against oxidative stress have important roles in atherosclerosis prevention.<sup>13</sup> Studies have shown that different forms of circulating bilirubin and its precursor, biliverdin, have the ability to remove the reactive forms of oxygen. They also prevent the oxidation of low-density-lipoprotein particles and monocyte chemotaxis all which are considered as stages of atherosclerosis.<sup>14,15</sup> 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 medicine, Nalanda Medical College and Hospital Patna, Bihar, India from Jan 2019 to Jan 2020, after taking the approval of the protocol review committee and institutional ethics committee.

### Inclusion criteria

- 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

### Methodology

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 were 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 were compared using student t-test and comparison between the frequencies was done by using chi-square test and Pearson correlation coefficient 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 in the age group between 50-60 years. The minimum age was 39 and the maximum age was 69 years. The mean age among the cases male and female respectively was  $62.76 \pm 8.2$  and  $63.85 \pm 8.3$  and controls group were  $61.85 \pm 8.4$  and  $62.38 \pm 8.5$  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	
Mean±SD	63.8±7.9	64.6±8.1	62.3±8.4	63.7±8.6	>0.05

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%)	0.327
Hypertension	43 (53.75%)	33 (41.25%)	0.148
Smoking	29 (36.25%)	27 (33.75%)	0.855
Family history of CVD	33 (41.25%)	25 (31.25%)	0.266
Obesity	21 (26.25%)	15 (18.75%)	0.177
Dyslipidemia	50 (62.25%)	33 (41.25%)	0.002

Test applied: chi-square test

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

Duration of CVD	Frequency	Percentage
Below 3 years	16	20%
3 - 5 years	40	50%
5 - 7 years	17	21.25%
Above 7 years	7	8.75%
Total	80	100%
<b>Mean±SD</b>	<b>4.4±2.8</b>	

The duration of CVD among the cases varied from 1 year to 10 years with majority of the subjects' duration was between 3 and 5 years and the mean duration were 4.4±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	0.588
SGPT (IU/L)	28	34	0.205
GGT (IU/L)	33	30	0.306

Test applied: 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).

**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	0.921
Direct bilirubin (mean±SD)	0.44±0.13	0.34±0.14	0.23±0.05	<0.001	0.879
Indirect bilirubin (mean±SD)	0.74±0.27	0.67±0.15	0.62±0.09	<0.001	0.926

Test applied: Pearson Correlation coefficient

## 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. 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.<sup>16</sup> 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.<sup>17</sup> 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.<sup>18</sup>

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.<sup>19</sup>

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 62.76±8.2 and 63.85±8.3 and controls group were 61.85±8.4 and 62.38±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.<sup>20</sup>

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.<sup>21</sup> 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.<sup>22,23,24</sup> A recent study in patients with peripheral arterial disease (PAD) revealed similar results showing a clear association between low bilirubin concentrations and PAD.<sup>25</sup> 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.<sup>26</sup> 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.<sup>16</sup> Several authors have suggested that bilirubin plays a potential role in inhibition of lipid oxidation.<sup>27</sup> 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.<sup>28</sup> In a previous study, the 3-year incidence of coronary artery disease was significantly lower in patients with Gilbert syndrome.<sup>29</sup> 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.*<sup>21</sup>

### 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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