ORIGINAL ARTICLE

Relationship between Serum Bilirubin levels and Coronary Artery Disease

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ABSTRACT

BACKGROUND AND OBJECTIVES: Bilirubin, the bile pigment was initially considered as a toxic waste but later known to be a physiological antioxidant, with protective effect on prevention of coronary artery disease (CAD) and atherosclerosis. CAD, being a burden in the both developed and developing countries, it is very essential to find the parameters that prevents and provokes the CAD. Thus this study was planned to find the association between serum bilirubin level and coronary artery disease. **METHODS:** This study was done among the patients attending outpatient department of general medicine in Vinayaka Mission's Kirupananda variyar Medical College & Hospital, Salem during the period December 2015 to January 2016. 100 cases of coronary artery disease were included and 100 controls were included after matching age and other co-morbid conditions. After taking written informed consent, detailed history, general and systemic examinations were done along with blood investigations. Data were analyzed using SPSS 16. **RESULTS:** The mean age of patients with CAD and non CAD was found to be 61.87±15.9 and 56.47±11.03 years. Mean serum total bilirubin was reported to be 0.8967±1.7 and 0.4774±.15 among subjects with CAD and non CAD, respectively, which was also found to be statistically significant (0.017< 0.05). **CONCLUSION:** Serum bilirubin could be used to foresee the risk of CAD in case of high risk population and it can be measured easily in the clinical laboratory and applied in medical practices.

Keywords: Serum Bilirubin, Caronary Artery Disease, CAD

INTRODUCTION

Bilirubin, the principle bile pigment and also it is the end product of heme catabolism. Previously there was a belief that the bilirubin was a toxic waste. However, contrary to earlier there which is highly a potent one recent research proved that bilirubin is physiological antioxidant.[1,2] Atherosclerosis[3] as well as coronary artery diseases[4] were protected by high levels of bilirubin. Coronary Artery Disease (CAD), which usually occurs as the result of impairment of lipid oxidation and oxygen radicals, which results in Arterial plaque formation, atherosclerosis and inflammation.

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Bilirubin, with its antioxidant properties, it protects the atherosclerotic process by preventing oxidized Low Density Lipoprotein (LDL) formation. Bilirubin is also capable of providing potent scavenging effect of peroxyl radicals. Such capability arises out of increase in the circulatory bilirubin. The circulatory bilirubin plays a physiologic role to protect against the diseases where oxygen and peroxyl radicals are involved. Smoking, blood cholesterol and hypertension are leading risks that contribute for the ischemic heart disease. Bilirubin, being an antioxidant, has dietary as well as endogenous protective characteristics. Therefore, more the serum bilirubin concentration higher the prevention of LDL oxidation, eventually the risk of ischemic heart disease is reduced.[5-7] Thus this study was planned with the intention to find the association between serum bilirubin and coronary artery disease.

OBJECTIVES

The primary objective of this study is to find the association between serum bilirubin level and coronary artery disease.

MATERIALS AND METHODS

This descriptive analytical case control study was done among the patients attending outpatient department (OPD) of general medicine in Vinayaka Mission's Kirupananda Variyar Medical College & Hospitals , Salem during the period December 2015 to January 2016 and all patients who presented in OPD, during the same period was included in the study. After excluding the patients who were not fulfilling the criteria, 100 cases of coronary artery disease were included and 100 controls were included after matching age and other co-morbid conditions.

Inclusion criteria

Cases*:* Patients who attended general medicine outpatient department in Vinayaka Mission's Kirupananda Variyar Medical College & Hospitals, who had coronary artery disease which was evidenced by electrocardiogram (ECG), abnormalities and enzyme changes within past 5 years were included as cases. Also, patients who underwent Coronary bypass surgery or percutaneous coronary interventions, significant stenosis on coronary angiogram and an unequivocally positive stress ECG within the past 5 years were included in the study as cases.

Controls: Controls were taken from the same department, who has no history of Coronary Artery Disease and also they were matched with age and other co-morbid conditions.

Exclusion criteria

Known cases of liver disease, alcoholism, fever with jaundice, cerebro vascular accident, chronic kidney disease, anemia, Malignancy, CAD with failure, hemodynamic instability, autoimmune disease, chronic obstructive pulmonary disease, chronic or current infections and patients on hepatotoxic and anti-inflammatory drugs in the past 30 days, were excluded from the study. After taking written informed consent for their participation, in the study, from all the study subjects, detailed history including duration of Coronary Artery Disease, symptoms, family history of CAD, smoking, alcohol intake, past history of jaundice were taken. Followed by general examination with blood pressure (BP), Body Mass Index (BMI) and systemic examinations were done. Blood investigations like Liver function test (LFT) including serum total bilirubin, Direct bilirubin, Indirect bilirubin, liver enzymes (AST, ALT, SAP), Fasting lipid profile (FLP) including Total cholesterol, LDL and HDL and 12 lead ECG were done. Total serum bilirubin was measured in the laboratory by spectrophotometry method. In the Jendrassik-Grof allied methods, total bilirubin (including direct bilirubin) is reacted with diazotized sulfanilicacid in an acidic medium to form azobilirubin. In the absence and in the presence of “accelerator” substances most commonly caffeine and sodium benzoate, although several others have been proposed-direct and total Bilirubin, respectively were quantified. The absorbance of the azo pigment thus developed is then measured as such for direct bilirubin, or for total bilirubin, after treatment with alkaline tartrate solution, which hits the absorption maximum of the azo pigment toward longer wavelengths.

Statistical analysis

All statistical analyses were performed using the SPSS (Software package used for statistical analysis) package, version 16. The Mean values of all parameters in subgroups were calculated by independent sample t-test. To find the association between various parameters and the outcome of CAD odds ratio and 95% confidence interval was calculated. p-value of less than 0.05 was considered to be statistically significant.

OBSERVATIONS

The mean age of study participants with CAD was found to be 61.87±15.9 years and among those who were without CAD was found to be 56.47±11.03 years and also it was found to be statistically

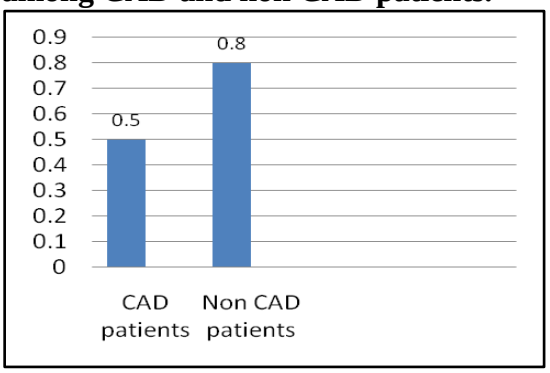
significant (0.006 < 0.05). The mean Body mass index among the CAD group and non- CAD group was found to be 23.17±4.4 and 23.23±3.3, respectively, which was not statistically significant. Among the 100 patients with CAD, there were 72 patients with anterior wall myocardial infarction and 28 patients with inferior wall myocardial infarction. The mean duration of CAD was reported to be 3.16±2.6 years. Mean serum total bilirubin was reported to be 0.8967±1.7 among subjects with coronary artery disease and 0.4774±.15 among the subjects without coronary artery disease. This was found to be statistically significant (0.017< 0.05). Direct bilirubin was found to be 0.0787±.08 and 0.0786±.08 among CAD and non-CAD subjects respectively (Figure 1). Also, it was found to be statistically not significant. Indirect bilirubin was found to be 0.4047±.04 and 0.39±.051 among CAD and non-CAD subjects respectively, which was found to be statistically not significant. The liver enzyme, AST was reported as 10.83±0.51 and 11±0.46 among patients with CAD and non CAD, respectively. There was no statistical significance found. Similarly, ALT was reported as 11.61±1.4 and 10.94±1.1 among patients with CAD and non CAD, respectively, with no statistical significance.

Table 1: Particulars regarding bilirubin, lipid profile and liver enzymes among subjects with and without coronary artery disease

Variables	Patients with CAD (%)	Patient without CAD (%)	p value
Mean age	61.87±15.9 yrs	56.47±11.03 yrs	.006*
BMI	23.17±4.4	23.23±3.3	.08
Total bilirubin	0.4774±.15	0.8967±1.7	.017*
Direct bilirubin	0.0787±.08	0.0786±.08	.993
Indirect bilirubin	0.4047±.04	0.39±.051	.42
Liver enzymes			
AST	10.83±0.51	11±0.46	.08
ALT	11.61±1.4	10.94±1.1	.16
Lipid profile			
TC	163.95±9.1	153.49±12.4	.002*
LDL	127.92±12.8	105.42±8.1	.000*
HDL	42.96±2.9	52.06±4.3	.000*

*statistically significant

Figure 1: Mean total serum bilirubin among CAD and non CAD patients.



In the lipid profile, mean total cholesterol was found to be 163.95±9.1 and 153.49±12.4 among patients with CAD and non CAD, respectively, with statistical significance (0.002< 0.05). Mean LDL was found to be 127.92±12.8 and 105.42±8.1, which was found to be statistically significant (.000<0.05). Also High Density Lipoprotein (HDL), which is commonly called as good cholesterol was reported to be high among patients without CAD (52.06±4.3) and low among CAD patients (42.96±2.9). Also, this was found to be statistically significant (.000<0.05).

In this study, there were more number of male subjects with 36% and 28.5% belonging to coronary artery disease and non-coronary artery disease group, respectively and 14% and 21.5% of females were there in CAD and non-CAD group, respectively with an odds ratio (OR) 1.94 (1.08 to 3.5) and also it was found to be statistically significant (0.02 < 0.05). There were 26.5% of subjects, with diabetes mellitus and among them 13% and 13.5% were belonging to CAD and non-CAD group, respectively. Also 30% of subjects, with hypertension were participated in this study with 15% in each CAD and non-CAD group, respectively. Total of 19% subjects with family history of CAD was included. Also 21.5% of smokers were included and among them 10.5% and 11% were belongs to CAD and non-CAD group, respectively. Odds ratio and 95% confidence interval were not calculated for diabetes, hypertension, family history of CAD and smoking, as

they were matched while selecting the subjects.

Table 2: Particulars regarding risk factors other than bilirubin among subjects with and without coronary artery disease

Variables	No. of patients with CAD (%)	No. of patient without CAD (%)
Sex		
Male	72 (36 %)	57 (28.5 %)
Female	28 (14 %)	43 (21.5 %)
Diabetes mellitus		
Present	27 (13.5 %)	26 (13 %)
Absent	73 (36.5 %)	74 (37 %)
Hypertension		
HTN	30(15 %)	30(15 %)
Non HTN	70(35 %)	70(35 %)
Family history of CAD		
Present	19(9.5 %)	19(9.5 %)
Absent	81(40.5 %)	81(40.5 %)
History of smoking		
Present	21 (10.5 %)	22 (11 %)
Absent	79 (39.5 %)	78 (39 %)

DISCUSSION

Factors which alter the bilirubin level in the blood include protein binding and acidosis. However, it is uncertain whether these factors were responsible for the cardio protective potential of bilirubin, because the first factor, Protein binding in turn modified by albumin concentration in plasma and acidosis. These factors are expected to affect the balance between bound unconjugated bilirubin instrumental and free (diffusible) form, instrumental in changing the penetration of unconjugated bilirubin into cells. Likewise, the second factor, hypoxia, is altered by membrane integrity. The alteration in membrane integrity actually modulates transferring capacity of bilirubin. This complex interaction in turn affects the antioxidative capacity of different bilirubin forms. Therefore it is imperative to assess how the bilirubin antioxidative capacity is altered by circulating concentration of blood pH, free bilirubin and circulating albumin.

Prevention of Atherosclerosis: Prevention of atherosclerosis by bilirubin was explained by several mechanisms. The widely accepted mechanism includes Lipid oxidation inhibition by bilirubin and Enhanced Heme Oxygenase (HO) activity of bilirubin.

Lipid oxidation inhibition by bilirubin: Among the Lipoproteins, notably LDL, is extremely prone to oxidation. The uptake of oxidized LDL by intimal macrophages is the key step in atherogenesis. This process eventually results in the accumulation of lipid-rich foam cells. It is possible that bilirubin protects lipids and lipoproteins against oxidation as bilirubin possess anti-oxidant capacity. The advantage of this process is that it protects the vascularity against atherogenesis. Therefore low bilirubin concentration is associated with increase in the oxidized lipoproteins and lipids. This increased oxidation of lipids results in enhanced formation of atherogenic plaque in blood vessels.[8]

Enhanced Heme Oxygenase (HO) activity of bilirubin: Heme Oxygenase activity of bilirubin is due to the changes that occurs as a result of increased elimination of heme**,** enhanced production of CO (Carbon Monoxide)**,** enhanced iron and increased production of biliverdin**.**

Decrease in concentration of HO causes vascular smooth muscle cell proliferation this in turn eventually leads to stenosis. Increased heme oxygenase activity of bilirubin results in antiatherogenic and cardio protective effects and also it prevents neointimal formation by inhibiting vascular smooth muscle cell proliferation. Thus any variation in the concentration of any of the above metabolites alters the pathophysiology of atherosclerosis.[9]

Antioxidant role of Bilirubin: Several studies have shown that different circulating forms of bilirubin like free bilirubin, albumin-bound bilirubin, unconjugated bilirubin and conjugated bilirubin are powerful antioxidants. Besides they are able to protect human LDL (Low Density Lipoprotein) against peroxidation.[10]

The two reasons for plasma bilirubin to reduce the risk of atherogenesis in high

concentration include involvement of oxidized LDL in the formation of atherogenic plaques and under physiological conditions the ability of bilirubin to serve as a potent lipid chain-breaking antioxidant.[11]

It is established fact that bilirubin is a physiological antioxidant. This fact has been established over the years of experiments in humans and animals.

Yamaguchi et al[12] reported that the oxidative metabolities of bilirubin (biotripyrrins) were present in the urine of healthy human's and also they isolated the metabolites of bilirubin from ascorbic acid-deprived rats, which were treated with endotoxin.[13] In turn, feeding of ascorbic acid resulted in the reduction of bilirubin metabolites and also reduced the hepatic concentration of HO (heme oxygenase) mRNA principally stimulated by endotoxins[13]. Another study done by Schwertner et al[14] reported that lower bilirubin concentration results in higher incidence of coronary artery disease, vice versa higher bilirubin subjects had lower occurrence of coronary artery disease. It has also been reported that below normal serum bilirubin level is associated with the presence of ischemic heart disease. Hopkins et al[15] found that patients with early familial CAD have an average total serum bilirubin of 8.9±6.1 μmol/L and the average level in healthy control subjects is 12.4±8.1 μmol/L, which is significantly higher than coronary artery disease patients.

Briemer et al[16] reported that low concentrations of serum bilirubin are associated with increased risk of ischemic heart disease. Madhavan et al[17] reported, on prolonged investigations found that multiple risk factors of CAD like smoking, LDL-cholesterol, obesity and diabetes were inversely correlate with plasma bilirubin concentration. They further reported that the protective factors like HDL-cholesterol, lower FEV1, and lower serum albumin are directly correlating with serum bilirubin. Hulea et al[18] reported a genetic variation in bilirubin concentration in individuals with early CAD displaying lower bilirubin than unaffected persons. On the basis of above findings, lower bilirubin was found to be an independent risk factor for CAD. Between CAD morbidity and bilirubin concentration an inverse correlation was demonstrated. The results of this study were consistent with studies done by Levinson et al[19] and Djousse et al[20]. In these studies similar inverse correlation have been shown not only between serum bilirubin concentrations and coronary artery disease, but also between bilirubin and peripheral vascular disease, carotid initima media thickness and stroke.

CONCLUSION

Coronary artery disease patients were found to have lower bilirubin level and patients without Coronary artery disease were found to have high levels of bilirubin, in this study. This shows that bilirubin plays a protective role against coronary artery disease; in turn assessment of serum bilirubin could very well be used to foresee the risk of coronary artery disease in case of high risk population. The clinical relevance relates to potential preventive as well as therapeutic approaches and the diagnostic relevance emphasis the plasma bilirubin concentration, as a provisionally new marker of atherogenic risk which can be measured in the clinical laboratory and applied in medical practices. Also, in order to prevent coronary artery disease, drugs that increase the bilirubin in moderate level can be used in future.

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