

## Research Paper



# The Relationship Between the Bilirubin Levels and Coronary Artery Disease in Patients With Stable Coronary Disease

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## ABSTRACT

**Objectives:** Coronary artery disease (CAD) is an atherosclerotic disease that is a major cause of mortality worldwide. Bilirubin has been considered an antioxidant that protects patients against atherosclerosis. Therefore, the present study examines the relationship between serum bilirubin levels with CAD.

**Methods:** In this cross-sectional, analytical study, the patients with suspected CAD were electively scheduled for angiography. Their blood samples were measured for total, direct, and indirect bilirubin levels. Following angiography, comparisons were made between the two groups of CAD and non-CAD subjects.

**Results:** Of 140 studied patients, 73(52.1%) were male and 67(47.9%) were female. The mean age of subjects was  $55.32 \pm 10.71$  years which was statistically significantly significant ( $P < 0.001$ ). The mean total, direct, and indirect bilirubin levels between the groups were  $0.74 \pm 0.57$  ( $P = 0.79$ ),  $0.22 \pm 0.07$  ( $P = 0.77$ ), and  $0.48 \pm 0.45$  ( $P = 0.77$ ) mg/dL, respectively, which were not significant. The mean total ( $0.77 \pm 0.57$  vs  $0.71 \pm 0.57$ ) and indirect bilirubin levels ( $0.53 \pm 0.53$  vs  $0.44 \pm 0.35$ ) were higher in men than women and mean direct bilirubin was similar between men and women ( $0.23 \pm 0.07$  vs  $0.22 \pm 0.07$ ). There was a statistically significant relationship between male and female groups in total and indirect bilirubin ( $P = 0.05$ ).

**Discussion:** No significant relationship was found between bilirubin levels and CAD either in terms of protective or aggravating role.

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## Introduction

Coronary artery disease (CAD) is a type of atherosclerotic disease that occurs when plaque builds up in the coronary arteries, the blood vessels that supply the heart with oxygen-rich blood which leads to chest pain (angina), heart attack (myocardial infarction), or sudden cardiac death [1, 2]. The prevalence of CAD in the general population is estimated at 6.3%, with a lower rate in women (5.2%) compared to men (7.5%) [3]. However, the number of CAD cases is increasing in developing countries [4].

The high prevalence of cardiovascular diseases in society underscores the importance of recognizing risk factors and screening susceptible individuals for preventative and treatment strategies [5]. While numerous key risk factors for CAD have been identified, such as age, gender, diabetes, high blood pressure, smoking, abnormal lipid levels, obesity, homocystinuria, kidney dysfunction, physical inactivity, an unhealthy diet, and family history or genetics [6, 7], there may be other factors that potentially heighten the risk of CAD. An example is plasma bilirubin levels, which possess protective and preventative characteristics against coronary atherosclerosis [8, 9].

Bilirubin, a pigment derived from the breakdown of heme, possesses significant endogenous antioxidant properties [10]. These properties mitigate lipid peroxidation and impede the advancement of atherosclerosis [11]. Even mild elevations in circulating bilirubin levels show promise in preventing and reducing the prevalence of CAD and other conditions characterized by oxidative stress, such as type II diabetes mellitus and cancer [12]. Recent research suggests that bilirubin may have a role in predicting the prognosis of CAD [13]. According to a prior investigation, elevated total bilirubin levels could independently forecast adverse cardiac events in patients with ST-segment elevation myocardial infarction who receive primary percutaneous coronary intervention [14].

According to growing evidence derived from empirical studies, inflammation, and bilirubin play crucial roles in the development of coronary atherosclerosis and ischemia. Furthermore, considering the transitional stage of economic and urban development that Iran is undergoing, an increase in the prevalence of cardiovascular diseases has been observed. There is a scarcity of research conducted on the association between serum bilirubin levels and CAD in Iran. Consequently, this investigates the correlation between serum bilirubin levels and CAD.

## Materials and Methods

In this cross-sectional, analytical study, the patients with suspected CAD (based on history or non-invasive tests) who were electively scheduled for angiography at Taleghani Hospital (Urmia, Iran) after obtaining written consent were included in the study.

The blood samples collected from the participants were obtained after a minimum of 12 h of overnight fasting. Before conducting angiography, the levels of total, direct, and indirect bilirubin in the blood samples were measured using the diazo method with diazotized sulfanilic acid and a colorimetric technique. Additionally, the levels of total cholesterol, triglyceride, low-density lipoprotein, high-density lipoprotein, urea, and creatinine were measured using an enzymatic colorimetric method. Liver function tests were conducted on all patients, focusing specifically on the levels of serum total, indirect, and direct bilirubin. Resting 12 lead electrocardiogram was performed to identify evidence of ischemia and a bedside screening echocardiogram was conducted on admission to assess ejection fraction, left ventricle dimensions, and wall motion abnormalities.

Angiography was performed according to the Judkins technique. Angiography films were studied separately by two interventional cardiologists at the center. The intensity of CAD was expressed in percentage and the number of involved vessels was recorded as follows: 1) Less than or equal to 50%; 2) 50% to 70%; and 3) More than 70%.

A complete history was taken from the patients, including all risk factors (age, gender, diabetes mellitus, hypertension, dyslipidemia, smoking) and drug intake history. Patients with hepatocellular disorders, acute coronary syndrome in the past three months, liver problems (liver function tests >2-fold normal level), kidney problems (creatinine >2 mg/dL), malignant disease, erythrocyte diseases, connective tissue diseases and history of alcohol consumption were excluded from the study.

After the angiography, the patients were divided into two groups (CAD and non-CAD) based on the angiography results. The criterion for entering into the CAD group is occlusion of at least 50% of the coronary artery lumen, including left anterior descending, left main coronary artery, left circumflex branch, right coronary artery, or their main branches. Finally, total, direct, and indirect bilirubin levels were compared between the groups.

## Statistical analysis

Quantitative variables were reported as Mean±SD and qualitative variables were provided as numbers (percentages) in the form of appropriate tables and graphs. To compare the mean of bilirubin between patients with and without CAD, if the data was normal, the independent t-test was used, and if it was not normal, the Mann-Whitney test was used. The normality of the data was tested using the Smirnov-Kolmogorov test. The  $P \leq 0.05$  was considered statistically significant. The data were then imported into the SPSS software, version 20 for analysis.

## Results

A comparison of demographic and disease-related variables between the two groups is provided in Table 1. Of the 140 studied patients, 70 were in the group of patients with CAD involvement and 70 were in the group of patients without CAD. The mean age of subjects was  $55.32 \pm 10.71$  years (minimum=30 and maximum=77) which was statistically significant ( $P < 0.001$ ). Meanwhile, 73(52.1%) patients were male and 67(47.9%) were female in both groups. A significant relationship

was observed only in the diabetes history variable between the two groups ( $P = 0.02$ ).

According to Table 2, the mean total bilirubin level between the groups was  $0.74 \pm 0.57$  mg/dL with a minimum amount of 0.2 and a maximum amount of 4.4 mg/dL. No significant difference was observed between the groups ( $P = 0.79$ ).

According to Table 3, the mean direct bilirubin level between the groups was  $0.22 \pm 0.07$  mg/dL with a minimum amount of 0.1 and a maximum amount of 0.5 mg/dL. No significant difference was observed between the groups ( $P = 0.77$ ).

According to Table 4, the mean indirect bilirubin levels between the groups was  $0.48 \pm 0.45$  mg/dL with a minimum amount of 0.1 and a maximum amount of 4.1 mg/dL. No significant difference was observed between the groups ( $P = 0.77$ ).

The mean total ( $0.77 \pm 0.57$  vs  $0.71 \pm 0.57$ ) and indirect bilirubin level ( $0.53 \pm 0.53$  vs  $0.44 \pm 0.35$ ) was high in men compared to women and mean direct bilirubin was simi-

**Table 1.** Comparison of demographic and disease-related variables between the two studied groups

Variables		No. (%) / Mean±SD		P
		CAD	Non-CAD	
Gender*	Male	32(45.7)	41(58.5)	0.17
	Female	38(54.3)	29(41.5)	
Age**		$58.64 \pm 9.35$	$52.63 \pm 10.40$	<0.001
Diabetes mellitus*	Yes	22(31.4)	10(14.2)	0.02
	No	48(68.6)	60(85.8)	
Hypertension*	Yes	42(60)	35(50)	0.3
	No	28(40)	35(50)	
Dyslipidemia*	Yes	6(8.57)	6(8.57)	1
	No	64(91.43)	64(91.43)	
Smoking*	Yes	15(21.42)	16(22.8)	1
	No	55(78.58)	54(77.2)	

CAD: Coronary artery disease; SD: Standard deviation.

\*Chi-square, \*\*Independent t-test.

**Table 2.** Comparison of mean total bilirubin level between the two groups (independent t-test)

Total Bilirubin	Mean±SD	Minimum (mg/dL)	Maximum (mg/dL)	Range of Changes (mg/dL)	P
CAD group	$0.75 \pm 0.6$	0.3	4.4	4.1	0.79
Non-CAD group	$0.72 \pm 0.54$	0.2	4	3.8	
Total	$0.74 \pm 0.57$	0.2	4.4	4.2	

CAD: Coronary artery disease.

**Table 3.** Comparison of mean direct bilirubin level between the two groups (independent t-test)

Direct Bilirubin	Mean±SD	Minimum (mg/dL)	Maximum (mg/dL)	Range of Changes (mg/dL)	P
CAD group	0.23±0.8	0.1	0.5	0.4	0.77
Non-CAD group	0.22±0.6	0.1	0.5	0.5	
Total	0.22±0.07	0.1	0.5	0.4	

CAD: Coronary artery disease.

lar between men and women (0.23±0.07 vs 0.22±0.07). There was a statistically significant relationship between male and female groups in total and indirect bilirubin (P=0.05) (Table 5).

## Discussion

An increased level of serum bilirubin has been linked to a reduced likelihood of experiencing coronary heart disease and cardiovascular disease [15]. Moreover, this investigation discovered that the average age of individuals in the CAD group, as well as the prevalence of diabetes in that same group, were notably higher compared to the non-CAD group. This finding suggests that age and diabetes are significant risk factors in the development of CAD. According to a previous study, coronary heart disease is a complex condition arising from the presence of coronary atherosclerosis, with advanced age, hyperlipidemia, hypertension, smoking, and diabetes serving as its primary risk factors [16]. These findings are in line with the studies conducted by Abbasi Ranjbar et al. [17]

and Yu et al. [18], which demonstrate that individuals with higher angiography scores exhibit older age and a higher prevalence of diabetes compared to the rest of the study participants.

The two groups in our study exhibited no significant correlation regarding gender, hypertension history, smoking, and dyslipidemia history. This finding contradicts a study conducted by Omid et al. [19], which demonstrated that males tend to develop cardiovascular disease at an earlier age and have a greater likelihood of CAD development than females. Conversely, the research conducted by Nabat Chian et al. [20] indicated that the CAD group consisted of a larger number of men and had a higher prevalence of hypertension and smoking compared to the non-CAD group. Furthermore, another study revealed that being male is a significant risk factor for CAD [21].

**Table 4.** Comparison of mean indirect bilirubin level between the two groups

Indirect Bilirubin	Mean±SD	Minimum (mg/dL)	Maximum (mg/dL)	Range of Changes (mg/dL)	P
CAD group	0.51±0.55	0.1	4.1	4	0.92
Non-CAD group	0.45±0.32	0.2	1.5	1.3	
Total	0.48±0.45	0.1	4.1	4	

CAD: Coronary artery disease.

Notes: Independent t-test.

**Table 5.** Comparison of total, direct, and indirect bilirubin levels between men and women (independent t-test)

Bilirubin	Mean±SD		P
	Men	Women	
Total bilirubin	0.77±0.57	0.71±0.57	0.05
Direct bilirubin	0.23±0.07	0.22±0.07	0.38
Indirect bilirubin	0.53±0.53	0.44±0.35	0.05

The current investigation discovered that the average concentration of total bilirubin was higher in the group with CAD compared to the group without CAD. However, no statistically significant association was observed between the two groups. These findings are in line with a prior study that indicated no significant variation in the mean total bilirubin levels between individuals with and without coronary artery disease [22], suggesting that measuring serum bilirubin levels may not serve as a reliable predictor of coronary vessel disease. Nonetheless, other research has demonstrated that total bilirubin could potentially contribute to CAD prevention by increasing high-density lipoprotein-cholesterol levels and reducing inflammation [5]. Furthermore, there is evidence suggesting that the severity of coronary atherosclerosis in patients with stable CAD is negatively correlated with the level of total serum bilirubin, independent of other factors [23]. Additionally, a study conducted by Akboga revealed an inverse relationship between bilirubin and the extent of coronary artery involvement, which can be attributed to the antioxidant and anti-inflammatory properties of bilirubin [23].

The present investigation revealed a higher presence of both direct and indirect bilirubin in the CAD group compared to other groups; however, no statistically significant association was detected between the two groups. This finding is in line with a previous study [20] that also found no significant relationship when measuring direct or indirect bilirubin between CAD and non-CAD groups. In contrast to our study, studies conducted by Salari et al. [24] and Taban et al. [5] demonstrated a significant correlation between direct bilirubin levels and the severity of coronary artery involvement. Additionally, our results demonstrated a significant relationship between total and indirect bilirubin levels in both men and women, with women exhibiting lower levels compared to men. In contradiction to this finding, a study conducted by Lan et al. [25] reported higher levels of serum total bilirubin and direct bilirubin in males compared to females. These dissimilarities between genders can be attributed to the influence of estrogen on bilirubin levels [26].

## Conclusion

The findings from this study indicate no notable correlation between bilirubin levels and the development or exacerbation of CAD; however, evaluating bilirubin levels may hold significance when considering factors, such as the number of affected vessels, the intricacy of vessels, and major hospital occurrences in patients with higher bilirubin levels. Consequently, bilirubin levels

may act as a predictive element, in conjunction with other influential factors, in determining the number of coronary arteries affected and the severity of their involvement.

## Study limitations

There are notable limitations to our study. The number of patients included was low. Therefore, it is advised that future studies should have a larger sample size and consider utilizing a wider range of clinical and laboratory parameters. Accordingly, it may be possible to establish bilirubin levels as a treatment objective, and as an indicator for assessing the severity of CAD and managing incidents within hospitals. Additionally, conducting further research could lead to the discovery of new biomarkers for identifying cardiovascular risk factors, foreseeing cardiovascular diseases, and monitoring outcomes. Consequently, it is recommended that additional studies be undertaken to validate the findings of our current study.

## Ethical Considerations

### Compliance with ethical guidelines

This study was approved by the Ethics Committee of [Urmia University of Medical Sciences](#) (Code: IR.UMSU.REC.1398.043).

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### Authors' contributions

Conceptualization and study validation: Hamidreza Samimagham; Supervision: Mahmood Khayatian and Mahsa Rahimzadeh; Implementation, data analysis and data interpretation: Elham Ghazanfari; Writing and review: Hossein Montazerghaem; Final Approval: All authors.

### Conflict of interest

The authors declared no conflict of interest.

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