To evaluate the Correlation between Serum Bilirubin Levels and Coronary Artery Disease patients

Dr.Pradeep Kumar Jain[1], Dr.Aekta Jain[2]

[1]M.D (Gen MED), F.I.C.M, F.I.C.P, A.I.F.S.C, Professor, Ananta Institute Of Medical Sciences & Research Centre, DistSiyol Udaipur, Rajasthan, India
[2]M.B.B.S, D.C.H

Corresponding author:
Dr.Aekta Jain, Email: dr.aekta87@gmail.com

ABSTRACT

Aims: To evaluate the correlation between serum bilirubin levels and coronary artery disease patients.

Material and methods: A cross-sectional study was conducted in the Department of medicine. Total of 100 subjects were included in the study with 50 cases and 50 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 entire study subjects were divided into two groups of 50 cases (with CVD) and 50 controls. The mean age among the cases male and female respectively was 62.6 ± 5.8 and 63.2 ± 7.9 and controls group were 61.9 ± 9.6 and 62.9 ± 7.7 years male and female respectively. 60% were male and 40% female in case group and 62% patients were male and 38% patients were female in control group. 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 1 years to 10 years with majority of the subjects' duration was between 3 and 5 years and the mean duration was 4.7 ± 2.7 years. 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 confirmed a significant inverse association between the reduced serum bilirubin levels and the occurrence of Coronary artery disease. Thus total bilirubin may serve as a protective biomarker of CAD.

Keywords: Coronary artery disease, Risk factor, Serum bilirubin

INTRODUCTION

In recent years, the incidence of cardiovascular disease has increased gradually in developing nations.[1] Coronary artery disease has received most attention from medical workers because of its high fatality rate, especially the acute onset of coronary artery disease. The current scenario necessitates the recognition of novel risk factors and screening of individuals who are at risk of developing CAD.[2] In spite of detailed studies on many established risk factors

like diabetes mellitus, hypertension, smoking, dyslipidemiaetc, the studies on serum bilirubin and its pre disposition to CAD are worth a consideration. Postulated cardio protective effects of bilirubin are Has antioxidant properties that inhibit oxidation of LDL and lower LDL levels[3] Preserves vascular nitric oxide that maintains vessel wall elasticity Has anti-inflammatory benefits and inihibits platelet activation and aggregation properties that prevent thrombus formation suppresses matrix metalloproteinases and maintains vascular integrity Several studies have concluded inverse correlations between CAD and total serum bilirubin levels.[4] Also inverse correlation between serum bilirubin and smoking, increased LDL cholesterol, diabetes and increased BMI have been documented. Lesser serum bilirubin levels are associated with Protein Kinase C activation, inflammation, and oxidative stress, which are known mediators of endothelial and micro vascular dysfunction. [5] 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.

Many factors increase the risk of CAD, including low serum bilirubin level, which is associated with increased risk of CAD.[6]Several studies have shown that bilirubin, the final product of hem metabolismhas potent antioxidant capacity.[7] 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.[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] 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.[14,15] 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.

MATERIAL AND METHODS

A cross-sectional study was conducted in the Department of medicine, after taking the approval of the protocol review committee and institutional ethics committee. After taking informed consent detailed history was taken from the patient or the relatives. The technique, risks, benefits, results and associated complications of the procedure were discussed with all patients. Patients with evidence of coronary artery disease by ECG, ECHO were included in this study. Patients with symptoms of congestive cardiac failure, Chronic kidney disease, Chronic liver disease, autoimmune diseases and COPD and malignancy were excluded from the study.

Total of 100 subjects were included in the study with 50 cases and 50 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 50 cases (with CVD) and 50 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 41 and the maximum age was 72 years. The mean age among the cases male and female respectively was 62.6±5.8 and 63.2±7.9 and controls group were 61.9±9.6 and 62.9±7.7 years male and female respectively. 60% were male and 40% female in case group and 62% patients were male and 38% 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: Demographic profile of the patients

Age group	Cases=50		Controls=50	
	Males=30	Females=20	Males=31	Females=19
Mean±SD	62.6±5.8	63.2±7.9	61.9±9.6	62.9±7.7

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: risk factors for CVD Patients

Risk factors	Cases (n=50)	Controls (n=50)	P value
Diabetes	16 (32%)	14 (28%)	0.33
Hypertension	27 (54%)	21 (42%)	0.15
Smoking	18 (36%)	17 (34%)	0.86
Family history of CVD	21 (42%)	32 (32%)	0.27
Obesity	13 (26%)	10 (20%)	0.18
Dyslipidemia	31 (62%)	21 (42%)	0.003

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

Duration of CVD	Number of patients =50	%	Mean±SD
Below 3 years	10	20%	
3 - 5 years	25	50%	
5 - 7 years	11	22%	4.7±2.7
Above 7 years	4	8%	

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 4.7±2.7 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±0.03	1.21±0.22	<0.001
Direct bilirubin	0.23±0.05	0.49±0.15	<0.001
Indirect bilirubin	0.71±0.14	0.88±0.18	<0.001
SGOT (IU/L)	10	17	0.59
SGPT (IU/L)	17	15	0.21
GGT (IU/L)	23	18	0.31

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: Correlation between serum bilirubin levels and the ejection fraction among the CVD patients.

Serum bilirubin	>60 % (n= 12)	50-60 % (n=28)	<50 % (n=10)	P value	r value
Total bilirubin (mean±SD)	1.5±0.25	0.85±0.19	0.73±0.19	<0.001	0.91
Direct bilirubin (mean±SD)	0.41±0.14	0.33±0.16	0.21±0.06	<0.001	0.88
Indirect bilirubin (mean±SD)	0.72±0.23	0.65±0.17	0.59±0.07	<0.001	0.93

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.[16] 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.[17] A particular enzyme namely the hemeoxygenase (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.[18]

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 Schwertneret al.[19]

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 62.6±5.8 and 63.2±7.9 and controls group were 61.9±9.6 and 62.9±7.7 years male and female respectively. 60% were male and 40% female in case group and 62% patients were male and 38% 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.[20]

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.[21] So it seems that higher bilirubin level has a protective effect against coronary artery stenosis (CAS).

The present study among 50 CAD patients and 50 healthy controls confirmed the results of several previous epidemiological studies that low serum bilirubin levels were associated with increased risk for coronary events.[22,23,24] A recent study in patients with peripheral arterial disease (PAD) revealed similar results showing a clear association between low bilirubin concentrations and PAD.[25] 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-glucuroniltransferasa enzyme in liver. Estrogens also decrease LDL level, increase HDL level and reduce LDL oxidation.[26]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.[16]Several authors have suggested that bilirubin plays a potential role in inhibition of lipid oxidation.[27] 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.[28] In a previous study, the 3-year incidence of coronary artery disease was significantly lower in patients with Gilbert syndrome.[29] 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.[21]

CONCLUSION

This study confirmed a significant inverse association between the reduced serum bilirubin levels and the occurrence of Coronary artery disease. Thus total bilirubin may serve as a protective biomarker of CAD.

REFERENCES

1. Hansson GK, Robertson AK, Söderberg - Nauclér C. Inflammation and atherosclerosis. Annu Rev Pathol. Mech Dis. 2006 Feb 28;1: 297-329.
2. Louise D. Bilirubin protects against heart disease. Medical Xpress. 2012.
3. Mayer M. Association of serum bilirubin concentration with risk of coronary artery disease. Clin Chem. 2000 Nov 1; 46(11):1723-7.
4. Schwertner HA, Jackson WG, Tolan G. Association of low serum concentration of bilirubin with increased risk of coronary artery disease. Clin Chem. 1994 Jan 1;40(1):18-23
5. Erdogan D, Gullu H, Yildirim E, Tok D, Kirbas I, Ciftci O, ET AL. Low serum bilirubin levels are independently and inversely related to impaired flow-mediated vasodilation and increased carotid intimamedia thickness in both men and women. Atherosclerosis. 2006 Feb 1; 184(2):431-7.
6. Lingenhel A, Kollerits B, Schwaiger JP, Hunt SC, Gress R, Hopkins PN, et al. Serum bilirubin levels, UGT1A1 polymorphisms and risk for coronary artery disease. ExperGerontol J. 2008; 43(12): 1102-107.
7. Stocker R, Glazer AN, Ames BN. Antioxidant activity of albumin-bound bilirubin. Proceedings of the National Academy of sciences of the United States of America 1987; 84: 5918-922.
8. Gullu H, Erdogan D, Tok D, Topcu S, Caliskan M, Ulus T, et al. High serum bilirubin concentrations preserve coronary flow reserve and coronary microvascular functions. ArteriosclerThrombVasc Biol. 2005; 25(11): 2289-294
9. Hopkins PN, Wu LL, Hunt SC, James BC, Vincent GM, Williams RR. Higher serum bilirubin is associated with decreased risk for early familial coronary artery disease. ArteriosclerThrombVasc Biol. 1996; 16(2): 250-55.
10. Giral P, Ratziu V, Couvert P, Carrie A, Kontush A, Girerd X, et al. Plasma bilirubin and gamma-glutamyltransferase activity are inversely related in dyslipidemic patients with metabolic syndrome: relevance to oxidative stress. Atherosclerosis 2010; 210(2): 607-13.
11. Hansson GK, Robertson AK, Soderberg-Naucler C. Inflammation and atherosclerosis. Annu Rev PatholMech Dis. 2006; 1: 297-329.
12. Berliner JA, Navab M, Fogelman AM. Atherosclerosis: basic mechanisms. Oxidation, inflammation, and genetics. Circulation 1995; 91(9): 2488-496.
13. Ghem C, Sarmento-Leite RE, de Quadros AS, Rossetto S, Gottschall C. Serum bilirubin concentration in patients with an established coronary artery disease. Int Heart J. 2010; 51(2): 86-91.
14. Neuzil J, Stocker R. Free and albumin-bound bilirubin are efficient co-antioxidants for alphatocopherol, inhibiting plasma and low density lipoprotein lipid peroxidation. J Biol Chem. 1994; 269(24): 16712-6719.
15. Rigato I, Ostrow JD, Tiribelli C. Bilirubin and the risk of common non-hepatic diseases. Trends Mol Med. 2005; 11(6): 277-83.
16. Mayer M. Association of serum bilirubin concentration with risk of coronary artery disease. Clin Chem. 2000 Nov 1;46(11):1723-7
17. Morita T. Hemeoxygenase and atherosclerosis. ArteriosclerThrombVasc Biol. 2005;25(9):1786- 95.
18. Neuzil J, Stocker R. Free and albumin-bound bilirubin are efficient co-antioxidants for alpha- tocopherol, inhibiting plasma and low density lipoprotein lipid peroxidation. J Bio Chem. 1994;269(24):16712-9.
19. Schwertner HA, Jackson WG, Tolan G. Association of low serum concentration of bilirubin with increased risk of coronary artery disease. Clin Chem. 1994 Jan 1;40(1):18-23

20. Ghem C, Sarmento-Leite RE, de Quadros AS, Rossetto S, Gottschall CA. Serum bilirubin concentration in patients with an established coronary artery disease. Int Heart J. 2010;51(2):86- 91.
21. Taban SM, Golmohammadi A, Parvizi R, Kezerlou AN, Separham A, Hosnavi Z. The relation of serum bilirubin level with coronary artery disease based on angiographic findings. Crescent J Med Biol Sci. 2015;2(4):130-4.
22. Troughton JA, Woodside JV, Young IS, Arveiler D, Amouyel P, Ferrières J, et al. Bilirubin and coronary heart disease risk in the Prospective Epidemiological Study of Myocardial Infarction (PRIME). Eur J Cardiovasc Prevent Rehabilitation. 2007 Feb;14(1):79-84
23. Novotny L, Vitek L. Inverse relationship between serum bilirubin and atherosclerosis in men: a meta- analysis of published studies. ExpBiol Med (Maywood). 2003 May;228(5):568-71.
24. Djoussé L, Rothman KJ, Cupples LA, Levy D, Ellison RC. Effect of serum albumin and bilirubin on the risk of myocardial infarction (the framingham offspring study). Am J Cardiol. 2003;91(4A):485-8.
25. Rantner B, Kollerits B, Anderwald-Stadler M, Klein-Weigel P, Gruber I, Gehringer A, et al. Association between the UGT1A1 TA-repeat polymorphism and bilirubin concentration in patients with intermittent claudication: results from the CAVASIC study. Clin Chem. 2008 May 1;54(5):851-7.
26. Freeman R. Hormone replacement therapy (estrogen and progesterone): is it necessary for heart disease prevention?. Preventive Cardiol. 2000 Jan;3(1):21- 4.
27. Stocker R, Glazer AN, Ames BN. Antioxidant activity of albumin-bound bilirubin. Proceed National Academy Sci. 1987 Aug 1;84(16):5918-22.
28. Ishizaka N, Ishizaka Y, Takahashi E, Yamakado M, Hashimoto H. High serum bilirubin level is inversely associated with the presence of carotid plaque. Stroke. 2001 Feb 1;32(2):580-3
29. Vítek L , JirsaJr M, Brodanová M, Kaláb M, Mareček Z, Danzig V, et al. Gilbert syndrome and ischemic heart disease: a protective effect of elevated bilirubin levels. Atherosclerosis. 2002 Feb 1;160(2):449-56.