Interaction Between Serum Bilirubin Level and Lipid Profile¶

SERUM BİLİRUBİN DÜZEYİ VE LİPİD PROFİLİ ARASINDAKİ İLİŞKİ

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Summary

Lipid oxidation, a which is accepted as an important element of arterial plaque formation and atherosclerosis, is involved in the pathophysiology of cardiovascular diseases (CVD). Due to the fact that bilirubin has antioxidant properties, it has recently been suggested that increased physiological concentrations of serum bilirubin may have a protective role in the atherosclerotic process. In addition an inverse relationship between circulatory total bilirubin and increased risk of CVD has been reported. However, information on this topic remains scarce. The aim of this study was to evaluate the relationships between serum bilirubin and lipid profile in middle-aged 250 subjects (135 female).

Serum bilirubin concentrations were measured with colorimetric method and divided into three groups : < 0,4, 0,47-0,53 and > 0,7 mg/dl. Lipid profile parameters which were studied include total cholesterol (TC) , triglycerides (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), apolipoprotein B (Apo B), apolipoprotein A-I (Apo A-I) and lipoprotein (a) [Lp (a)]. When calculated by Pearson correlation coefficients, there was no correlation between bilirubin and lipid profile in three groups. One-way ANOVA variance analysis was also indicated that there was no significant difference between lipid profiles in three groups.

Consequently, it may be said that there is a relationship between bilirubin and CVD, compatible with previous studies, but we have not observed any relationship between bilirubin and lipid profile.

Key Words: Bilirubin, Lipoproteins, Lipids, Cardiovascular disease

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Özet

Arteriyel plak oluşumu ve aterosklerozun önemli bir öğesi olarak kabul edilen lipid oksidasyonu, kardiyovasküler hastalıkların (KVH) patofizyolojisinde rol oynar. Son zamanlarda, bilirubinin antioksidan özelliklerinden dolayı, serum bilirubininin artmış fizyolojik konsantrasyonlarının arteriosklerotik süreçte koruyucu olduğu ileri sürülmektedir. Dolaşımdaki total bilirubin ve artmış KVH riski arasında ters bir ilişki vardır, ancak bu konudaki bilgiler sınırlıdır. Bu çalışmanın amacı orta yaşlardaki 250 insanda (135'i kadın) serum bilirubini ve lipid profili arasındaki ilişkiyi değerlendirmektir.

Kolorimetrik yöntemle ölçülen bilirübin konsantrayonları 0.4'ün altındakiler, 0.47-0.53 arasındakiler ve 0.7'den büyük olanlar şeklinde üç gruba ayrıldı. Lipid profil parametreleri olarak total kolesterol (TC), trigliserit (TG), düşük dansiteli liprotein kolesterol (LDL-C), yüksek dansiteli lipoprotein kolesterol (HDL-C), apolipoprotein B (Apo B), apolipoprotein A-I (A-I) ve lipoporotein (a)) [Lp (a)]. Pearson korelasyon katsayıları hesaplandığında, bu deneklerin bilirubin düzeyleri ve lipid profili arasında önemli bir fark bulunamadı. One-way ANOVA varyans analizi de üç grubun lipid profili arasında önemli bir fark olmadığını gösterdi.

Daha önce yapılan çalışmalarda, bilirubin ve KVH arasında bir ilişkinin olduğu bildirilmiştir. Sadece bilirübin ve lipid profili arasındaki ilişkiyi incelediğimiz çalışmamızda bilirübin ve lipid profili arasında bir ilişki saptamadık.

Anahtar Kelimeler: Bilirubin, Lipoproteinler, Lipidler, Kardiyovasküler hastalıklar.

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Bilirubin is a catabolic product of hemoglobin which is formed in cells of the reticuloendothelial system (1). More recent evidence suggests that bilirubin is a potent physiological antioxidant that may provide important protection against atherosclerosis, CVD and inflammation (2-5). In several studies it has been that different circulating forms of bilirubin are powerful antioxidants: Free bilirubin, albumin-bound bilirubin, conjugated bilirubin and unconjugated bilirubin were all noted to be effective scavengers of peroxyl radicals (6-11). Furthermore, bilirubin, especially with albumin, appears to be cytoprotective (12,13).

Lipids and lipoproteins are important risk factors for CVD. On the basis of the known involvement of oxidized low density lipoprotein (LDL) in formation of atherogenic plaques and the ability of bilirubin to serve as a potent lipid chain-breaking antioxidant under physiological concentrations of plasma bilirubin may reduce atherogenic risk (2, 14, 15). A low concentration of bilirubin might also

prevent solublization of cholesterol and its clearance through the bile, thereby increasing serum cholesterol concentrations (16). Low bilirubin was associated with several cardiovascular risk factors, in particular smoking, low concentrations of high-density lipoprotein cholesterol, low forced expiratory volume in 1 second and low serum albumin (4).

Lipoprotein (a) [Lp(a)] refers to particles formed by the covalent binding of the glycoprotein apoprotein (a) to apoprotein B of LDL by disulfide linkage. Increased plasma concentration of Lp(a) has been associated with increased risk for premature coronary disease in numerous retrospective case-control studies among white subjects and in most prospective studies (17). It has been found that the ability of nonlipid risk factors to increase risk associated with Lp(a) was dependent on the presence of a moderately high total/HDL-C ratio (17, 18).

The aim of this study was to evaluate the relationships between serum bilirubin and lipid profile as cardiovascular risk factors in medium-aged 250 subjects (135 female).

Material and Methods

In order to evaluate the effects of serum bilirubin on blood lipids and lipoproteins we measured the serum lipid profile, together with serum lipoprotein (a) and serum total bilirubin levels in 250 middle-aged subjects. We divided the subjects into three groups based on serum bilirubin concentrations: < 0,4 (group 1) , 0,47-0,53 (group 2) and > 0,7 mg/dl (group 3).

Of the patients 115 were males and 135 females, their mean age range was 40 to 60 years old. They were admitted the hospital for clinically different complaints. The following were exclusion criteria: patients on known lipid altering medications, diabetics, patients with chronic kidney disease, liver disease and CVD. Patients who refused to participate were also excluded.

The patients were fasting overnight and in order to avoid effects of diurnal variation specimens were collected between 0900 and 1500 hours. Blood for lipoprotein studies was drawn, without preservatives, from the antecubital vein. Sera were obtained by centrifugation at room temperature. Bilirubin, cholesterol, HDL-C and triglyceride assays were stored at 4^{o}C and determined within 48h. Aliquots were frozen at -70^{o}C for apo B, Apo A-I and Lp(a) measurement. Samples were stored at -70^{o}C by using small-volume storage vials that were thawed only once at the time of assay to avoid the differential loss of Lp(a) antigenicity seen at lower storage temperatures.

Bilirubin, total cholesterol, HDL-C, triglycerides, Apo B, Apo A1 and Lp(a) were assayed by using kits by automated clinical chemistry analyzers according to the manufacturer's instructions (Roche/Hitachi 912, Roche Diagnostics GmbH, D-68298 Mannheim, Germany). LDL-C concentrations were obtained by using the Friedewald calculation.

The SPSS statistical software package was used for data analysis (SPSS 10.0 for windows, 1999 SPSS Inc.) To evaluate potential confounding or interrelations between bilirubin and lipid profile, we used Pearson's correlation and One-way ANOVA. Two tail tests were used for significance (p= 0,005).

Results

Descriptive statistics for the three groups are given in Table 1.

When calculated by Pearson correlation coefficients, the correlation was not found to be significant between total serum bilirubin levels and lipid profile in these subjects (Table 2). One-way ANOVA variance analysis indicated that any significant difference was not found between bilirubin and lipid profile in three groups (Table 3).

Discussion

In the present study, we aimed to evaluate whether total bilirubin and serum lipid profile levels are connected with each other in 250 middle-aged subjects. We divided

Table 1. Summary of descriptive statistics for three groups

	Group 1 (n= 121)			*Group 2 (n= 49)*			*Group 3 (n= 80)*		
mg/dl	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD
TG	34	796	157 ± 111	34	488	123 ± 86.09	24	503	127±86,74
TC	45	384	201 ± 52,57	118	305	193 ± 46,00	78	346	195±48,26
LDL-C	30	245	123 ± 41,08	58	221	119 ± 41,87	46	241	122±40,25
HDL-C	25	100	47 ± 12,14	23	71	48 ± 12,00	20	79	48 ±12,54
APOB	50	192	108± 28,22	55	183	104 ± 28,05	45	198	102±31,44
APOA-I	11	225	132 ± 29,19	84	192	133 ± 23,96	35	220	130±32,00
LP(a)	,00	210	27,84 ± 36,34	1,00	112	21 ± 21,69	0,00	124	19±19,62
TBİL	,06	,39	,31 ± 0.009	,47	,69	,56± 0.07	0,70	1.44	0,93±0,2

Table 2. Correlation coefficients between total bilirubin and lipid profile levels in 250 middle-aged subjects

		TG	*TC*	*LDL-C*	*HDL-C*	*APO B*	*APO A-I*	*Lp (a)*
Pearson correlation Coefficients	Total Bilirubin	-0, 169	-0,050	0,031	-0,054	-0,039	-0,163	0,015
Sig (2-tailed)	Total Bilirubin	0,007	0,435	0,631	0,395	0,536	0,010	0,815

Table 3. Results of One Way ANOVA variance analysis between lipid profile parameters in three groups

Variable		*Sum of Squares*	*df*	*Mean Square*	*F*	*Sig.*
	Between Groups	447,534	2	223,767	,262	,770
	Within Groups	203948,102	239	853,339		
APOA-I	Total	204395,636	241			
	Between Groups	1884,714	2	942,357	1,098	,335
	Within Groups	205097,825	239	858,150		
APOB	Total	206982,539	241			
	Between Groups	2984,741	2	1492,371	,599	,550
	Within Groups	595440,796	239	2491,384		
TC	Total	598425,537	241			
	Between Groups	31,186	2	15,593	,104	,901
	Within Groups	35914,404	239	150,269		
HDL-C	Total	35945,590	241			
	Between Groups	723,075	2	361,537	,213	,809
	Within Groups	406494,682	239	1700,815		
LDL-C	Total	407217,757	241			
	Between Groups	4083,912	2	2041,956	2,429	,090
	Within Groups	200951,630	239	840,802		
LP(a)	Total	205035,542	241			
	Between Groups	60711,854	2	30355,927	3,112	,046
TG	Within Groups	2331202,032	239	9753,983		
	Total	2391913,886	241			

the subjects into the three groups based on serum bilirubin concentrations: < 0,4 (group 1) , 0,47-0,53 (group 2) and >0,7 mg/dl (group 3). We have not observed any significant difference between lipid profile in the three groups.

Several studies were performed to determine if serum bilirubin, when combined with various lipid and lipoprotein risk factors, enhances our ability to predict coronary artery disease (CVD) (3-5). Several studies have noted an inverse relationship between the presence CVD and circulatory total bilirubin (3-11).

In 1994, Schwertner et al. (3) were the first to observe a significant inverse correlation between total bilirubin concentrations and the prevalence of CVD. Subsequently, Hopkins et al. (5) noted that patients with early familial CVD have an average total serum bilirubin of 8,9 ± 6,1 μmol/L compared with 12.4 ± 8.1 μmol/L in healthy control subjects. Those investigators reported that there was a strong inverse association between serum bilirubin and risk for early familial CVD (5).

In a prospective study of 7685 middle-aged British men, Breimer et al. (4) observed a U-shaped relationship between serum bilirubin and risk of ischemic heart disease. U-shaped relationship means that, with the rising bilirubin level, the incidence of CVD declines, but when the values are close to the upper normal range, the incidence of CVD rises again. According to their findings, these authors concluded that U-shaped relationship could be interpreted

as support for the role of endogenous antioxidants in the etiology of CVD (4).

In 1998, Schwertner (19) examined the association between cigarette smoking and serum bilirubin antioxidant concentrations in 715 middle-aged men undergoing coronary angiography. This study's data showed that subdividing the subjects according to maximum percent stenosis on angiography (< 10, 10-49, 50-100%) revealed a significant inverse association between smoking and bilirubin (< 0,01) within each subset. It was reported that cigarette smoking might increase the risk of CVD by lowering antioxidant concentrations and raising oxidized lipid and lipoprotein concentrations (19).

Serum bilirubin is derived primarily from the degradation of hemoglobin. Heme oxygenase (HO) is the rate-limiting enzyme of bilirubin production. It is a microsomal enzyme, present in both central and peripheral tissues, that converts heme to biliverdin and CO (20). Biliverdin is subsequently reduced to bilirubin by the cytosolic enzyme biliverdin reductase (21). An inducible form of HO (HO-1) is expressed at a low concentration in vascular endothelial and smooth muscle cells. It is markedly induced by heme, metals oxidative stress, inflammatory mediators, oxidized LDL and hypoxia. Several experiments have suggested that HO-1 is a stress response protein that plays an important function in cell defense mechanisms against oxidative injury. HO-1 activity is responsible for increased CO and bilirubin formation as well as iron release in pathological conditions such as CVD, hypoxia, ischemic-reperfusion and hypertension (20). The complex interactions between HO expression, the circulating concentrations of its substrate and products and the effect of these components, specifically of bilirubin on the vascular, on lipid metabolism and on the cardiovascular system will hopefully be the focus of extensive research in the coming years (2).

We have studied the patients admitted the hospital for clinically different complaints. In addition, we have excluded patients taking lipid altering medications, diabetics, persons with chronic kidney disease, liver disease and CVD. According to our findings, there was no association between bilirubin concentrations and any of lipid profile parameters. However, we determined a significant difference between TG levels in three groups of female (p= 0,005) and a poor inverse correlation between TG and bilirubin levels in female subjects (=-0,24), but we couldn't explained it.

If CVD were associated with a higher production of free radicals, increased consumption of bilirubin might occur as secondary result of the CVD (5). Our current findings support the view that increases in serum bilirubin concentrations within the normal range might not be in association with serum lipid profile as risk factors for CVD under non-pathological conditions.

In a study of Levinson 1, lipoprotein lipids and apo B from 254 male patients were compared with bilirubin as a risk factor for CVD. This investigator observed that a highly significant correlation was found between bilirubin and apo B, but not with TC, TG or HDL-C. It was concluded that the bilirubin was a weaker global marker than the lipoproteins and was interacted with apo B (1).

Ko GT et al. (22) examined the relationship between serum bilirubin and CVD risk factors such as age, sex, smoking, glycemic status, obesity and lipid indices in 1508 Hong Kong Chinese. These investigators found that serum bilirubin concentration was inversely correlated with fasting insulin, triglyceride, very-low-density lipoprotein and glycated hemoglobin level (22).

In 1998, Li Y and Zhao S (23) investigated the effects of serum bilirubin on blood lipids and lipoproteins in 237 subjects. Those authors' findings showed that TC and LDL-C were inversely correlated with serum bilirubin.

Antioxidant activity and cardioprotective potential might be attributable to any of the bilirubin forms, including free unconjugated bilirubin, protein bound unconjugated bilirubin, delta bilirubin or mono/diconjugated bilirubin (7,24,25). Under physiological conditions, the predominant circulatory form of bilirubin is the unconjugated, albumin-bound form. Some of conditions, such as protein binding, acidosis, hypoxia and extent of hemolysis, modify the relative proportions of this form of bilirubin in the blood and affect the cardiopotential of bilirubin (2).

Consequently, it may be said that there was a relationship between bilirubin and CVD compatible with previous studies, but we have not observed any relationship between bilirubin and lipid profile.

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