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Association of serum total bilirubin with traditional and novel cardiovascular risk factors in apparently healthy subjects

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ABSTRACT

Background. Bilirubin is considered as one of the strongest endogenous antioxidants. Recent studies suggest that high total bilirubin levels are associated with lower cardiovascular disease (CVD) risk. The aim of this study was to evaluate a the relationship between serum total bilirubin and traditional as well as new risk factors for CVD in apparently healthy subjects.

Materials and methods. Study included 60 non-smoking, non-obese individuals (30 females, 30 males) with normal fasting glucose, aged 25–40 years. In all subjects basic anthropometric indicators (weight, WHR, BMI), blood pressure and laboratory tests: serum total bilirubin (Bil-T), lipid profile, CRP, plasma glucose, apolipoproteins AI and B and 25-hydroxyvitamin D were performed. Subjects were divided into two groups of relative CVD risk, specified by total bilirubin levels: low-risk (≥ 13.7 μmol/L) and high-risk (< 13.7 μmol/L).

Results. Total bilirubin ranged 6.16–32.15 μmol/L. In the study group 58% of subjects had Bil-T values above 13.7 μmol/L. Higher values occurred more frequently in men (70%) and in subjects aged 30–40 years (71%). Statistically significant relationship between Bil-T, and traditional and new risk factors for CVD was found. Bil-T correlated negatively with non-HDL-C, LDL-C, systolic blood pressure as well as with apoB: apoAI ratio, apolipoprotein B, TC:HDL-C and TC. A weak positive correlation was found between serum Bil-T and HDL-C. Additionally, negative correlations with CRP and positive with 25-hydroxyvitamin D were observed in women. In subjects with low CVD risk, the prevalence of low LDL-C concentration (< 2.59 mmol/L) was nearly three-fold higher compared with high-risk groups. Furthermore, in low-risk group the prevalence of serum CRP < 1 mg/L and HDL-C levels > 1.55 mmol/L was 2-fold and 3-fold higher, respectively, and higher incidence of low apoB:apoAI values (<0.6) was observed.

Conclusions. Serum total bilirubin may play an important role in reducing the risk of CVD, therefore its assay seems to be valuable for more accurate assessment of cardiovascular risk in young, non-obese, non-diabetic individuals.

Key words: total bilirubin, antioxidants, cardiovascular disease, cardiovascular risk factors

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Introduction

Cardiovascular disease is the leading cause of death worldwide. According to World Health Organization (WHO) in 2008 CVD was the cause of death of over 17 million people, including 7 million due to coronary heart disease (CHD) and 6 million due to stroke, which accounted for 30% of all deaths. It is estimated that in 2030 the mortality will increase up to 23 millions [1]. In Poland CVD is the cause of death in 43% of men and 54% of women [2].

Atherosclerosis is the main pathogenetic factor for CHD and stroke. It is a degenerative and inflammatory process, occurring mainly in medium and large arteries, associated with accumulation of fatty substances, fragments of cells, calcium, mucopolysaccharides and collagen fibers in the intima. Progression of atherosclerosis leads to partial or complete closure of the lumen, which causes organ ischemia and clinical symptoms. Many factors, both not modifiable (for example: gender, age, family history) and modifiable (smoking, low physical activity, obesity, dyslipidemias etc.), are relevant in the

pathogenesis of atherosclerosis and CVD. In the last decades particular attention is paid to the importance of the low grade inflammation and reactive oxygen species (ROS) in the endothelial dysfunction.

Proinflammatory cytokines (IL-6 and TNF-α, IL-1) and growth factors produced by macrophages infiltrating the vessel wall intensify endothelial dysfunction by stimulating the production of ROS [3]. Angiotensin II, which impairs the efficiency of the heart as a result of activation of the renin-angiotensin-aldosterone system, also shows the additional impact on the increased production of ROS. Increased ROS cause oxidative stress, which leads to the activation of genes dependent redox potential. These multipotential genes are responsible for the production of proinflammatory cytokines which enhance the inflammatory reactions in the positive feedback mechanism. IL-1β and TNF-α have the ability to stimulate the production of adhesion molecules, procoagulatory factors and other inflammatory mediators by endothelium and other cells. Moreover, IL-6 induces the expression of hepatic genes encoding acute phase reactants: C-reactive protein (CRP) and plasma amyloid-A (SAA) [4].

Bilirubin is a product of the oxidative metabolism of heme in mammals, originating primarily from hemoglobin. Heme catabolism occurs in the microsomal fraction of the reticuloendothelial system of the spleen, by the enzyme called heme oxygenase [5]. Bilirubin is excreted in bile as polar compounds: mono- and di-glucuronide derivatives, formed after previous esterification with glucuronic acid [6]. Concentration of bilirubin in the blood is widely used as an indicator of liver and biliary tract function.

Heme degradation and synthesis of bilirubin are considered very important elements of the cell antioxidant defense. During this process heme molecules with prooxidative properties are neutralized (Fig. 1). Released iron, which may be a stimulant for the production of free radicals, is immediately bound by ferritin. Under normal concentrations, unbound bilirubin has scavenging properties to singlet oxygen. For certain peroxidases, in particular horseradish peroxidase and prostaglandin H synthase, in the presence of hydrogen peroxide or organic hydroxides bilirubin acts as a reducing agent, thereby reducing the number of potential oxidizing factors [7]. Bilirubin, as an antioxidant, can neutralize free radicals generated *in vivo* as well as exogenous ones, for example in tobacco smoke. Studies in recent years show an inverse relationship between bilirubin and BMI, total cholesterol and LDL-C, suggesting that high levels of bilirubin can be a factor in reducing the risk of cardiovascular disease. The aim of this study was to evaluate the relationship between serum total bilirubin and traditional as well as new risk factors for CVD in young, healthy subjects.

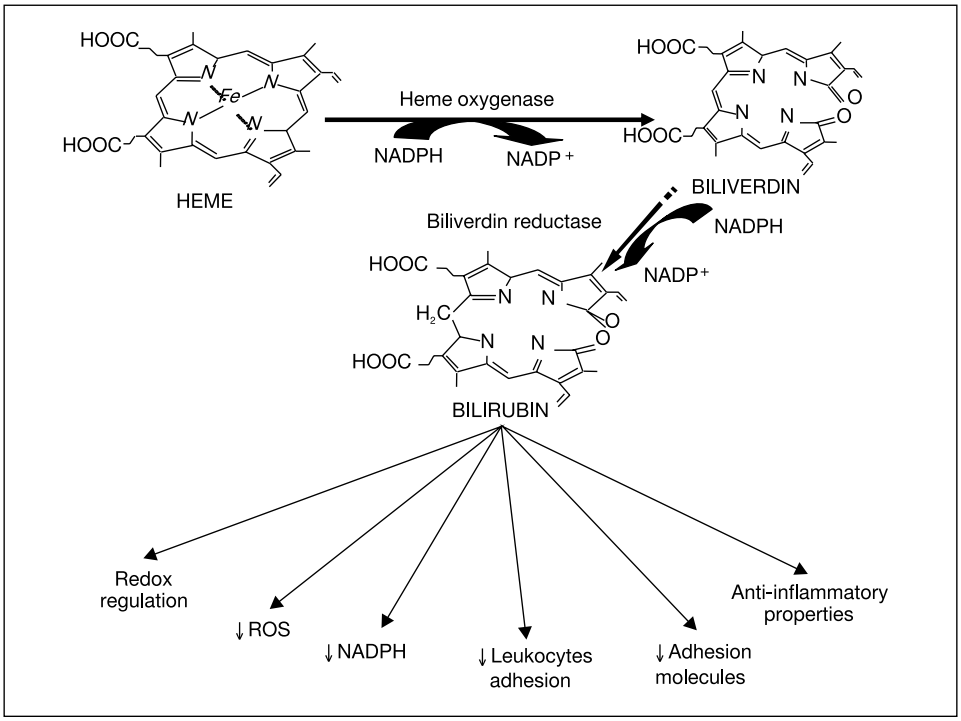

Figure 1. Antioxidative properties of bilirubin (modified according to [7])

Subjects, materials and methods

Study included 60 non-obese (BMI < 30 kg/m^2), non-smoking subjects (30 women, 30 men) aged 25–40 years with normal fasting glucose (3.33––5.49 mmol/L). Anthropometric measurements (weight, BMI, WHR) and medical history of chronic diseases were performed. Blood pressure was measured twice in 1–2 minutes intervals on dominant hand with the automatic blood pressure monitor Omron M6 Comfort (Omron Healthcare, Kyoto, Japan) which is clinically validated. Serum and fluoride plasma were collected in the morning (7.00–9.00 a.m.) after 10–12 hours of fasting. Immediately after collection the samples were centrifuged in low temperature (4°C). After centrifugation, serum samples were divided into small aliquots — one part was used for the routine laboratory measurements performed immediately on automatic analyzers and others were frozen in –70°C to avoid peptides degradation into further apolipoproteins and 25-hydroxyvitamin D assays. Serum samples were thawed only once in the room temperature, directly before measurements. Laboratory tests: glucose, total bilirubin (T-Bil), C-reactive protein (CRP), total cholesterol (TC), HDL-cholesterol (HDL-C), triglycerides (TG), apolipoproteins AI and B (apoAI, apoB) and 25-hydroxyvitamin D (25(OH)D) were performed on Architect ci8200 (Abbott Laboratories, Abbott Park, USA) and Cobas e411 (Roche Diagnostics, Indianapolis, USA) analyzers. LDL cholesterol (LDL-C), non-HDL cholesterol (non-HDL-C) and atherogenic indexes (TC:HDL-C, apoB:apoAI) were calculated using proper equations. The routine laboratory parameters were considered normal if they were as follows: plasma glucose 3.33–5.49 mmol/L (60–99 mg/dL); T-Bil 3.42––20.52 μmol/L (0.2–1.2 mg/dL); CRP < 1 mg/L, 25(OH)D ≥ 30 ng/mL. Reference values for lipid profile comply with the current ESC/EAS guidelines [8]. All laboratory measurements were performed in the Department of Laboratory Medicine, Nicolaus Copernicus University Collegium Medicum in Bydgoszcz, Poland.

Statistical analysis was performed using STATISTICA 10.0 software (StatSoft Inc. 2012). Data were presented as mean ± standard deviation (normal distribution) or median and 25th–75th percentile (non-Gaussian distribution). Differences between study groups were measured by t-Student, U-Mann-Whitney and ANOVA Kruskal-Wallis tests. P value < 0.05 was considered statistically significant.

The study was approved by the Bioethics Committee at the NCU Collegium Medicum in Bydgoszcz, Poland (No. KB 627/2010) and complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects. An informed written consent was obtained from all participants involved in this study.

Results

Table 1 shows clinical and biochemical characteristics of the study group. Total bilirubin (T-Bil) concentration ranged 6.16–32.15 μmol/L (0.36–1.88 mg/dL). In women significantly higher values of HDL-C, apoAI, 25(OH)D and lower values of anthropometric indices, plasma glucose, TG and atherogenic indexes were found, compared to men. Despite higher median of T-Bil in men, compared to women, the difference was not statistically significant (p > 0.05).

Relevant correlations between T-Bil and selected parameters were observed (Tab. 2). In the whole group total bilirubin showed moderate negative correlation with TC, apoB, apoB:apoAI and TC:HDL-C as well as strong inverse relationship with non-HDL-C (R = –0.54; p < 0.001), LDL-C (R= –0.56; p < 0.001) and systolic blood pressure (R = –0.59; p < 0.001). Weak but statistically significant positive correlation with HDL-C (R = 0.27; p < 0.05) was found. Similar association between T-Bil and lipid parameters was observed in both women and men, however, it seemed to be stronger in men. Moreover, correlations with CRP (R = –0.46; p < 0.05), and 25(OH)D (R = 0.41; p < 0.05) in women, and diastolic blood pressure (R = –0.6; p <0.001) and WHR (R = –0.39; p < 0.05) in men were noticed.

Subjects were divided into two groups of cardiovascular risk (Tab. 3) specified by total bilirubin concentration according to Endler et al. study [9]: low risk ≥ 13.7 μmol/L (≥ 0.8 mg/dL) and high risk < 13.7 μmol/L (< 0.8 mg/dL). In study group 58% of subjects had low CVD risk and this state occurred more frequently in men (70%) and individuals aged 30––40 years (71%). Significantly lower levels of systolic blood pressure, TC, LDL-C, non-HDL-C, apoB and atherogenic indexes were found in subjects with T-Bil ≥ 13.7 μmol/L.

In the low CVD risk group a higher prevalence of potential anti-atherogenic factors was observed (Fig. 2 and 3). In subjects with T-Bil ≥ 13.7 μmol/L prevalence of LDL-C < 2.59 mmol/L (< 100 mg/dL) and CRP < 1 mg/L, considered as a indicators of low cardiovascular risk, was four-fold (p = 0.005) and two-fold (p = 0.03) higher, respectively, compared with individuals with lower T-Bil levels. Moreover, high values of HDL-C > 1.55 mmol/L (> 60 mg/dL) occurred almost three times more frequently (p = 0.025) in the low CVD risk group. As far as novel lipid risk factors are concerned, it was found that non-HDL-C < 3.37 mmol/L (< 130 mg/dL) was more prevalent (p = 0.006) in the low CVD risk group as well as the tendency to low apoB (< 0.6 g/L) and apoB:apoAI ratio (< 0.6) (both p = 0.05).

Table 1. Clinical and biochemical characteristics of the study group

Variables	All (n = 60)	Women (n = 30)	Men (n = 30)	p*
Age (years)	30 (27–34)	30 (27–35)	30 (27–33)	ns
BMI [kg/m^2]	22.3 (20.8–25)	21 (20–22.3)	24.4 (21.7–25.9)	< 0.001
WHR	0.79 (0.75–0.86)	0.76 (± 0.04)	0.86 (± 0.04)	< 0.001
SBP [mm Hg]	118 (112–126)	117 (110–126)	121 (115–127)	ns
DBP [mm Hg]	78 (74.5–81)	76 (± 6.5)	78 (± 3.4)	ns
Glucose [mmol/L]	5.10 (4.77–5.35)	4.88 (4.61–5.10)	5.33 (5.10–5.49)	< 0.001
CRP [mg/L]	0.6 (0.3–1.4)	0.5 (0.3–1.2)	0.8 (0.5–1.5)	ns
T-Bil [μmol/L]	14.4 (10.8–19.1)	13.6 (9.6–18.8)	16.7 (12.3–19.5)	ns
TC [mmol/L]	4.44 (3.94–4.71)	4.59 (3.96–4.87)	4.27 (3.83–4.66)	ns
HDL-C [mmol/L]	1.46 (1.19–1.58)	1.54 (1.37–1.68)	1.28 (1.11–1.53)	0.0008
TG [mmol/L]	0.88 (0.69–1.09)	0.83 (0.63–0.98)	0.96 (0.75–1.41)	0.028
LDL–C [mmol/L]	2.46 ± 0.53	2.49 ± 0.45	2.44 ± 0.61	ns
Non-HDL-C [mmol/L]	2.90 ± 0.56	2.87 ± 0.47	2.93 ± 0.65	ns
TC:HDL-C	2.92 (2.63–3.61)	2.89 ± 0.47	3.36 ± 0.84	0.009
ApoAI [g/L]	1.42 (1.31–1.57)	1.57 ± 0.21	1.34 ± 0.15	<0.001
ApoB [g/L]	0.70 ± 0.13	0.65 (0.58–0.76)	0.71 (0.62–0.80)	ns
ApoB:ApoAI	0.49 ± 0.12	0.44 ± 0.1	0.54 ± 0.13	0.002
25(OH)D [ng/mL]	19 ± 7.6	23 ± 6.7	15 ± 6.6	< 0.001

Results are presented as: mean ± SD or median (25–75%)
*Differences men vs. women; ns – not significant; SBP/ DBP– systolic/diastolic blood pressure

Table 2. Correlation between total bilirubin and selected CVD risk factors

Variables	All (n = 60)	Women (n = 30)	Men (n = 30)
WHR	ns	ns	R = –0.38; p < 0.05
SBP	R = –0.59; p < 0.001	R = –0.62; p < 0.001	R = –0.64; p < 0.001
DBP	ns	ns	R = –0.60; p < 0.001
CRP	ns	R = –0.46; p < 0.05	ns
TC	R = –0.46; p < 0.001	ns	R = –0.75; p < 0.001
HDL-C	R = 0.27; p < 0.05	ns	R = 0.59; p < 0.05
LDL-C	R = –0.56; p < 0.001	R = –0.46; p < 0.05	R = –0.75; p < 0.001
Non-HDL-C	R = –0.54; p < 0.001	R = –0.41; p < 0.05	R = –0.78; p < 0.001
TC:HDL-C	R = –0.45; p < 0.001	R = –0.38; p < 0.05	R = –0.76; p < 0.001
apoB	R = –0.44; p < 0.001	R = –0.37; p < 0.05	R = –0.61; p < 0.001
apoB:apoAI	R = –0.37; p < 0.05	ns	R = –0.60; p < 0.001
25(OH)D	ns	R = 0.41; p < 0.05	ns

ns — not significant; SBP/ DBP— systolic/diastolic blood pressure

Discussion

In recent years several population-based studies indicated a relationship between total bilirubin concentration and cardiovascular disease risk in general population. The NHANES 1999–2004 Study reported that 1.71 μmol/L (0.1 mg/dL) increase in bilirubin concentration was associated with 9% reduced odds of stroke and with 10% reduced odds of an adverse stroke outcome [10]. In the Framingham Heart Study subjects with UGT1A1*28 allele, which encodes hepatic bilirubin uridine diphosphate-glucuronosyltransferase

Table 3. Comparison of measured variables in two groups of CVD risk, defined by total bilirubin concentration

	Total bilirubin [μmol/L]		
Variables	**< 13.7 high CVD risk (n = 25)**	**≥ 13.7 low CVD risk (n = 35)**	**p**
Age (years)	31 (28–34)	29 (27–34)	ns
BMI [kg/m^2]	22.3 ± 2.7	23.1 ± 2.6	ns
WHR	0.81 ± 0.07	0.81 ± 0.06	ns
SBP [mm Hg]	125 (118–127)	115 (108–123)	0.015
DBP [mm Hg]	79 (74–82)	77 (75–80)	ns
Glucose [mmol/L]	5.05 (4.88–5.27)	5.16 (4.77–5.38)	ns
CRP [mg/L]	1.0 (0.5–1.5)	0.5 (0.3–1.0)	ns
TC [mmol/L]	4.69 (4.56–4.89)	4.22 (3.73–4.45)	< 0.001
HDL-C [mmol/L]	1.40 (1.11–1.55)	1.48 (1.24–1.61)	ns
TG [mmol/L]	0.85 (0.72–1.08)	0.90 (0.69–1.09)	ns
LDL-C [mmol/L]	2.79 ± 0.49	2.23 ± 0.42	< 0.001
Non-HDL-C [mmol/L]	3.22 ± 0.55	3.37 ± 0.44	< 0.001
TC:HDL-C	3.40 ± 0.81	2.90 ± 0.55	0.0026
ApoAI [g/L]	1.45 (1.36–1.56)	1.38 (1.31–1.59)	ns
ApoB [g/L]	0.76 (0.68–0.87)	0.65 (0.58–0.71)	0.0032
ApoB:ApoAI	0.53 ± 0.13	0.46 ± 0.11	0.024
25(OH)D [ng/mL]	19.6 (14.82–22.9)	16.9 (12.07–24.24)	ns

Results are presented as: mean ± SD or median (25–75%)
ns — not significant; SBP/ DBP— systolic/diastolic blood pressure

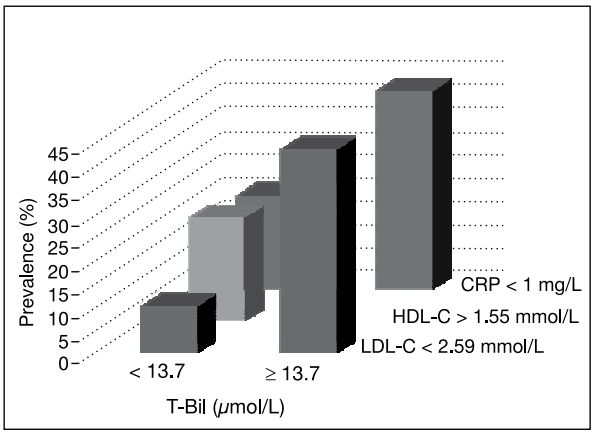

Figure 2. Prevalence of low CRP, LDL-C and high HDL-C values depending on total bilirubin concentration

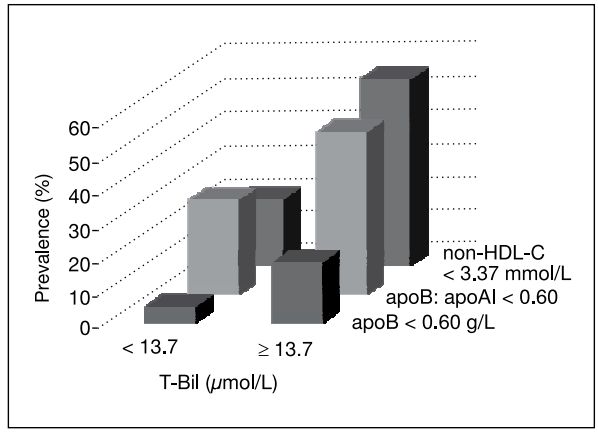

Figure 3. Prevalence of low non-HDL-C, apoB and apoB:apoAI values depending on total bilirubin concentration

and is responsible for higher bilirubin concentration, had significantly lower CVD and coronary heart disease risk (CHD) [11]. Moreover, the prevalence of CHD in patients with Gilbert syndrome is 2%, while in general population it approximates to 12% [12]. In the study by Horsfall et al. [13] the risk of any CVD event, myocardial infarction and death resulting from any cause was 18%, 34% and 33%, respectively, higher in statin-treated patients with bilirubin level of 5 μmol/L (0.3 mg/dL) compared with those with a similar CVD risk profile and median bilirubin concentration of 10 μmol/L (0.6 mg/dL). It was emphasized that clini-

cians should pay particular attention to patients with bilirubin levels < 6 μmol/L (< 0.35 mg/dL). According to Endler et al. [9], subjects with T-Bil > 13.7 μmol/L (> 0.8 mg/dL) have a 40% reduction in prevalence of CHD after adjustment for factors such as: age, smoking, diabetes, hypertension, HDL, triglycerides, and BMI. However, this association was strong in men, while in women it was not significant.

In our study a strong relationship between serum total bilirubin and traditional as well as novel CVD risk factors, mainly lipid parameters and blood pressure, was observed in both women and men. Kamisako et al., in the study including 270 Japanese men, indicated positive correlation of higher T-Bil levels with lower concentration of chylomicron remnants which have high proatherogenic properties [14]. In patients with Gilbert syndrome, concentration of total cholesterol, triglycerides, IDL, VLDL and LDL was significantly lower compared with controls [15]. In the study by de Sauvage Nolting et al. [16] significantly lower T-Bil levels were found in patients with CVD and familial hypercholesterolemia compared with controls (0.57 vs. 0.61 mg/dL; $p < 0.05$). After statin treatment a significant decrease in TC, LDL-C and TG as well as an increase in HDL-C and total bilirubin concentration was reported. A negative correlation between T-Bil and BMI, LDL-C and TC was observed by McArdle et al. [17]. Total bilirubin concentration was negatively associated with glycated hemoglobin (HbA_{1c}) independently of plasma glucose, age, obesity, and inflammation in non-diabetic Japanese [18]. Moreover, lower T-Bil levels in smoking subjects and negative correlation with CRP in smoking men and non-smoking women were found.

Recent studies suggest that bilirubin shows cardioprotective effects in coronary arteries. In acute myocardial stress, including infarction with ST elevation, an increase in total bilirubin and many cytokines and neurohumoral mediators levels is detected. Elevated T-Bil is considered to be a compensation for increased production of inflammatory mediators. In conditions of severe stress, activation of the heme oxidase is observed. The degree of HO-1 activation is closely linked to the intensity of the inflammatory response, caused by myocardium necrosis [19]. In this process, there is a severe degradation of heme particles and increased production of T-Bil. Heme degradation products and bilirubin have a protective effect due to their antioxidant properties. Therefore, determining the concentration of total bilirubin might be useful in the evaluation of CVD risk and its outcomes in apparently healthy subjects as well as in cardiological patients. Due to the limitations of our study, including relatively small study group, the results need to be confirmed in a large population-based study.

Conclusions

The significant correlation between total bilirubin and traditional as well as novel CVD risk factors, which is presented in our study, denotes its potential role as a new additional risk indicator in young, non-obese, non-diabetic subjects. Higher T-Bil concentration is associated with lower values of proatherogenic factors and higher values of cardioprotective agents which indicates that the determination of total bilirubin should be included for more accurate cardiovascular disease risk assessment .

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