# ROLE OF SERUM BILIRUBIN AS A MARKER OF CORONARY ARTERY DISEASE

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## ABSTRACT

### BACKGROUND
Serum bilirubin is having protective effect as an antioxidant with capacity to remove reactive species of oxygen. Studies have suggested that an increased bilirubin level promotes protection against atherosclerosis. We have evaluated the level of serum bilirubin in patients with suspected coronary artery disease and its correlation with severity of lesion as assessed by modified Gensini score on angiography.

### MATERIALS AND METHODS
A total of 540 patients have been enrolled in the study. Patients with diagnosis of acute coronary syndrome, stable angina, history of typical angina, Treadmill test positive were enrolled in the study. All patients were confirmed to have normal liver and renal function and were taken written consent for coronary angiography. The severity of lesion on angiography was assessed by modified Gensini score. Based on angiography results, the patients have been divided into CAD and control group.

### RESULTS
The no. of patients in CAD group were (n=380) and control group were (n=160). Mean age of presentation in CAD group was 51.40±10.31 yrs. compared to controls 49.80±10.01 yrs. (p=0.1). Males were 70.26% in CAD group and 62.5% in controls (p = 0.08). The serum bilirubin was significantly lower in cases than in controls (0.56±0.28 mg/dL vs. 0.79±0.30 mg/dL, p <0.0001). There was a significant difference between other parameters assessed, i.e. RDW CV (14.46±0.74 vs. 13.72±0.85, p <0.0001), RDW SD (45.61±4.42 vs. 44.41±5.68, p = 0.0086), serum uric acid (7.10±2.06 mg/dL vs. 5.96±1.96 mg/dL, p <0.0001). Univariate analysis have been done followed by multivariate logistic regression analysis for assessing the independent risk factors for CAD. Serum bilirubin, RDW, Sex, Age and Diabetes were found to be independent predictors of presence of CAD. Serum bilirubin negatively correlated with presence of CAD (n=540, r=-0.46, p<0.0001). The cutoff value of serum bilirubin based on Receiver Operating Characteristic curve (ROC) analysis was 0.52 mg/dL for presence or absence of CAD with sensitivity of 80.3% and specificity of 89.1%. The correlation of low bilirubin with severity of CAD was (n=380, r=-0.34). The low serum bilirubin in smokers compared to nonsmokers (both in cases and control groups) did not attain statistical significance. The effect of smoking on level of bilirubin was minimal.

### CONCLUSION
Low Serum bilirubin predicts the presence of CAD and is negatively correlated with the severity of CAD.

### KEYWORDS
Serum Bilirubin, Coronary Artery Disease, Gensini Score.

## BACKGROUND
Serum bilirubin is having protective effect as an antioxidant with capacity to remove reactive species of oxygen. Studies have suggested that an increased bilirubin level promotes protection against atherosclerosis. We have evaluated the level of serum bilirubin in patients with coronary artery disease and its correlation with severity of lesion as assessed by modified Gensini score on angiography. The primary intention was to see the level of bilirubin in patients with CAD compared to controls and the correlation of level of bilirubin with the lesion severity, i.e. as bilirubin level decreases whether the severity of lesion increased on angiography by Gensini score.

## MATERIALS AND METHODS
The study has been conducted between August 2014 to March 2015 at Department of Cardiology, Osmania General Hospital, Hyderabad. It is a prospective observational study.

## Inclusion Criteria
Patients with age more than 18 years and a diagnosis of acute coronary syndrome, chronic stable angina, treadmill test positive and atypical angina with conventional risk factors have been enrolled in the study.

## Exclusion Criteria
Patients with a previous diagnosis of coronary artery disease, post percutaneous coronary intervention, coronary artery bypass surgery, anaemia, liver disease, prosthetic valve disease, chronic kidney disease were excluded from the study. Patient with right ventricular myocardial infarction and failure, those with cardiogenic shock, those with previous heart failure were excluded from the study. Each patient has been enrolled in the study after a written and informed consent. Patients were confirmed to have normal liver and renal function tests before the enrolment.

## Coronary Angiography
Coronary artery disease by angiography is defined as lumen narrowing by more than 50% in diameter. The severity of lesion on angiography assessment was done by the Modified Gensini Score. The coronary arterial tree is divided into eight proximal segments and the severity of lesion is graded as lumen narrowing of <50% -1, 51-75% -2, 76-99% -3 and 100% occlusion as 4. The total score is sum of the lesion scores assessed in eight proximal segments. The minimum value is 4 and maximum of 32. Mild disease is score of 1-6, moderate is 7-13 and severe is >13.

## Patient Examination
Each patient underwent clinical examination, electrocardiographic analysis and echocardiographic evaluation of the heart, biochemical analysis before coronary angiography. After coronary angiography patients were divided into those with coronary artery disease and those without coronary artery disease (controls). Quantitative serum bilirubin (total) has been analysed in the patients of both groups. Method of bilirubin measurement was by 2, 5-dichlorophenyldiazonium (DPD) method and the sample was collected within 24 hours of patient admission. The heart function was evaluated by 2D-echo and those with pericardial effusion, severe LV dysfunction and dilated chambers and RV dilation leading to altered LFT bilirubin were excluded from the study.

## Statistical Analysis
Statistical analysis were carried out using MedCalc (Belgium). Continuous variables are expressed as mean±Standard Deviation (SD). Categorical variables are expressed as percentages. Comparison of continuous variables between two groups by Student's t-test. Receiver Operating Characteristic (ROC) curves for serum bilirubin values were plotted to determine the optimal cutoff point for use in clinical decision making. Multivariate logistic regression analysis was used to identify the independent predictors of angiographic CAD. A p value of <0.05 is considered significant statistically. Tables, Bar diagrams, Pie diagrams have been illustrated wherever necessary.

## RESULTS
A total of 540 consecutive patients were enrolled in the study. Post coronary angiography, patients were divided into CAD group and without angiographic CAD group (controls). Patients in CAD group and those in controls were 380 and 160, respectively. Mean age of presentation in CAD group was 51.40±10.3 years compared to controls 49.80±10.01 years, (p=0.1). Males were 70.26% in CAD group and 62.5, 62% in controls (p=0.08). Most common comorbidity was hypertension seen in 221 (58.16%) patients followed by smoking in 199 patients (52.37%) in the CAD group. The cases and controls were matched according to age, sex and comorbidities (Figure 1).

The serum bilirubin was significantly lower in cases than in controls (0.56±0.28 mg/dL vs. 0.79±0.30 mg/dL, p <0.0001). There was a significant difference between other parameters assessed, i.e. RDW CV (14.46±0.74 vs. 13.72±0.85, p <0.0001), RDW SD (45.61±4.42 vs. 44.41±5.68, p=0.0086), serum uric acid (7.10±2.06 mg/dL vs. 5.96±1.96 mg/dL, p <0.0001) (Table1) (Figure 2).
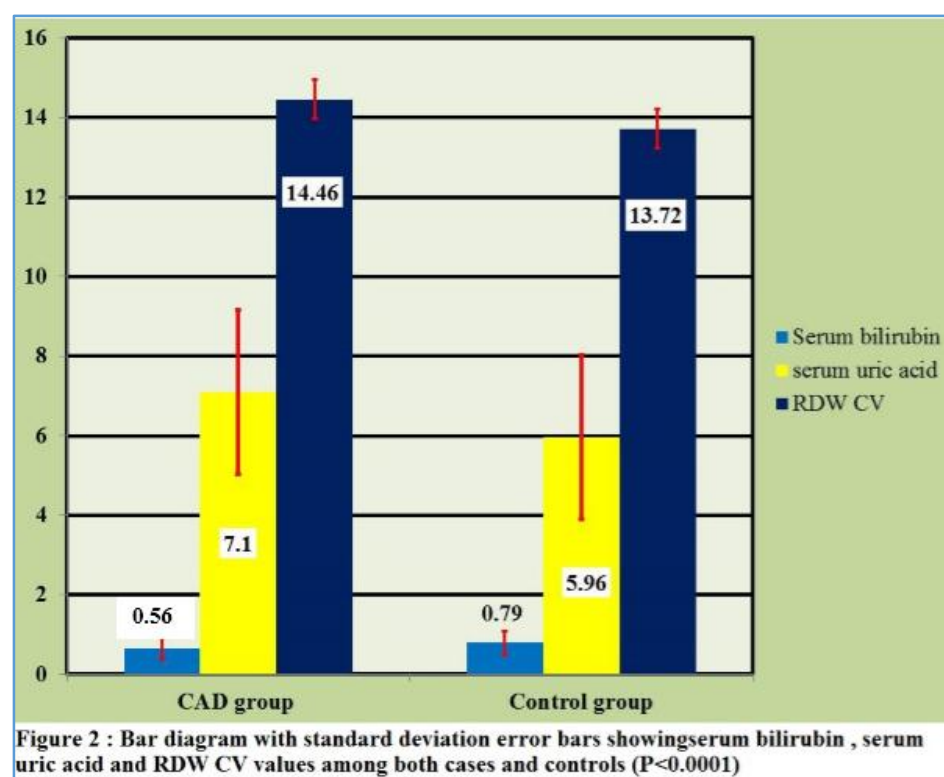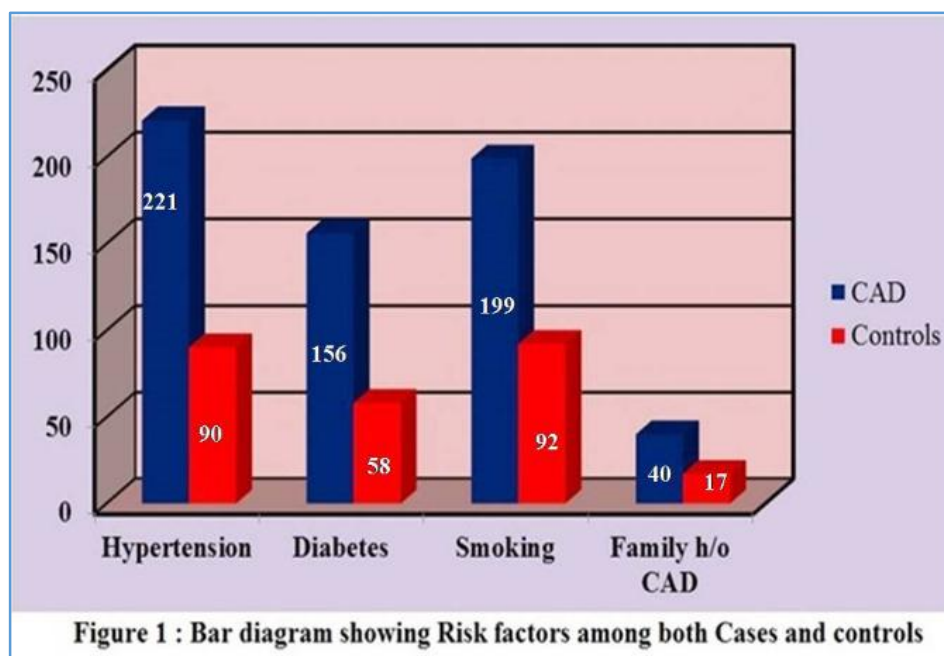
| Characteristic | CAD (n=380) | Control Group (n=160) | P value |
|---|---|---|---|
| Age (yrs.) | 51.4±10.31 | 49.80±10.01 | 0.10 |
| Males, n (%) | 267 (70.26) | 100 (62.5) | 0.0824 |
| Females, n (%) | 113 (29.74) | 60 (37.5) | 0.0782 |
| M:F | 2.36:1 | 1.66:1 | |
| Hypertension, n (%) | 221 (58.16) | 90 (56.25) | 0.6819 |
| Diabetes, n (%) | 156 (41.05) | 58 (36.25) | 0.2982 |
| Smoking, n (%) | 199 (52.37) | 92 (57.50) | 0.2753 |
| Family h/o CAD, n (%) | 40 (10.53) | 17 (10.62) | 0.9752 |
| RDW CV (%) | 14.46±0.74 | 13.72±0.5 | <0.0001 |
| Serum uric acid, mg/dL | 7.10±2.06 | 5.96±1.96 | <0.0001 |
| Serum bilirubin, mg/dL | 0.56±0.28 | 0.79±0.30 | <0.0001 |
| *Table 1* | | | |

Yrs.- years, M- males, F- females, n- number, h/o- history. CAD- coronary artery disease, RDW- red blood cell distribution width.
CV- coefficient of variant, SD- standard deviation, mg-milligram, dL- decilitre.

Figure 1 : Bar diagram showing Risk factors among both Cases and controls



Figure 2 : Bar diagram with standard deviation error bars showingserum bilirubin , serum uric acid and RDW CV values among both cases and controls (P<0.0001)

Univariate analysis was done followed by multivariate logistic regression analysis for assessing the independent risk factors for CAD. Serum bilirubin, RDW, sex, age and diabetes were found to be independent predictors of presence of CAD and not severity of CAD.

Serum bilirubin negatively correlated with presence or absence of CAD (n=540, r=-0.46, p<0.0001) and the severity of CAD (n=380, r=-0.34, p<0.0001), moderate correlation noted.

The cutoff value of serum bilirubin based on Receiver Operating Characteristic Curve (ROC) analysis was 0.52 mg/dL for presence or absence of CAD with sensitivity of 80.3% and specificity of 89.1% (AUC=0.8) (Figure 3).

The low serum bilirubin in smokers compared to nonsmokers both in cases (r=-0.0476, p=0.3816) and control groups is not statistically significant thereby ruling out the effect of smoking on bilirubin levels, which has high reactive oxygen species burden reducing the antioxidant levels (r=-0.117, p=0.176). Studies have shown that smokers have low serum bilirubin levels compared to the nonsmokers because of increased consumption of the antioxidants for counteracting the reactive oxygen species as a result of smoking on endothelial system. This was not shown in our study.
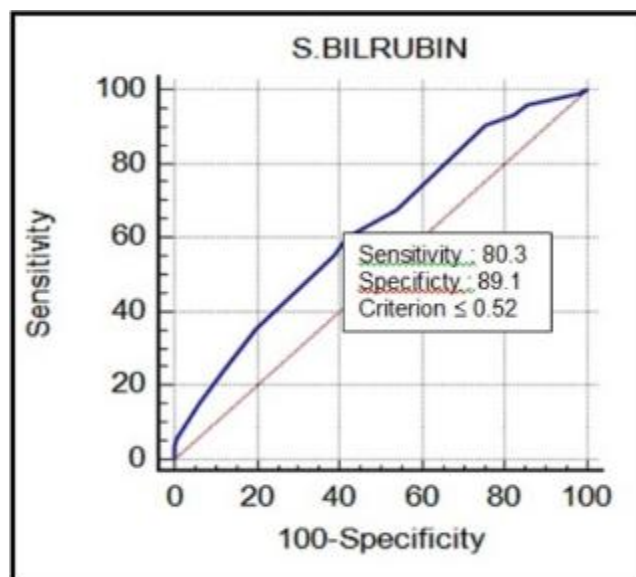
*Figure 3. ROC Curve of Serum Bilirubin for the Presence or Absence of CAD (AUC=0.8)*

## DISCUSSION

Atherosclerosis is the main aetiology underlying the Coronary Artery Disease (CAD), the major cause of mortality worldwide both in developed and developing countries.[1] Antioxidants are the predominant adaptive responses by the arterial vasculature in response to the oxidative stress thereby preventing the atherosclerosis.[2] Bilirubin, a toxic waste product formed during heme catabolism is in fact a potent physiological antioxidant that provides important protection against atherosclerosis and inflammation.[2,3] Heme Oxygenase (HO) particularly the HO-1 enzyme, a stress inducible one involved in the heme catabolism is an important function in cell defense mechanism against oxidative injury. The products of the catabolic reaction, i.e. bilirubin, carbon monoxide and iron have a protective role (e.g.- LDL peroxidation and chemotaxis of monocytes are prevented by bilirubin).[2] The other important role of bilirubin, the natural antioxidant 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.[4]

Plasma bilirubin inversely correlated with risk factors of CAD- smoking, diabetes and obesity, thus emphasising the oxidative stress underlying in them[5] (we did not see the correlation in our study). Inverse relationship between the presence of CAD and circulatory total bilirubin was first observed by Schwertner et al.[6]

Male gender is one of the most important risk factors for CAD. The same was found in our study.[7] Males were predominant in cases more than the controls. We matched the cases and controls with regards to age, sex and comorbidities thereby removing the confounding factors responsible for the lowering of bilirubin as a result of the oxidative stress and other mechanisms. The study by Hopkins et al[8] and the Ghem et al[7] differed from ours by the fact that the cases and controls are not matched and had a higher prevalence of the risk factors in the CAD group. According to the Third National Health and Nutrition Examination Survey done between 1988 and 1994, serum bilirubin levels were significantly higher in men compared to women.[9] The low levels of bilirubin in the CAD group compared to the controls with predominant males may emphasise the increased oxidative stress and thereby the low values in them.

Our data correlated with previous studies that the prevalence of CAD correlated with decreased levels of bilirubin. Total bilirubin level less than 0.52 mg/dL behaved as an independent risk factor for presence of CAD on multiple regression analysis along with RDW, age, sex and diabetes. On Receiver Operating Curve analysis, the Area Under the Curve (AUC) was 0.8 with sensitivity and specificity of 80.3% and 89.1%, respectively.

The present study also showed a significant negative correlation with the severity of CAD as assessed by the modified Gensini score, not found in the study by Ghem et al.[7]

Bilirubin is a novel marker of atherosclerosis and negatively correlates with severity of atherosclerotic burden. The statins[10] and rapamycin[11] drugs increase the HO-1 enzyme levels and thereby contribute to the increase in the bilirubin and hence the antioxidative effect.

Serum uric acid is a metabolite of purine metabolism. Most epidemiological studies have suggested that there is an association between raised serum uric acid and CAD.[12,13,14] Two theories are in existence regarding the role of uric acid; 1) It is a by-product from the lysosomal degradation of glycoprotein-urate complexes and binds with the lysosomal membrane via hydrogen bond causing membrane lysis.[15] 2) Mediator of inflammation through the direct activation of the complement factors, free radical injury of the vessel wall.[16] Elevated uric acid is linked with obesity,[17] hypertension,[18] male gender.[19] The serum uric acid levels increased in the CAD group with decrease in serum bilirubin levels, thus showing the oxidative stress and the free radicals burden. Red Blood Cell Distribution Width (RDW) is used in the differential diagnosis of anaemia.[20] The hepcidin molecule is responsible for the increased RDW in patients with CAD.[21] RDW values were significantly raised compared to the controls.

## LIMITATIONS
It is a single centre study. Inherent selection bias of the case control study. Interobserver variability has not been done.

## CONCLUSIONS
1. The inverse relationship between total bilirubin and the CAD is well established even in the presence of other risk factors, thereby emphasising its protective role as an antioxidant.
2. A total bilirubin level of less than or equal to 0.52mg/dl predicts the presence or absence of CAD with a sensitivity of 80.3% and specificity of 89.1%.
3. Serum bilirubin inversely correlated with severity of CAD.

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