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RESEARCH PAPER

Evaluation of serum bilirubin and its role as an antioxidant in coronary artery disease

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Background and aims: Bilirubin was considered a metabolic waste product of heme with no physiological function. Later it was found that bilirubin may possess an antioxidant role due to its biliverdin bilirubin redox cycle. Considering the etiopathology of coronary artery disease (CAD), which involves lipid peroxidation, the present study aims to find an association between established cases of CAD and serum bilirubin levels. Materials and methods: This case-control study includes 50 diagnosed cases of CAD and 50 healthy age and sex-matched individuals as control. Subjects with haemolytic disease, liver disease, renal disease, gout, and thyroid disorder, and those on antioxidant supplementation or lipid-lowering therapy were excluded from the study. The serum bilirubin level is estimated in all subjects in the dry chemistry automated analyzer Vitros 250 system. Data were analyzed by using Microsoft Excel. Results: The mean value of serum bilirubin in CAD cases was 0.46±0.14 mg/dl with the range 0.2-0.9 mg/dl, whereas, in the control group, it was 0.70±0.17 mg/dl with the range of 0.4-1.2mg/dl. The low level of serum bilirubin in CAD cases was found to be significant (p<.001). Conclusion: A significant association between low serum bilirubin and CAD cases was observed. The association of low serum bilirubin levels with CAD may reflect its consumption in endogenous antioxidant activity. The inverse correlation between plasma bilirubin concentration and CAD may have important clinical and diagnostic implications. In this regard, further studies are undoubtedly needed to establish the sensitivity and specificity of serum bilirubin as a marker of cardiovascular morbidity.

Keywords: Lipid profile; endogenous antioxidant; biliverdin bilirubin redox cycle.

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INTRODUCTION

Coronary artery disease (CAD) is a giant killer in developed countries and is rapidly becoming one in developing countries.[1,2] Innumerable clinical studies, experimental works, and biochemical evaluations have been continuing in search of a better understanding, prevention, and treatment of this dreadful disease. All these efforts to know the disease in all its perplexities are justified, considering that it affects a sizeable proportion of the population in the prime period of life, thus, restricting its use to the family and society.

CAD is a multifactorial disease. Extensive research and epidemiological studies in different parts of the world have shown a positive relationship between the incidence of CAD with plasma cholesterol, low-density lipoprotein (LDL) cholesterol, very low-density lipoprotein (VLDL) cholesterol, and triglycerides. But the critical risk factors, lipids, and lipoproteins do not account for the disease in 30% to 40% of the population with CAD.[3] Besides, the major CAD risk factors do not predict subsequent myocardial infarction accurately. They do not fully explain the social class difference in Indians as Indians have a high incidence of CAD, which is not fully explained by conventional risk factors.[4] The search for CAD risk factors that might explain these variants has been stimulated by the

evidence that free radicals are involved in the pathogenesis of atherosclerosis,[5] and those antioxidants, both dietary and endogenous, may be important protective factors.[6]

The generation of reactive oxygen metabolites representing free radicals and highly reactive non-free radicals like H2O2 and O2 is integral to our daily life. They are harmful to biomolecules and are implicated in the causation and progress of several disease processes like CAD, cancer, diabetes, etc. Usually, the human body has an abundant supply of antioxidants, and it is seen that this abundant supply of antioxidants counteracts oxidative stress caused by free radicals, which delay or inhibit oxidation and neutralize the free radicals. In nature, therefore, when there is a balance of oxidative stress and the antioxidant supply, there is perfect harmony, and no tissue destruction occurs. However, if there is an imbalance, i.e., either an excess of free radicals or a deficiency of antioxidant supply, tissue damage can occur, or a disease process ensues.

The concept of investigation of antioxidants for the prevention of atherosclerosis has arisen from observational trials that demonstrated an inverse relationship between the consumption of antioxidant vitamins and the risk of cardiovascular events.[7] Recently, attention has been given to the antioxidant role of bilirubin on CAD. Bilirubin, an abundant bile pigment that causes jaundice, has long lacked any clear physiologic role. It is produced from the enzymatic reduction of biliverdin, a product of heme oxygenase activity by biliverdin reductase. More recent evidence suggests that bilirubin is a potent endogenous antioxidant that can protect cells from a 10,000-fold excess of H2O2. Barnanoet al.,[8] reported that bilirubin is a major physiologic antioxidant cytoprotectant. Thus, cellular depletion of bilirubin markedly augments tissue levels of reactive oxygen species and causes apoptotic cell death. Depletion of glutathione, generally regarded as a physiological antioxidant cytoprotectant, elicits a lesser increase in reactive oxygen species and cell death. The influential antioxidant role of bilirubin is reflected by an amplification cycle whereby bilirubin is oxidized to biliverdin and then recycled back to bilirubin by biliverdin reductase.

This redox cycle may constitute the principal physiologic function of bilirubin. Bilirubin could be involved in CAD in several ways[9,10] and could protect lipids and lipoproteins against oxidation.

Stringer et al.3 andYlaet al.[11] hypothesized that oxidized lipids and lipoproteins are known to be atherogenic. Low bilirubin concentrations could be associated with an increase in oxidized lipids and lipoproteins. A low concentration of bilirubin might also prevent the solubilization of cholesterol and its clearance through the bile, which in turn increases serum cholesterol concentration.[12, 13]

Considering the etiopathology of CAD, which involves lipid peroxidation and the antioxidant role of bilirubin, the present study was undertaken to determine if there is any association between established cases of CAD and serum bilirubin levels. A better understanding of such factors may influence the nature of therapeutic intervention in patients with or even before the development of CAD.

MATERIALS AND METHODS

This case-control study was conducted in a tertiary care hospital in Assam. Fifty newly diagnosed cases of CAD were included in this study, and 50 healthy age and sex-matched individuals were taken up from a normal population group as control. Subjects with haemolytic disease, liver disease, renal disease, gout, and thyroid disorder and those on antioxidant supplementation or lipid-lowering therapy were excluded from the study.The relevant history taking, physical examination, and laboratory investigation were done in all the cases.

After taking consent, a blood sample was collected from each participant.Serum Bilirubin (Total Bilirubin)was estimated by the Diazo method in the dry chemistry automated analyzer Vitros 250 system.The photosensitive and unstable bilirubin sample was protected from direct exposure to light and assayed immediately after collection.All other relevant biochemical parameters are also estimated in the Vitros 250 system.

Student's t-test was applied using Microsoft excel to assess if there were any statistically significant differences in parameters between the cases and controls.

RESULTS

The serum bilirubin level in the control group was 0.70±0.17 mg/dl. In CAD cases, there was a fall in serum bilirubin level with a mean ± SD value of 0.46±0.12 mg/dl (Table 1).

Table 1 Range, mean and SD of serum bilirubin level in CAD cases and controls

Study group	No. of cases			Serum bilirubin (mg/dl)					
				Range			Mean ± SD		
	Male	Female	Overall	Male	Female	Total	Male	Female	Overall
CAD cases	37	13	50	0.2-0.9	0.3-0.7	0.2-0.9	0.46±0.14	0.43±0.13	0.46±0.12
Control	32	18	50	0.4-1.2	0.4-0.8	0.4-1.2	0.72±0.18	0.65±0.12	0.70±0.17

SD- Standard Deviation; CAD-coronary artery disease

Again, serum bilirubin level was lower in females than in males both in CAD cases and controls (0.43±0.13 mg/dl in females and 0.46±0.14 mg/dl in male CAD cases; 0.65±0.12 mg/dl in females and 0.72±0.18 mg/dl in the male control group) as shown in **Figure 1.**

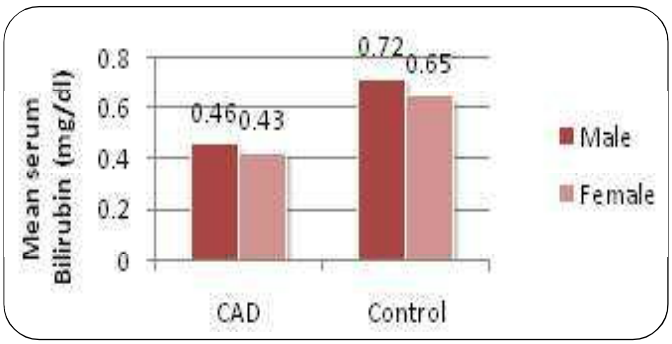

Figure 1 Sex-wise distribution of serum bilirubin in coronary artery disease and control group

There was no influence of age on serum bilirubin level in the control group. Similarly, no age-specific trend was observed in CAD cases **(Figure 2)**.

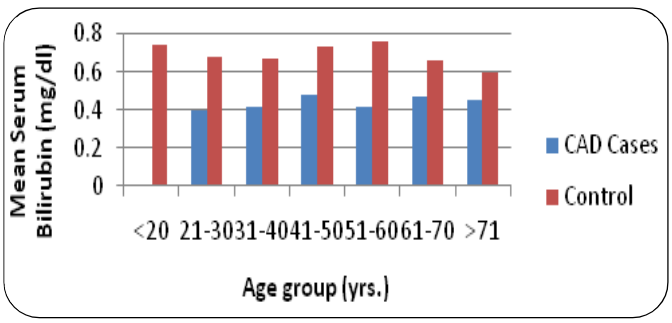

Figure 2 Age-wise distribution of serum bilirubin in coronary artery disease (CAD) cases and control group

The current study found that in the CAD group, 44 cases (88%) had serum bilirubin in the low normal range (0.1-0.6 mg/dl). Only 6 cases (12%) had serum bilirubin in the high normal range (0.61-1.2 mg/dl), while in the control group, 17 cases (34%) had serum bilirubin in the low normal range, and 33 cases (66%) had in the high normal range **(Figure 3 and 4).**

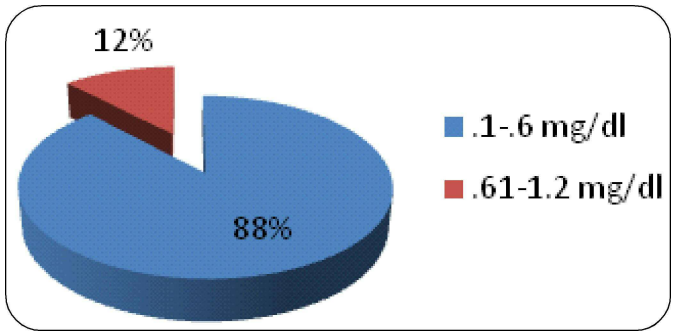

Figure 3 Incidence of high and low normal serum bilirubin levels in coronary artery disease (CAD) cases

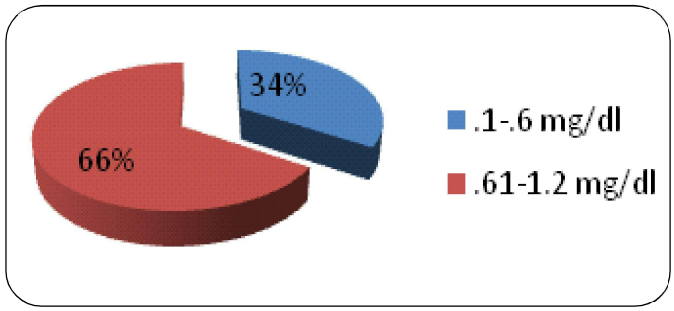

Figure 4 Incidence of high and low normal serum bilirubin levels in the control group

Statistical analysis of the study showed that the p-value is highly significant (<.001) for serum bilirubin in CAD and control groups (Table 2).

Table 2 Statistical analysis and the significance of the result of serum bilirubin in study groups

Study group	No. of observation	Serum bilirubin mg/dl	Degree of freedom	't' value	p-value	Significance
CAD	50	0.46±0.14	98	7.7	<.001	Very highly significant
Vs						
Control	50	0.70±0.17				

CAD - coronary artery disease

In the present study, the mean value of serum bilirubin in CAD cases was 0.46±0.14 mg/dl with the range 0.2-0.9 mg/dl, whereas, in the control group, it was found to be 0.70+0.17 mg/dl with the range of 0.4-1.2mg/dl. The low serum bilirubin level in CAD cases was significant compared to the controls on statistical analysis (p<.001). George Endler et al.,[14] also found that serum bilirubin was lower in CAD patients than in controls (p<.001). They found mean serum bilirubin in CAD cases 0.6mg/dl with a range of 0.44-0.78 mg/dl against 0.71 mg/dl mean value with a range of 0.5-0.96 mg/dl in controls. Ladislav and Vitek[15] also found a close negative relationship between serum bilirubin and atherosclerosis (p<.001). Troughton et al.[16] also reported that bilirubin was significantly lower in cases of CAD (Geometric mean in CAD cases 0.46 mg/dl, range 0.31-0.72 mg/dl compared with 0.53 mg/dl mean value, 0.36-0.75 mg/dl range in controls).

Schwertner et al.,[17] suggested that the relation of increased CAD risk with low bilirubin reflected its consumption in endogenous antioxidant activity. Thus, it is possible that in individuals with a low intake of exogenous antioxidants, these endogenous antioxidants might compensate for the deficiencies and act as markers of dietary antioxidant deficiency. The antioxidants may be operating in a complex way modulated by each other. Thus, exogenous, and endogenous antioxidants might be operating both in the early phase of the development of atherosclerosis and in the period closer to the onset of clinical events.

Low serum bilirubin concentration is independently and inversely associated with an increased risk of CAD by various research workers.[4,17] It was observed that the strength of association between bilirubin and CAD appears to be like that of HDL cholesterol and CAD. The process by which bilirubin exerts its effect is not known. However, the endogenous antioxidant property of serum bilirubin has been proposed as a possible mechanism. These findings have been recently confirmed in a prospective study of middle-aged British men with ischaemic heart disease and several case-control studies involving individuals with CAD.

To explain possible mechanisms of bilirubin action in preventing atherosclerosis, several mechanisms have been suggested to play a potential role in the atherogenic and cardioprotective effects of bilirubin. The most popular one is bilirubin-mediated inhibition of lipid oxidation. Lipoproteins, particularly LDL cholesterol, are highly susceptible to oxidation, and it is known that the atherogenic process involves the uptake of oxidized LDL by intimal macrophages leading to the accumulation of lipid-rich foam cells. Given the antioxidant property of bilirubin, it is possible that bilirubin protects lipids and lipoproteins against oxidation and thereby offers protection against atherogenesis. Again bilirubin is also effective in the solubilization of cholesterol and aiding in its clearance through bile.[12,13]

The current study shows that serum bilirubin level was lower in females than in males in healthy controls and CAD cases. In the CAD group, it was 0.43±0.13 mg/dl with a range of 0.3-0.7 mg/dl in females against 0.46±0.14 mg/dl with a range of 0.2-0.9 mg/dl in males. In the control group, females had serum bilirubin 0.65±0.12 mg/dl with a range of 0.4-0.8 mg/dl against 0.72±0.18 mg/dl with a range of 0.4-1.2 mg/dl for males. This is consistent with the observation made by George Endler et al.[14] They found serum bilirubin level of 0.71mg/dl (range 0.51-0.96 mg/dl) for male CAD cases against 0.6 mg/dl (range 0.44-0.78 mg/dl) for female CAD cases (p<.001). In the control group, values are 0.75 mg/dl (range 0.51-1.1 mg/dl) for

males and .6 mg/dl (range 0.44-0.80 mg/dl) for females ($p<.001$). The lower serum bilirubin in women may reflect the influence of estrogen,[18] possibly related to increased bilirubin excretion by induction of UDP glucuronyl transferase in the liver, as suggested in animal studies.[19]

Limitations: Limitations of this study are single-centre small-sized study, and serum bilirubin is estimated only at the time of admission of cases. A large-sized prospective study estimating serum bilirubin at different times would have been better for evaluating its predictive value.

CONCLUSION

The present study found that patients with CAD have lower serum bilirubin concentrations than the control group. The association of low serum bilirubin levels with CAD may reflect its consumption in endogenous antioxidant activity. The protective role of bilirubin in healthy people may involve its antioxidant, anti-inflammatory, and cellular protective properties. The inverse correlation between plasma bilirubin concentration and CAD may have important clinical and diagnostic implications. The clinical relevance relates to potential preventive and therapeutic approaches. In contrast, the diagnostic relevance concerns the diagnostic utility of atherogenic risk that can be measured easily in the clinical laboratory and applied in medical practice. In this regard, further studies are undoubtedly needed to establish the sensitivity and specificity of serum bilirubin as a marker of cardiovascular morbidity.

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