

Original Article

The Relation of Serum Bilirubin Level with the Severity and Complexity of Coronary Artery Disease and Long-term Outcomes in the Patients Undergoing Primary Percutaneous Coronary Intervention

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Background and aims: Bilirubin has been considered an antioxidant that protects against atherosclerosis. The aim of this study was to evaluate the relationship of serum bilirubin level with the severity and complexity of coronary artery disease (CAD) and long-term outcome in the patients undergoing primary percutaneous coronary intervention (PCI).

Materials and Methods: This prospective cohort study was performed on 136 patients with STEMI undergoing PCI in Heshmat Hospital, Rasht, Iran during 2 years. The complexity was calculated by using dedicated software of the syntax score and the severity of CAD was determined based on the number of vessel involvement. Also, long-term outcomes were evaluated by major adverse cardiac events (MACE) after one year.

Results: The mean total bilirubin level was 0.95 ± 0.69 mg/dl; 60.3% of the patients were in the low range of syntax score (0-22) and 38.2 % had two vessels involved. In examining the relationship between serum bilirubin level with the severity and complexity of CAD and MACE, there was no statistically significant association ($p > 0.05$); but, the majority of the patients with high bilirubin levels had a lower number of vessels involved and the complexity of the vessels; Also, MACE was observed in the patients with low bilirubin levels.

Conclusions: According to the results, attention to serum bilirubin level could be important in CAD and the protective effect of bilirubin can be used for new therapeutic decisions. Further studies on a larger sample of patients have been recommended to confirm and complete these findings.

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Introduction

Coronary artery disease (CAD) is one of the most common causes of death worldwide. In 2010, about 7 out of total 53 million deaths were due to ischemic heart disease [1]. Heart muscle as an oxidative tissue and with constant activity is one of the tissues susceptible to oxidative damage caused by reactive oxygen species such as superoxide, hydrogen peroxide and hydroxyl [2]. Oxidative stress plays an important role in the pathogenesis of degenerative diseases or chronic such as atherosclerosis, diabetes and cancer [3, 4]. Oxidative stress as a result of an imbalance between the production of free radicals and reactive oxygen species on the one hand and the antioxidant defense system on the other hand. In other words, aerobic biological systems in order to cope with free radicals and reactive oxygen species, antioxidant defense mechanisms designed to neutralize the deleterious effects of these factors attacker, or to a minimum [4].

New scientific studies have suggested that bilirubin is a potential physiological antioxidant that can be an important protective role against atherosclerosis, CAD and inflammation take over [5]. 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 metabolism has 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 [9-12]. Atherosclerosis and inflammation are associated with free oxygen and peroxy radicals' formation [13, 14]. Arterial protective responses and adjustment against oxidative stress have important roles in atherosclerosis prevention [15]. 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 [16-20]. Considering the high prevalence of CAD and impact of bilirubin level on severity of the disease and its consequences, this study was performed to assess bilirubin levels in the patients with STEMI who underwent primary percutaneous coronary intervention (PCI), its relation with the complexity and severity of coronary vessel involvement and to determine its long-term effects in such patients.

Materials and Methods

The study was performed as a prospective cohort on 168 patients with STEMI who underwent primary PCI in the Heshmat Hospital (Rasht, Iran) during 2 years. The patients with hepatocellular disorder ($LFT \geq 2$

fold above the normal level), patients suffering from malignancy, those who had an alcohol abuse history and severe heart failure ($EF \leq 30\%$), acute coronary syndrome in past three months, kidney problem (creatinine >2 mg/dl), erythrocyte diseases and connective tissue diseases were excluded from the study. After performing the exclusion criteria, 136 patients were registered. All the patients with diagnosis of STEMI, including: having chest-pain for more than 20 minutes, ST segment elevation of more than 1 mm in at least 2 adjacent leads on electrocardiography, or the patients with new LBBB, underwent primary PCI within 12 hours of the diagnosis.

All these patients received stat dose of 325 mg aspirin and 600 mg clopidogril. Maintenance dose: 150 mg clopidogril for one week, then 75 mg clopidogril for the period of a month up to 1 year, according to their stent type [21]. Angiography was done by femoral approach and a dose of 5000 IU heparin was given to all patients before the procedure. Successful PCI was defined as $<50\%$ residual of in-segment stenosis.

The angiography films were studied and the number of vessels with $\geq 50\%$ involvement and minimum diameter of 1.5 mm was determined. Syntax score was calculated separately for each patient according to the software (<http://syntax.score.com>).

Total bilirubin level was measured by the standard method of photometry in the serum of all patients and then the patients were divided into two groups, according to their bilirubin levels: patients with high total bilirubin (>1 mg/dl) and the patients with a low level total

level (≤ 1 mg/dl). Demographic information, including age, gender, body mass index (BMI), diabetes mellitus, and hypercholesterolemia were initially obtained in specified forms. All the patients were followed up after a year by making phone calls, free visits and echocardiography. Long-term outcomes were evaluated by major adverse cardiac events (MACE), including death and myocardial infarction (re-MI), stroke and stent thrombosis. Death count was determined by death due to cardiovascular etiologies or death with no specific cause. Stent thrombosis was defined as complete occlusion on angiography.

Re-MI was defined as creatine kinase-MB ≥ 2 folds above the upper normal limit. ST-segment elevation and stroke were described as physical disability related to neurologic issues. This study was confirmed by the Ethics Committee of Guilan University of medical sciences research and technology assistance and informed consent forms were obtained from each patient.

Statistical analysis

After collecting, the data were analyzed by descriptive and inferential statistics (Kolmogorov-Smirnov, t-test, Chi-square test, Fisher exact test and logistic regression). P-value ≤ 0.05 was considered statistically significant.

Results

Demographic characteristics of the patients with a high total bilirubin level and low total bilirubin level are shown in table 1. The findings revealed that the average age of patients being studied was 58.71 ± 13.13 years. The average bilirubin level was 0.95 ± 0.69 mg/dl. Most of the patients were male (70.6%), with bilirubin levels of less

than 1 mg/dl (70.6%), left ventricular ejection fraction of 30-45% (50%), mild complexity of coronary vessels, with a syntax score of 0.22 (60.3%), two vessels disease (38.2%), did not suffer from diabetes mellitus (75%) and hypercholesterolemia (70.6%).

After assessment of the relation between serum bilirubin level and demographic information associated with the disease, only left ventricular ejection fraction has a statistically significant relation with the serum total bilirubin level ($p < 0.03$). Although no significant relation was observed between the severity of coronary vessel involvement, coronary vessel complexity and MACE, but the findings revealed that the majority of the patients with high serum total bilirubin levels had less number of coronary vessels involved (43.5% with one affected vessel) and less vessel complexity (syntax score 0-22 63.6%). MACEs did not occur in the patients with a high total bilirubin level, but were observed in 7.6% of the patients with a low total bilirubin level. In a regression logistic analysis (with Enter method) all demographic variables associated with the disease were analyzed. In this analysis, bilirubin level was not a predictive factor for any of the three variables: severity of coronary vessels involvement, complexity of coronary vessels and MACE; but BMI and left ventricular ejection fraction were known as predictive factors for severity of coronary vessels involvement, as well as hypercholesterolemia as a predictive factor for complexity of coronary vessels (Tables 2 and 3).

Discussion

Serum bilirubin level, as a potentially antioxidant endogenous compound, is related to a number of diseases associated with oxidative stress, including atherosclerosis cancer and neurodegenerative diseases [22]. This study was performed in order to determine the relation between serum bilirubin level with the complexity and severity of coronary vessels involvement and its long-term impacts on the patients suffering from STEMI underwent PCI. This study is among the few studies, which have explored the relation between total bilirubin levels and MACE, one year after PCI, and also its relation with complexity of vessels in the patients suffering from Acute STEMI. No statistical significant was observed in the present study during exploring the relation between severity of coronary vessel involvement and total bilirubin level, but most patients with high serum bilirubin level had less affected vessels. In this regard, the results of study conducted by Einollahi et al, revealed that the importance of measuring serum bilirubin levels as a predictor of coronary vessel disease, had no significant difference in verge, total bilirubin amount between the affected and the healthy groups, although a mild reverse relation between serum bilirubin and total bilirubin levels was observed [22], which were congruent with the results of the present study in a study conducted by Sadeghi et al. to evaluate the relation between serum bilirubin levels with coronary diseases based on angiographic findings, a significant difference was observed between verge total bilirubin

levels in two groups: patients with normal angiography and patients with 3 vessels involved. A reverse relation was observed in this study between total bilirubin levels, number of vessels affected the severity of stenosis and type of coronary vessel [23]. In a study

conducted by Ghem et al. the average bilirubin level was higher in the control group as well. And bilirubin level reduction was related to severity and more prevalence of coronary vessel disease [15].

Table 1. Correlation of demographic and disease-related variables with bilirubin level in studied subjects

Variables	Low bilirubin N (%) 90 (66.2%)	High bilirubin N (%) 46 (33.81%)	P-value
Gender			
Female	26 (28.9)	14 (30.4)	Chi-square 0.85
Male	64 (71.1)	32 (69.6)	
Age (Mean±SD)	59.55±13.28	57.04±12.97	t-test 0.29
BMI (Mean±SD)	26.74±5.19	26.42±3.73	t-test 0.73
Hypercholesterolemia			
Yes	30 (33.3)	10 (21.7)	Chi-square 0.16
No	60 (66.7)	36 (78.3)	
Diabetes mellitus			
Yes	20 (22.2)	14 (30.4)	Chi-square 0.29
No	70 (77.8)	32 (69.6)	
LVEF			
Less than 30	10 (11.1)	2 (4.3)	Chi-square 0.03*
30-45	38 (42.2)	30 (65.2)	
More than 45	42 (46.7)	14 (30.4)	
Number of involved vessels			
1	28 (31.1)	20 (43.5)	Chi-square 0.17
2	34 (37.8)	18 (39.1)	
3	28 (31.1)	8 (17.4)	
Complexity of coronary vessel			
0-22	54 (60)	28 (63.6)	Fisher exact test 0.53
22.1-32	32 (35.6)	16 (36.4)	
More than 32	4 (4.4)	0	
MACE			
Yes	6 (6.7)	0	Chi-square 0.09
No	84 (93.3)	46 (100)	

*Significance level: P-value≤0.05; LVEF=Left ventricle ejection fraction; BMI=Body mass index

Table 2. Regression coefficients of severity of coronary vessels involvement predictors by Enter method

Predictors	B	SE	OR	95% Confidence Interval of OR		P-value
				Min	Max	
LVEF	Less than 30	-21.96	1.63	0.001	0.001	0.99
	30-45	-2.19	0.64	0.11	0.03	0.001*
	More than 45	Reference	-	-	-	-
BMI	0.13	0.07	1.14	0.99	1.029	0.05*

*Significance level: P-value \leq 0.05; LVEF=Left ventricle ejection fraction; BMI=Body mass index

Table 3. Regression coefficients of complexity of coronary vessels predictors by Enter method

Predictors		B	SE	OR	95% Confidence Interval of OR		P-value
					Min	Max	
Hypercholesterolemia	Yes	-1.37	0.69	0.25	0.07	0.97	0.04*
	No	Reference	-	-	-	-	-

*Significance level: P-value \leq 0.05

Likewise, in the present study, in the regression model, total bilirubin level was not known as a predictor for severity of coronary disease. In this regard, the results of Einollahi et al. study revealed bilirubin measurement can be important as a predictive index for CAD incidents [22]. Also, in the study conducted by Ghem et al, bilirubin was known as a new predictive marker for CAD [15]. Also, Erdogan et al. showed that high levels of bilirubin can serve as a predictive factor for CAD [24]. These findings are different from the results of the present study.

While studying the relation between coronary vessel complexity (according to syntax score) and serum total bilirubin level, no significant relation was found, but most patients with bilirubin levels had less complexity of the vessels. Also, total bilirubin level was not known as a predictor for complexity of

coronary vessels in the regression model. In this regard, the results of a study conducted by Sahin et al. on NSTEMI patients revealed that total bilirubin level was significantly higher in the group with a high syntax score, and a significant relation was observed between total bilirubin level and syntax score [25], which incongruent with the results of the present study. This incongruity may be justified by the difference in studying the patients group. In Turfan et al. study aligned with results of the present study, total bilirubin level in the group with a low Syntax score was significantly higher than other groups, and total bilirubin was known as an independent predictor for syntax score [26]. Also, in Nabatchian et al. study the results showed that measuring bilirubin as a marker for the prediction of CAD is important [5]. No significant relation was observed in reviewing the relation of MACE with serum

total bilirubin level, but no major cardiac events were seen in the patients with high bilirubin levels, but were observed in the patients with low bilirubin level. Total bilirubin was not known as a predictor for MACE as well. In this regard, in Sahin et al. study no significant relation was observed between the numbers of cardiac events in two groups with high and low bilirubin levels [25], which is congruent with the results of the present study. In contrast to the findings of this study, the results of Gul et al. revealed that number of hospital events was significantly higher in the group with high total bilirubin compared to the group with low total bilirubin. In regression analysis, it was observed a relation between high total bilirubin level and major hospital events. Total bilirubin was known as predictor for hospital events, but no significant relation was found between total bilirubin level and long-term outcomes [27]. In other study, it has been shown that total levels of bilirubin have a prognostic association with MACE in male patients with myocardial infarction [28].

One of the limitations of the present study was low number of the patients. It's recommended that larger studies using more samples and more extensive clinical/laboratory parameters be conducted. That may lead to the bilirubin levels considered as a treatment goal as well as an index for determining CAD severity and

controlling hospital events. Also, new markers for cardiovascular risk factor identification, predicting cardiovascular diseases and controlling outcomes could be identified; performing more studies is also recommended for confirming the results of the present study.

Conclusions

Although total bilirubin level in the present study was not known as a predictive factor for complexity and severity of coronary vessel involvement and MACE, but the results revealed that taking bilirubin levels under consideration according to less number of affected vessels, complexity of vessels and major hospital events in patients with higher bilirubin levels can be important. This predictive impact of bilirubin can be applied to new treatment plans. Furthermore, other factors such as, left ventricle ejection fraction, BMI and hypercholesterolemia are known as predictors of complexity and severity of coronary vessel involvement and MACE; planning the treatment and preventive interventions accordingly can be helpful for the patients.

Conflict of Interest

None declared.

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References

- [1]. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the Global Burden of Disease study 2010. *Lancet* 2012; 380(9859): 2095-128.
- [2]. Rahbar S, Ahmadiasl N. Effect of Long Term Regular Resistance Exercise on Heart Function and Oxidative Stress in Rats. *Ardebil University of Medical Sciences Journal* 2013; 12(3):256-64.
- [3]. Karajibani M, Montazerifar F, Hashemi M, Bolouri A, Dikshit M. Study of oxidative stress in

- patients with angina pectoris admitted to cardiac care unit (CCU) at khatam-al-Anbia hospital in zahedan. *Zahedan Journal of Research in Medical Sciences* 2013; 15(3): 20-5.
- [4]. Steghamati A, Zarban A, Doosti M. Evaluation of antioxidant status and oxidative stress index in patients with Type II diabetes mellitus. *Iran J Endocrinol Metabolism*. 2002; 3(4): 239-45.
- [5]. Nabatchian F, Einollahi N, Boroomand MA, Abbasi S. Can bilirubin be an indicator for predicting coronary artery disease? *Payavarde Salamat* 2014; 7(2): 91-100.
- [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. *Exper Gerontol 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. *Arterioscler Thromb Vasc 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. *Arterioscler Thromb Vasc Biol*. 1996; 16(2): 250-55.
- [10]. Han SS, Na KY, Chae DW, Kim YS, Kim S, Chin HJ. High serum bilirubin is associated with the reduced risk of diabetes mellitus and diabetic nephropathy. *Tohoku J Exper Med*. 2010; 221(2): 133-40.
- [11]. Chin HJ, Song YR, Kim HS, Park M, Yoon HJ, Na KY, et al. The bilirubin level is negatively correlated with the incidence of hypertension in normotensive Korean population. *J Korean Med Sci*. 2009; 24(Suppl 1): S50-6.
- [12]. 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.
- [13]. Hansson GK, Robertson AK, Soderberg-Naucler C. Inflammation and atherosclerosis. *Annu Rev Pathol Mech Dis*. 2006; 1: 297-329.
- [14]. Berliner JA, Navab M, Fogelman AM. Atherosclerosis: basic mechanisms. Oxidation, inflammation, and genetics. *Circulation* 1995; 91(9): 2488-496.
- [15]. Ghem C, Sarmiento-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.
- [16]. 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 Biol Chem*. 1994; 269(24): 16712-6719.
- [17]. Mayer M. Association of serum bilirubin concentration with risk of coronary artery disease. *Clin Chem*. 2000; 46(11): 1723-727.
- [18]. Nakayama M, Takahashi K, Komaru T. Increased expression of hemeoxygenase-1 and bilirubin accumulation in foam cells of rabbit atherosclerotic lesions. *Arteriosclerosis, Thrombosis, and Vascular Biology* 2001; 21(8): 1373-377.
- [19]. Stocker R. Antioxidant activities of bile pigments. *Antioxidants and Redox Signaling* 2004; 6(5): 841-49.
- [20]. Rigato I, Ostrow JD, Tiribelli C. Bilirubin and the risk of common non-hepatic diseases. *Trends Mol Med*. 2005; 11(6): 277-83.
- [21]. Braunwald E, Zipes DP, Libby P, Bonow R. *Braunwald's Heart Disease: A text book of Cardiovascular Medicine*. Vol 2. 7th. Philadelphia: Saunders. 2004.
- [22]. Einollahi N, Boromand MA, Nabatchian F, Abbasi S. The importance of measuring the concentration of serum bilirubin as a marker for predicting coronary artery disease. *J Lab Diagnostic*. 2015; 25: 6-13.
- [23]. Taban Sadeghi MR, Golmohamadi A, Parvizi R, Kezerlou Aghdam N, 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): 130-34.
- [24]. Erdogan T, Cicek Y, Kocaman SA. Increased serum bilirubin level is related to good collateral development in patients with chronic total coronary occlusion. *Internal Med J*. 2012; 51(3): 249-55.
- [25]. Sahin O, Akpek M, Karadavut S, Cosgun MS, Savas G, Sarli B, et al. Relation between Serum Total bilirubin Levels and Severity of Coronary Artery Disease in Patients with Non ST Elevation Myocardial Infarction. *J Am College Cardiol*. 2013; 62(18): 217-18.
- [26]. Turfan M, Duran M, Poyraz F, Yayla C, Akboga MK, Sahinarslan A, et al. Inverse relationship between serum total bilirubin levels and severity of disease in patients with stable coronary artery disease. *Coronary Artery Disease* 2013, 24(1): 29-32.
- [27]. Gul M, Uyarel H, Ergelen M, Akgul O, Karaca G, Turen S, et al. Prognostic value of total bilirubin in patients with ST-segment elevation acute myocardial infarction undergoing primary coronary intervention. *Am J Cardiol*. 2013; 111(2): 166-71.
- [28]. Baumann S, Huseynov A, Koepp J, Jabbour C, Behnes M, Becher T, et al. Comparison of serum uric acid, bilirubin, and C-reactive protein as prognostic biomarkers of in-hospital MACE between women and men with ST-segment elevation myocardial infarction. *Angiology* 2016; 67(3): 272-80.