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A. Erkan · B. Ekici · M. Uğurlu · G. İş · R. Şeker · S. Demirtaş · Ş. Korkmaz
Ufuk University School of Medicine, Ankara

The role of bilirubin and its protective function against coronary heart disease

Atherosclerotic cardiovascular disease is the leading cause of morbidity and mortality both in industrialized and developing countries. Atherosclerosis is a chronic inflammatory disease of the arterial wall, which commences as endothelial dysfunction and then proceeds to involve inflammation and deposition as well as peroxidation of lipids [1].

Bilirubin, a bile pigment formed as the end product of the breakdown of heme, is known to be an important endogenous antioxidant [2]. Owing to this property, it may limit lipid peroxidation [3] and retard the progression of atherosclerosis [4, 5]. Indeed, previous studies have reported an inverse relationship between serum bilirubin levels and the risk of coronary artery disease (CAD) [6, 7, 8, 9, 10]. Mildly elevated bilirubin levels in Gilbert's syndrome are associated with an ischemic heart disease risk of 2% compared to 12.1% in the general population [11]. A recent study revealed that total bilirubin levels independently predict adverse cardiac events in ST-segment elevation myocardial infarction (STEMI) patients who undergo primary percutaneous coronary intervention (PCI). Nevertheless, there was no association with long-term mortality [12].

Taking into account that atherosclerosis is a complex process that is initiated and accelerated by diverse risk factors, we aimed to test the antiatherosclerotic effects of bilirubin in a study population with multiple risk factors for CAD. Therefore, we investigated the relationship between serum bilirubin levels and the angiographic extent and severity of CAD in a group of patients with multiple cardiovascular risk factors.

Patients and methods

Study population

We reviewed 299 consecutive patients older than 18 years who underwent coronary angiography in 2010. All patients had symptoms suggestive of ischemia and/or they had a positive noninvasive stress test result. Of these patients, 221 were included in the study. Seventy-six of the patients had normal coronary angiograms and served as the control group. The remaining 145 patients with documented CAD and two or more cardiovascular risk factors constituted the final study group.

The study protocol was approved by the local ethics committee. Written informed consent was obtained from each study participant. Demographic, anthropometric, and clinical characteristics were recorded for each patient.

Patients with previous coronary revascularization (PCI or coronary artery by-pass graft surgery), significant valvular disease, cardiomyopathy, acute myocardial infarction in the previous 30 days, any sign of hepatobiliary disease including serum AST or ALT levels greater than two times the upper limit of normal (ULN), serum ALP or GGT levels greater than ULN, seropositivity for viral hepatitis, and renal failure as documented by an MDRD glomerular filtration rate less than 30 ml/min/1.73 m^2 were excluded from the study.

A Gensini score was determined for each angiogram as explained in the "Coronary angiography" section. The study group (n=145) was further classified according to the Gensini score as follows: group 1 if Gensini score was 1–19 (minimal CAD, n=82), and group 2 if Gensini score was 20 or higher (significant CAD, n=63).

Coronary angiography

Selective coronary angiography was performed using the Judkins technique and a standard angiographic system (GE Innova 2100-IQ, WI, USA). At least four views of the left anterior descending artery (LAD) and the circumflex artery (Cx) were obtained. At least two views of the right coronary artery were obtained.

Each angiogram was reviewed by two interventional cardiologists blinded to the study data. The extent and severity of CAD were evaluated using the Gensini scoring system as previously described [15]. The Gensini score for each angiogram was the average of the scores calculated by the two observers. In case of a discrepancy between the observers, the angiogram was rescored and the new average was assigned as the Gensini score.

Tab. 1 Baseline characteristics of patients according to CAD severity

	Controls	Minimal CAD	Significant CAD	p value
Gender (M/F)	29/47	48/34	45/18	**0.000**
Hypertension	35 (46%)	58 (71%)	46 (73%)	**0.001**
Hyperlipidemia	26(34%)	44(53%)	36(57%)	**0.011**
Diabetes mellitus	14(18%)	29(35%)	28(44%)	**0.003**
Smoking	22(28%)	38(46%)	31(49%)	**0.026**
Age (years ±SD)	53.7±12.6	64.5±10.3	65.4±10.6	**0.000**
Total bilirubin (mg/dl)	0.56±0.29	0.61±0.38	0.61±0.34	0.861
Direct bilirubin (mg/dl)	0.19±0.11	0.22±0.13	0.23±0.12	0.195
Total cholesterol (mg/dl)	191.1±40.6	189.6±42.3	176.8±38.4	0.085
LDL-cholesterol (mg/dl)	120.5±33.4	121.5±37.2	109.8±33.1	0.099
HDL-cholesterol (mg/dl)	46.9±12.7	43.5±13.5	40.1±12.4	**0.003**
Triglyceride (mg/dl)	128.2±70.9	153.8±74.0	148.3±70.6	**0.023**
GGT (U/l)	21.8±9.1	23.7±8.9	22.5±9.6	0.364
Uric acid (mg/dl)	5.0±1.3	5.7±1.6	5.6±1.3	**0.006**
Hemoglobin (gr/dl)	13.3±1.7	13.3±1.8	13.3±1.7	0.987
MPV (fl)	8.7±1.1	8.7±1.1	8.8±1.0	0.941
hsCRP (mg/l)	3.5±3.3	5.2±5.9	4.7±6.4	0.295
CrCl (ml/min/1.73m^2)	100.3±35.4	86.5±28.1	83.7±23.8	**0.004**
TC/HDL	4.3±1.3	4.7±1.6	4.7±1.5	0.174

CAD coronary artery disease, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *GGT* gamma-glutamyl transferase, *MPV* mean platelet volume, *hsCRP* high-sensitive C-reactive protein, *CrCl* creatinine clearance, *TC* total cholesterol, *SD* standard deviationNumbers in parentheses denote percentages

Laboratory tests

Blood samples were obtained from the antecubital vein after 12 h of fasting. Serum levels of total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, fasting blood glucose, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), and total and direct bilirubin were determined using standard biochemical methods in an automated system (Integra 800, Roche Diagnostics, USA). The serological status for viral hepatitis was also noted.

Statistical analysis

All analyses were performed with the PASW Statistics version 18 software package (Chicago, IL, USA). The normal distribution of variables was verified with the Kolmogorov–Smirnov test. Spearman's rho correlation was used when one or both of the variables were not normally distributed. Comparisons between the groups were made either with ANOVA and the Student's t test when the distribution was normal or with the Kruskal–Wallis test and Mann–Whitney U test when the distribution was not normal. Binary comparisons among the groups were made through Bonferroni correction. The chi square (χ^2) test was used to investigate whether distributions of categorical variables differed within groups. Multivariate logistic regression was performed to assess the potential confounding effects of age, gender, diabetes mellitus (DM), hypertension (HT), hyperlipidemia (HL), smoking, creatinine clearance, and biochemical parameters. All analyses were stratified according to the severity of the CAD. A p value less than 0.05 was considered statistically significant.

Results

General characteristics

In all, 299 patients were screened of whom 78 were excluded because of the presence of one or more exclusion criteria. Of the 221 patients enrolled, 122 (55.2%) were male. The mean age was 61.0±12.4 years (range, 26–86 years). The overall average Gensini score was 14.79±37.55. According to the Gensini score, 76 patients were assigned to the control group (normal coronary arteries). The remaining 145 patients constituted the study group, which was further divided into two groups. Eighty-two patients were assigned to group 1 (minimal CAD), and 63 patients were assigned to group 2 (significant CAD). Patients in the study group (groups 1 and 2) were older, more likely to smoke, and more likely to be male, diabetic, and hypertensive when compared to the control group (**Tab. 1**). Serum levels of HDL-cholesterol were higher in the control group, whereas uric acid and triglyceride levels were higher in the study group. Serum LDL-cholesterol levels were numerically lower in the significant CAD group when compared to the minimal CAD and control groups; however, there was no significant difference across the groups. MDRD was significantly lower in the study group than in the control group (**Tab. 1**). There were no significant differences between the groups with respect to statin, angiotensin converting enzyme inhibitor, or aspirin use (data not shown).

Serum bilirubin levels and the Gensini score

Total and direct serum bilirubin levels did not differ significantly between the control group, group 1, and group 2 (**Tab. 1**).

There was a moderate and significant positive correlation between direct bilirubin levels and the Gensini score (r=0.158, p=0.019). This correlation persisted after adjustment for baseline characteris-

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The role of bilirubin and its protective function against coronary heart disease

Abstract

Background. Atherosclerotic cardiovascular disease is the leading cause of morbidity and mortality both in industrialized and developing countries. Atherosclerosis is a chronic inflammatory disease of the arterial wall, which also involves deposition and peroxidation of lipids. Bilirubin, an important endogenous antioxidant, may limit lipid peroxidation and retard the progression of atherosclerosis. Previous studies have reported an inverse relationship between serum bilirubin levels and the risk of coronary artery disease (CAD). Taking into account that atherosclerosis is a complex process that is initiated and accelerated by diverse risk factors, we aimed to test the antiatherosclerotic effects of bilirubin in a population with multiple risk factors for CAD.
Methods. The study included 221 patients who underwent coronary angiography owing to symptoms suggestive of ischemia and/or positive noninvasive stress test results. Of the patients, 76 had normal coronary angiograms and served as the control group. The remaining 145 patients with documented CAD and two or more cardiovascular risk factors constituted the study group. The study group (n=145) was further classified according to the Gensini score as follows: group 1 if Gensini score was 1–19 (minimal CAD, n=82), and group 2 if Gensini score was 20 or higher (significant CAD, n=63). Biochemical assessments including total and direct serum bilirubin levels were carried out using standard methods in automated systems.
Results. All of the cardiovascular risk factors were found significantly more frequently in the study group (groups 1 and 2) than in the control group. Total and direct serum bilirubin levels did not differ significantly between the control group, group 1, and group 2. There was a moderate and significant positive correlation between direct bilirubin levels and the Gensini score (r=0.158, p=0.019). There was no significant correlation between total bilirubin levels and the Gensini score.
Conclusion. In conclusion, our findings suggest that in the presence of multiple risk factors, similar concentrations of serum bilirubin may not confer the same level of protection against CAD as in an individual with a more favorable risk profile. The relationship between direct bilirubin levels and the Gensini score is unlikely to be causative, given the established antiatherosclerotic effects of bilirubin.

Keywords

Atherosclerosis · Bilirubin · Coronary artery disease · Risk factors · CAD protective function

Rolle des Bilirubins und seiner Schutzfunktion vor koronarer Herzkrankheit

Zusammenfassung

Hintergrund. Die atherosklerotische Herz-Kreislauf-Erkrankung ist führende Ursache bei Morbidität und Mortalität sowohl in Industrie- als auch in Entwicklungsländern. Atherosklerose ist eine chronisch-entzündliche Erkrankung der Arterienwand, bei der eine Ablagerung und Peroxidation von Lipiden stattfindet. Bilirubin, ein wichtiges endogenes Antioxidans, kann die Lipidperoxidation einschränken und das Fortschreiten der Atherosklerose verzögern. In früheren Studien wurde über ein umgekehrtes Verhältnis zwischen Serumbilirubin und dem Risiko einer koronaren Herzkrankheit (KHK) berichtet. Unter Berücksichtigung dessen, dass die Atherosklerose ein komplexer Vorgang ist, der durch verschiedene Risiken ausgelöst und beschleunigt wird, war es Ziel der Studie, die antiatherosklerotischen Wirkungen von Bilirubin in einer Population mit mehreren Risikofaktoren für KHK zu untersuchen.
Methoden. 221 Patienten, bei denen aufgrund ischämieverdächtiger Symptome eine Koronarangiographie erfolgte und/oder ein positives Ergebnis beim nichtinvasiven Belastungstest vorlag, wurden in die Studie aufgenommen. Als Kontrollgruppe dienten 76 Patienten mit normalen Koronarangiogrammen. Die restlichen 145 Patienten mit dokumentierter KHK und 2 oder mehr kardiovaskulären Risikofaktoren bildeten die Studiengruppe. Die Studiengruppe (n=145) wurde nach dem Gensini-Score aufgeteilt: In der Gruppe I lag der Gensini-Score zwischen 1 und 19 (leichte KHK, n=82); in Gruppe II lag der Gensini-Score bei 20 oder darüber (signifikante KHK, n=63). Labortests von biochemischen Parametern, einschließlich Gesamt- und direktem Bilirubin, wurden mittels Standardtestmethoden mit automatisierten Laborsystemen durchgeführt.
Ergebnisse. Alle kardiovaskulären Risikofaktoren traten in der Studiengruppe (Gruppe I und II) im Vergleich zur Kontrollgruppe signifikant häufiger auf. Gesamt- und direktes Bilirubin unterschieden sich nicht signifikant zwischen der Kontrollgruppe, Gruppe I und Gruppe II. Es lag eine mäßige, statistisch signifikante positive Korrelation zwischen direktem Bilirubin und dem Gensini-Score vor (r=0,158; p=0,019). Eine signifikante Korrelation zwischen dem Gesamt-Bilirubin-Wert und dem Gensini-Score bestand nicht.
Schlussfolgerung. Die vorliegenden Ergebnisse weisen darauf hin, dass bei mehreren Risikofaktoren ähnliche Bilirubinspiegel im Serum nicht den gleichen Schutz gegen KHK verleihen wie vergleichsweise bei Individuen mit einem günstigeren Risikoprofil. In Anbetracht der antiatherosklerotischen Wirkungen von Bilirubin ist es unwahrscheinlich, dass das Verhältnis zwischen direktem Bilirubin und dem Gensini-Score ursächlich ist.

Schlüsselwörter

Atherosklerose · Bilirubin · Koronare Herzkrankheit · Risikofaktoren · KHK-Schutzfunktion

tics and traditional risk factors (r=0.153, p=0.026). There was no significant correlation between total bilirubin levels and the Gensini score.

Discussion

Bilirubin, a product of heme catabolism, is known to be a potent endogenous antioxidant [2]. It is well-known that oxidative stress contributes to the atherosclerotic process, particularly through peroxidation of lipids and formation of oxidized LDL. It has been postulated that bilirubin can limit lipid peroxidation [3], which is a crucial step in the atherosclerotic process. Indeed, previous stud-

ies have shown that bilirubin may retard the progression of atherosclerosis [4, 5]. Accordingly, several studies have demonstrated that serum bilirubin concentrations are inversely related to the presence and severity of atherosclerotic CAD [6, 7, 8, 9, 10, 11]. Moreover, bilirubin levels independently predict adverse cardiac events in STEMI patients who undergo primary PCI [12].

In a recent study by Wei et al. [13], total serum bilirubin levels were inversely correlated with the angiographic complexity of CAD. In the same study, direct and indirect bilirubin levels showed no correlation with the angiographic complexity of CAD.

One exception to these previous findings may be the PRIME study, which has described the relationship of serum bilirubin levels and cardiovascular risk as a U-shaped curve, implying that bilirubin exerts a protective effect, yet excessive concentrations may have a detrimental effect [14].

It is known that serum bilirubin levels are modified by genetic factors. Serum bilirubin concentrations are affected by a major locus at the chromosome 2q telomere. A gene in this locus encodes hepatic bilirubin uridine diphosphate-glucuronosyltransferase (UGT1A1). An allele of this gene, designated UGT1A1*28, decreases transcription of the gene. Individuals homozygous for UGT1A1*28 have higher serum bilirubin concentrations. In an analysis of 1,780 unrelated individuals from the Framingham Offspring cohort who had been followed up for 24 years, homozygote UGT1A1*28 allele carriers with higher serum bilirubin concentrations had significantly lower risk of cardiovascular disease [16].

Somewhat contrary to the antiatherosclerotic properties of bilirubin established in the literature, we found a moderate, statistically significant positive correlation between serum bilirubin levels and the Gensini score, which reflects the extent and severity of CAD. Nevertheless, serum bilirubin levels did not significantly differ across the control group and the study groups. In light of previous reports, it is difficult to explain the positive correlation between serum bilirubin levels and the extent and severity of CAD. To the best of our knowledge, this is the first study in the literature demonstrating a positive relationship between serum bilirubin levels and the severity of CAD. However, considering the well-established antioxidant and antiatherosclerotic effects of bilirubin, it is unlikely that the relationship between serum bilirubin levels and the Gensini score is a causal one. To the contrary, it may be asserted that bilirubin could have exerted its protective effect but have failed in the face of significantly more prevalent proatherosclerotic risk factors in the study population, i.e., advanced age, male sex, smoking, and higher blood pressure.

Serum LDL-cholesterol levels were numerically lower in the significant CAD group when compared to the minimal CAD and control groups despite similar rates of statin use across the groups. Nevertheless, this difference was not statistically significant; therefore this paradox is unlikely to have exerted a major effect on the results of the study.

Atherosclerosis and resultant coronary heart disease are the consequences of the complex interplay of many intertwining pathologic processes. While it is generally agreed that bilirubin is an antioxidant that retards the formation of atherosclerosis, our findings suggest that in the presence of multiple risk factors, similar concentrations of serum bilirubin may not confer the same level of protection against CAD as in an individual with a more favorable risk profile. It has been reported that the association of serum bilirubin levels and the lower risk of CAD is as strong as that of smoking, high blood pressure, and low HDL-cholesterol with higher risk of CAD, individually [10, 17]. Nevertheless, it is also well-known that the impact of risk factors is additive. In other words, higher bilirubin levels may neutralize any one of these risk factors, but may not suffice to halt the progression of atherosclerosis in the presence of multiple risk factors.

Study limitations

Our study has certain limitations, including the relatively small sample size. The lack of genetic analysis of UGT1A1*28 may also be considered as a limitation.

Conclusion

In conclusion, our findings suggest that in the presence of multiple risk factors, similar concentrations of serum bilirubin may not confer the same level of protection against CAD as in an individual with a more favorable risk profile.

Corresponding address

A. Erkan
Ufuk University School of Medicine
Mevlana Bulvarı 86-88, Balgat, 06520 Ankara
Turkey
aycanfahri@gmail.com

Conflict of interest. On behalf of all authors, the corresponding author states that there are no conflicts of interest.

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