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Original Research Article

Determining the Association between Serum Bilirubin Levels and Coronary Artery Disease: A Case Control Study

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Abstract

Aim: 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: The cross-sectional study was conducted for a period of a one year in Ford hospital and research centre Pvt. Ltd, Patna, Bihar, India. The entire study subjects were divided into two groups of 50 cases (with CVD) and 50 controls.

Results: It was seen from the table that majority of the subjects were in the age group between 55 and 65 years. The minimum age was 43 and the maximum age was 75 years. 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 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 4.4 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.

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.

Keywords: Coronary Artery Disease, Ejection Fraction, Risk Factor, Serum Bilirubin.

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 antioxidant 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]

Bilirubin is a heme degradation product. During the catabolism of hemoglobin, heme is converted into biliverdin by heme oxygenase which is acted upon by biliverdin reductase to form bilirubin. In liver cells, bilirubin is changed to a conjugated form for secretion in bile juice. [9] At concentrations found in human plasma, bilirubin acts as an antioxidant to scavenge peroxyl radicals as efficiently as alpha-tocopherol. [10,11] Both free and bound forms of bilirubin can inhibit the oxidation of low-density lipoproteins at physiological concentrations. [12,13] Oxidation of low-density lipoproteins is an important initial step in atherogenesis that can stimulate platelet aggregation and can alter vasomotor properties. [11] Gilbert's syndrome patients have a reduced risk of cardiovascular disease (CVD) which is associated with increased bilirubin levels and altered lipid and inflammatory profiles. [14] At pathological levels, unconjugated bilirubin inhibits cytotoxic T cell activity and proliferative responses to human peripheral blood mononuclear cells in patients with neonatal or obstructive jaundice. [15]

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.

Materials and Methods

The cross-sectional study was conducted in department of Medicine for a period of a one year in Ford hospital and research centre Pvt. Ltd, Patna, Bihar, India. The entire study subjects were divided into two groups of 50 cases (with CVD) and 50 controls.

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, HCV IgM 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

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

Age group	Cases		Controls		P value
	Males	Females	Males	Females	
40-45	2	1	2	0	0.624
46-50	3	1	4	1	
51-55	1	1	1	1	
56-60	11	3	12	4	
61-65	8	8	7	6	
66-70	5	2	5	1	
>70	2	2	5	1	
Total	32	18	36	14	
Mean±SD	64.9±8.6	65.5±8.2	63.7±8.4	62.8±8.5	

It was seen from the table that majority of the subjects were in the age group between 55 and 65 years. The minimum age was 43 and the maximum age was 75 years.

Table 2: Prevailing risk factors for CVD among study subjects

Risk factors	Cases (n=100)	Controls (n=100)	P value
Diabetes	16 (32%)	14 (28%)	0.323
Hypertension	28 (56%)	22 (44%)	0.160
Smoking	20 (40%)	18 (36%)	0.832
Family history of CVD	21 (42%)	16 (32%)	0.272
Obesity	14 (28%)	10 (20%)	0.188
Dyslipidemia	32 (64%)	21 (42%)	0.007

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

Duration of CVD	Frequency	Percentage	Mean±SD
<3 years	11	22	4.4±2.8
3 -5 years	24	48	
5 -7 years	11	22	
>7 years	4	8	
Total	50	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 4.4 years. The patients' CVD status was confirmed by history, ECG findings and ECHO reports.

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	0.88±0.07	1.25±0.22	<0.001
Direct bilirubin	0.24±0.06	0.49±0.07	<0.001
Indirect bilirubin	0.65±0.08	0.84±0.12	<0.001
SGOT (IU/L)	25	28	0.571
SGPT (IU/L)	29	33	0.219
GGT(IU/L)	31	29	0.313

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

Serum bilirubin	>60%(n=14)	50-60%(n= 26)	<50% (n=10)	Pvalue
Total bilirubin(mean±SD)	1.1±0.34	0.86±0.24	0.72±0.17	<0.001
Direct bilirubin(mean±SD)	0.42±0.12	0.34±0.06	0.22±0.08	<0.001
Indirect bilirubin(mean±SD)	0.73±0.27	0.65±0.08	0.62±0.06	<0.001

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 ($p < 0.05$).

Discussion

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. [16] 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. [17] 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. [18-20]

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. [21] It was seen from the table that majority of the subjects were in the age group between 55 and 65 years. The minimum age was 43 and the maximum age was 75 years. 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. [22] 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 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 4.4 years. The patients' CVD status was confirmed by history, ECG findings and ECHO reports. 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. Several authors have suggested that bilirubin plays a potential role in inhibition of lipid oxidation. [23] 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. [24] In a previous study, the 3-year incidence of coronary artery disease was significantly lower in patients with Gilbert syndrome. [25]

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 ($p < 0.05$).

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.

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