

건강한 한국인 성인 남자에서 혈중 총 빌리루빈 농도와 Framingham 위험 점수와의 관계

전혜진, 이상화, 최유경, 이홍수, 심경원

이화여자대학교 의과대학 이대목동병원 가정의학과

The Relationship Between Serum Total Bilirubin and Framingham Risk Score in Healthy Korean Men

Hyejin Chun, Sang Wha Lee, Yukyung Choi, Hong Soo Lee, Kyung Won Shim

Department of Family Medicine, Ewha Womans University Mokdong Hospital, Ewha Womans University School of Medicine, Seoul, Korea

Background: Serum total bilirubin has been considered a harmful substance inducing oxidative reaction; but recently, there have been reports of it possessing antioxidative, anti-inflammatory and protective features against cardiovascular diseases. The purpose of this study was to investigate the relationship between total bilirubin and the Framingham risk score.

Methods: This study involved 3,414 healthy Korean men who underwent a medical check-up in a health promotion center in 2008. We calculated the Framingham risk score using age, smoking status, systolic blood pressure, total cholesterol and high-density lipoprotein cholesterol.

Results: The mean age of the participants was 44.9 ± 8.8 years. The log transformed serum total bilirubin level and the Framingham risk score had a negative linear relationship in a simple linear regression analysis (R^2 : 0.018, $P < 0.001$). In a multivariable analysis model, as well, the log transformed serum total bilirubin level and the Framingham risk score had a negative linear relationship (R^2 : 0.384, $P < 0.001$).

Conclusions: Our study showed a statistically significant negative relationship between total bilirubin and the Framingham risk score. Total bilirubin had a stronger relationship with the Framingham risk score than other standard cardiovascular risk factors except smoking, and thus may be useful in predicting cardiovascular risk in the outpatient clinic.

Korean J Health Promot 2013;13(1):1-7

Keywords: Total bilirubin, Framingham risk score, Cardiovascular disease

INTRODUCTION

Bilirubin has been regarded as a harmful substance, generated in the metabolic processing of heme, that causes oxidation. However, there is much attention towards the hypothesis that bilirubin is related to cardiovascular dis-

ease, lung disease and vessel disease where infection is the main mechanism. This study is based on several recent findings that bilirubin has anti-inflammatory effects and can prevent oxidative damage as free radical scavengers.^{1,2)} Heme is converted to biliverdin and carbon monoxide by heme oxydase-1, and biliverdin is converted to bilirubin by biliverdin reductase.³⁻⁶⁾ During this process, bilirubin is reported to prevent the oxidation of lipids including low-density lipoprotein cholesterol (LDL-C),^{7,8)} to reduce oxidative stress^{9,10)} and to reduce reactive oxygen species.¹¹⁾ More studies are required for an accurate understanding of these functions.

■ Received : August 16, 2012 ■ Accepted : January 24, 2013

■ Corresponding author : **Sang Wha Lee, MD, PhD**
Department of Family Medicine, Ewha Womans University Mokdong Hospital, Ewha Womans University School of Medicine, 1071 Anyangcheon-ro, Yangcheon-gu, Seoul 158-710, Korea
Tel : +82-2-2650-6018, Fax : +82-2-2654-2439
E-mail : fmewha@naver.com

Framingham risk score (FRS) is one of a number of scoring systems used to estimate an individual's chances of developing severe coronary heart disease within the next 10 years with marking of age, smoking, systolic blood pressure, total cholesterol and high-density lipoprotein cholesterol (HDL-C) for each sex in adults aged 20-70 yrs without clinical cardiovascular disease or coronary heart disease.¹²⁾ Cardiovascular events, however, can still occur in the low predicted 10-year cardiovascular disease risk group because the FRS provides only an average probability of event occurrence. The National Cholesterol Education Program Adult treatment panel III (NCEP ATPIII) recommends that the FRS score be evaluated by classifying the group with low risk as having 10% or less cardiovascular risk at 10 years, intermediate risk 10-20% and high risk 20% or more.

Atherosclerosis causes hardening or furring of blood vessels due to the formation of plaque, a buildup of macrophage cells or debris containing lipids, calcium and various amounts of fibrous connective tissue. Several studies have been tried to prevent the progress of atherosclerosis by using several natural antioxidants. More studies are required regarding whether there is a possibility that bilirubin can prevent the incidence of cardiovascular disease as an antioxidant¹³⁻¹⁵⁾ and development of enzyme to suppress bilirubin metabolism may be useful for preventing cardiovascular disease in healthy adults.

This study aimed to examine the evaluation of risk of cardiovascular disease using total bilirubin level and FRS by clarifying the relationship between total bilirubin level and FRS in healthy participants who visited the health promotion center at one university hospital in Korea.

METHODS

1. Subjects of study

The number of participants for this study totaled 11,704 adults (6,066 men and 5,168 women) older than 20 years of age who received routine health examinations at the health promotion center at Ewha womans university Mokdong hospital from January 2009 to December 2009. To exclude patients with hepatobiliary tract abnormality, which can affect serum bilirubin metabolism, patients with viral hepatitis including positive antigen of hepatitis B and positive

antibody of hepatitis C; liver cirrhosis; hepatocellular cancer; biliary tract cancer and obstructive biliary tract disease such as biliary calculus, Dubin-Johnson syndrome and Gilbert syndrome were excluded. In addition, regardless of the FRS, patients with coronary artery diseases such as angina and myocardial infarction, the high risk group for cardiovascular diseases, patients with cerebrovascular disease such as stroke, patients suffering from chronic kidney failure and diabetic patients. The FRS of 3,414 males and 2,910 females satisfying target standards were calculated. There being no female groups corresponding to a high risk group, only male participants were the final subjects of this study. This current study was approved by the ethical committee at Ewha womans university school of medicine.

2. Data collection

Height was measured to 0.1 cm with subjects in examination gown, heels together and back straight to keep heels, hips, shoulders and head in line. Subjects were weighed to 0.1 kg in their gowns and after voiding before breakfast. Body mass index (BMI) was calculated by dividing the weight (kg) by the square of height (m). Blood pressure was measured at the brachial artery using an electronic blood pressure meter with the subject sitting in the examination chair after resting for over 10 minutes. If the value was out of the normal range, it was measured again after an additional 10-minute rest. High blood pressure was defined as systolic blood pressure of 140 mmHg or above or diastolic pressure of 90 mmHg or above or if subjects were diagnosed in the past with hypertension and were on blood pressure lowering agents.¹⁶⁾ Diabetes was defined as fasting glucose >126 mg/mL (measured at the time of examination) or if subjects were diagnosed to have diabetes and were on hypoglycemic agents or insulin.¹⁷⁾ The following information was collected via a self-administered questionnaire: demographics, psychosocial factors, history of chronic disease, self-assessed general health status, alcohol use, smoking status, nutrition intake and physical activity/energy expenditure. If they quit smoking 6 months prior to this study or had never smoked, they were classified as non-smokers, and if they had smoked during the last 6 months, they were regarded as smokers.¹²⁾

For blood test, subjects fasted for >8 hours and venous blood collected to measure total cholesterol, HDL-C, LDL-C, triglyceride (TG), total bilirubin, fasting blood glucose (FBG), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and hepatitis viral marker (hepatitis B surface antigen, anti-hepatitis B surface, anti-hepatitis B core and anti-hepatitis C virus).

The FRS was calculated totaling the score of each item-age (separately for males and females), systolic blood pressure or taking blood pressure lowering agents, a smoking history, HDL-C and total cholesterol. The risk of incidence of cardiovascular disease was calculated for 10 years hereafter.

3. Statistical analysis

The general characteristics of the participants were expressed as mean±SD. Total bilirubin does not form a normal distribution so that the value converted to log was analyzed. One-way layout variance analysis was conducted on the differences of each variable for risk groups obtained with FRS. The relation between log converted total bilirubin and FRS was analyzed using simple linear regression analysis. In addition, multiple linear regression

analysis was performed after controlling for diastolic pressure, heart rate, BMI, LDL-C, TG, AST, ALT and FBG. All analyses were performed on the PASW program (version 17.0 K for Windows; SPSS Inc., Chicago, IL, USA). For all analyses, results were statistically significant when *P* value was <0.05.

RESULTS

The demographic and descriptive characteristics of subjects are listed in Table 1. Subjects included 3,414 male adults who were classified into 3 groups according to the 10-year FRS with the break down being- 2,563 persons (75.1%) in the low risk group, 687 persons (20.1%) in the intermediate risk group and 164 persons (4.8%) in the high risk group, with the mean age being 42.3±7.2 years, 50.6±9.7 years and 52.9±10.6 years, respectively. The mean 10-year FRS for cardiovascular disease was 7.9±4.8. The average total bilirubin was 1.0±0.4 mg/dL. Total bilirubin of 3 groups according to FRS decreased to 1.0±0.4 mg/dL, 0.9±0.3 mg/dL, 0.9±0.3 mg/dL respectively. The number (percent) of subjects exceeding 1.2 mg/dL, the upper limit of the reference level for normal bilirubin, was 346 (10.13%).

Table 1. Baseline characteristics of study population and comparison of variations by Framingham risk categories^a

Characteristic	All (n=3414)	Low risk (n=2563)	Intermediate risk (n=687)	High risk (n=164)
Age, y	44.9±8.8	42.3±7.2	50.6±9.7 ^b	52.9±10.6 ^c
BMI, kg/m ²	24.7±2.8	24.5±2.8	25.3±2.8 ^b	25.7±2.6 ^b
SBP, mmHg	125.5±12.2	124.8±11.8	126.8±12.8 ^b	131.7±12.6 ^c
DBP, mmHg	75.1±9.3	74.7±9.2	76.0±9.4 ^b	78.2±9.3 ^c
HR, bpm	64.1±9.3	63.8±9.3	64.4±9.1	66.8±10.0 ^b
TC, mg/dL	199.3±33.4	194.1±31.5	211.6±30.2 ^b	229.4±43.1 ^c
LDL-C, mg/dL	122.0±29.3	118.0±28.2	132.3±26.6 ^b	142.8±36.3 ^c
HDL-C, mg/dL	52.0±11.3	53.1±11.6	49.5±10.0 ^b	45.0±8.4 ^c
TG, mg/dL	141.9±103.1	130.3±90.1	165.5±98.7 ^b	223.2±207.2 ^c
FBG, mg/dL	92.2±9.6	91.4±9.4	94.5±9.9 ^b	95.6±10.5 ^b
AST, IU/L	26.2±12.3	25.4±11.7	28.0±12.6 ^b	30.4±18.0 ^c
ALT, IU/L	31.8±22.4	30.7±21.8	34.2±22.3 ^b	38.1±29.2 ^b
FRS	7.9±4.8	6.1±4.2	12.8±0.8 ^b	15.5±0.2 ^c
Smoker, n (%)	1451 (42.5)	827 (57.0)	486 (33.5)	137 (9.5)
TB, mg/dL	1.0±0.4	1.0±0.4	0.9±0.3 ^b	0.9±0.3 ^b

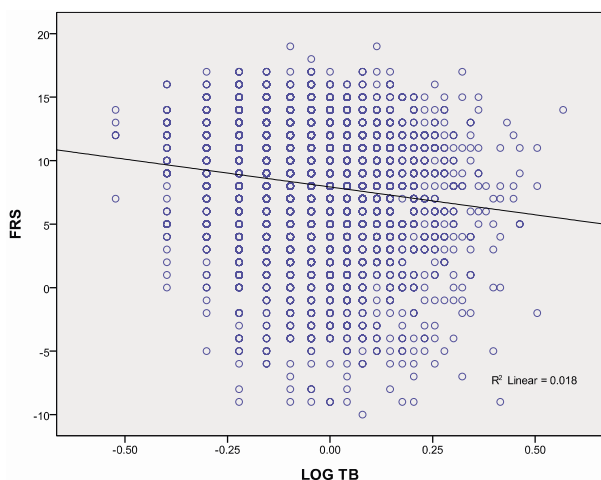
Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; FBG, fasting blood glucose; AST, aspartate aminotransferase; ALT, alanine aminotransferase; FRS, Framingham risk score; TB, total bilirubin.

^aData are presented as mean±SD unless otherwise indicated.

^bIndicates statistical significance (*P*<0.05) compared with the low risk group, and *p*-values were calculated by post hoc analysis of Turkey's multiple comparison test.

^cIndicates statistical significance (*P*<0.05) compared with the intermediate risk group, and *p*-values were calculated by post hoc analysis of Turkey's multiple comparison test.

Figure 1. Association between log-transformed total bilirubin and Framingham risk score



Graph shows a negative relationship between log-transformed total bilirubin and Framingham risk score by simple linear regression analysis.

$R^2=0.018$ by simple linear regression analysis.

FRS indicates Framingham risk score; LOG TB, log- transformed total bilirubin.

The variables which showed significantly higher average values in the intermediate and high risk groups than in the low risk group were age, systolic blood pressure, diastolic blood pressure, total cholesterol, LDL-C, TG and AST. HDL-C decreased significantly according to the FRS groups. While BMI, FBG, ALT and total bilirubin showed significantly higher values at greater than intermediate risk levels compared to the low risk group, no significant difference was identified between intermediate and high risk groups. In the case of heart rate, only the high risk group showed significantly higher difference from the low risk group ($P<0.05$).

Figure 1 shows that log converted total bilirubin has a statistically significant negative correlation with FRS by simple linear regression analysis ($R^2: 0.018$, $P<0.001$). The multiple linear regression analysis conducted after controlling for risk factors of cardiovascular disease not included in FRS items such as diastolic blood pressure, heart rate, BMI, LDL-C, TG, AST, ALT, FBG and smoking, shows that log converted total bilirubin has as negative correlation with FRS ($R^2: 0.384$, $P=0.001$). All values show independent correlation with FRS (Table 2).

All participants were classified into quartiles according to the total bilirubin level- first quartile included total bilirubin levels from 0.2 mg/dL to 0.8 mg/dL, 2nd quartile

from 0.9 mg/dL to 1.4 mg/dL, 3rd quartile from 1.5 mg/dL to 1.8 mg/dL and 4th quartile from 1.9 mg/dL to 3.8 mg/dL. As a result of obtaining the degree of risk corresponding to intermediate and high risk of FRS with logistic regression analysis based on the quartile of total bilirubin by classifying total subjects into 4 groups according to total bilirubin level, the 3rd quartile, 2nd quartile and 1st quartile recorded odds ratios (95% confidence interval) of 1.24 (1.105-1.751), 1.59 (1.171-1.985) and 2.12 (1.375-3.152), respectively (Table 3).

DISCUSSION

Bilirubin is a byproduct of heme metabolism and is regarded to play a role as a physiological antioxidant.¹⁾ Several studies reported that bilirubin showed a negative correlation with incidence rates for cardiovascular diseases and respiratory diseases such as chronic obstructive lung disease and lung cancer.^{1,18)} In the experiment on the effects of bilirubin in prevention of atherosclerosis as a natural inhibitor of vascular smooth muscle cell proliferation, heme oxygenase-1-derived bilirubin ameliorated post-ischemic myocardial dysfunction. Exogenously administered bilirubin significantly restored myocardial function and minimized both myocardial infarct size and mitochondrial damage on reperfusion.^{19,20)} With regard to cardiovascular disease including coronary artery disease, this study examined whether total bilirubin can be used as a new indicator for the risk of cardiovascular disease.

Emerging evidence has shown that total bilirubin might be an important enzyme in the pathogenesis of cardiovascular diseases. However, the mechanisms by which total bilirubin prevent atherosclerosis are not fully understood. Several lines of recent evidence suggest that an association between total bilirubin and atherosclerosis is plausible. Possible mechanisms between total bilirubin and atherosclerosis are prevention of lipid oxidation by bilirubin, activation of heme oxidizing enzyme, decreased infection and anti-complement reaction. First, there is an assumption that bilirubin itself has antioxidant effects, preventing oxidation of lipid and lipoprotein and preventing progression of atherosclerosis.^{1,21)} Second, there is a theory that a heme oxidizing enzyme is activated leading to an increase in its metabolites such as bilirubin, carbon monoxide, iron and biliverdin and an increase in the removal of heme with each step preventing a generation of atherosclerosis.

Atherosclerosis is prevented through the expansion of blood volume due to decreased hemoglobin density and decreased cell disruption in heme with decomposition of heme by heme oxidizing enzyme.^{22,23)} In addition, carbon monoxide works to regulate the activation of endothelial cells and the aggregation of platelet, as well as, the regulation of blood vessel elasticity. This also increases and helps vasodilatation.^{22,24,25)} With activation of the heme oxidizing enzyme, serum iron increases and the iron accumulating in the tissue decreases. Iron accumulated in the liver or kidney may cause chronic infection and tissue damage, which may be associated with atherosclerosis.^{1,26)} Accordingly, the density of total bilirubin increases as a result of activation of the heme oxidizing enzyme and plays an important role in preventing atherosclerosis. Third, according to one experimental result, bilirubin and biliverdin play a role in reducing infection and in the immune reaction by interrupting complement related reactions.²⁷⁾ That is, total bilirubin works as an intrinsic factor for cell protection through anti-complement reactions.²⁸⁾ While there are several assumptions as shown above, more studies are required to accurately understand the mechanism. No study has been conducted yet regarding whether keeping total bilirubin density at relatively high normal levels in healthy adult is related to the prevention of cardiovascular disease. And no study has been conducted up to now, comparing the association with FRS, rather than cardiovascular related risk factors.

Multiple linear regression analysis performed in this study revealed a negative correlation between total bilirubin and FRS, verifying that total bilirubin is an independent risk factor of cardiovascular diseases. A higher correlation between total bilirubin and FRS rather than other variables means that total bilirubin can be used as a factor estimating the risk of cardiovascular diseases. Rather than comparing each risk factor- age, obesity, diabetes, smoking, high blood pressure, increased LDL-C and decreased HDL-C, the existing risk factors for atherosclerosis and cardiovascular diseases, the use of FRS may be more helpful as it indicates those most likely to develop cardiovascular diseases and those most likely to benefit from prevention. Furthermore, FRS can be scored by a simple calculation. For this reason, FRS is used to determine who should be offered preventive medications such as antiplatelet agents and blood pressure and lipid

lowering medications.

Under the assumption that the risk for cardiovascular diseases is the highest at the 1st quartile of total bilirubin level, the degree of relative risk to be included at intermediate or high FRS groups were obtained in the 3rd, 2nd and 1st quartiles compared to the 4th. The 3rd quartile group recorded 1.24 times, the 2nd quartile 1.59 times and the 1st quartile 2.12 times, respectively. Accordingly, it was confirmed that the total bilirubin level decreased with FRS, resulting in the progression of atherosclerosis.

This study is meaningful in that it is the first study that clarified the relationship between total bilirubin and FRS through a cross-sectional study targeting healthy Korean adult males and connected this to the risk for cardiovascular diseases. This study clarified that the risk of cardiovascular diseases may increase in adult males who have low normal total bilirubin levels, and that total bilirubin can be used as an independent risk factor for cardiovascular diseases. While it was not included in the results of this study, the correlation between total bilirubin and FRS was analyzed statistically among adult females in the low and intermediate risk groups. The results showed a statistically significant correlation in simple and multiple linear regression analyses.

This study is limited in that its subjects were participants at the health promotion center of one university hospital, making it an unlikely representation of the general population; that it was a cross-sectional study, requiring a prospective study in the future; that targeting relatively healthy and adult males lead to more subjects in the low risk group compared to the intermediate and high risk groups in terms of FRS; that information on exercise and alcohol use was insufficient and could not be controlled and that the self-administered questionnaire on living habits was filled out by the patient themselves so that its accuracy might be low.

Further prospective studies are required regarding whether increasing total bilirubin within the normal range will be actually beneficial for preventing cardiovascular diseases.

요 약

배경: 총 빌리루빈은 산화작용을 하는 해로운 물질로 생각되어 왔으나 최근 항산화, 항염증 효과 및 과산화기 제거

기능이 밝혀지면서 심혈관계 보호인자로서 주목을 받고 있다. Framingham 위험점수는 20-79세 성인에서 향후 10년간 관상동맥 심혈관 질환의 위험도를 평가할 수 있는 점수로 본 연구는 총 빌리루빈과 Framingham 위험 점수와의 관계를 밝히고자 한다.

방법: 검진센터의 검진자 중 심혈관계 질환이 없는 성인 남자 3,414명을 대상으로 연령, 흡연, 수축기 혈압, 총 콜레스테롤, 고밀도 지단백 콜레스테롤을 점수화하여 Framingham 위험 점수를 계산하였다. 로그 치환한 총 빌리루빈과 Framingham 위험점수와의 관계는 단순선형회귀분석을 시행하여 분석하였고 이후 다중선형회귀분석을 시행하였다.

결과: 로그치환 혈중 총 빌리루빈과 Framingham 위험점수는 단순선형회귀분석 결과 음의 상관관계를 보였고(R^2 : 0.018, $P < 0.001$) 이완기 혈압, 맥박수, 체질량 지수, 공복혈당, 중성지방, 저밀도 콜레스테롤, aspartate aminotransferase, alanine aminotransferase, 흡연 유무를 통제한 후 분석한 다중선형회귀분석 결과 음의 상관관계를 보였다(R^2 : 0.384, $P < 0.001$).

결론: 총 빌리루빈 수치와 Framingham 위험점수와의 음의 상관관계가 있음을 확인하였고 이는 총 빌리루빈 수치가 심혈관계 질환의 독립적인 위험인자이며 심혈관계 위험도를 예측하는 유용한 인자로 활용될 수 있음을 의미한다.

중심단어: 총 빌리루빈, 프래밍엄 위험도 점수, 심혈관 질환

REFERENCES

- Schwertner HA, Jackson WG, Tolan G. Association of low serum concentration of bilirubin with increased risk of coronary artery disease. *Clin Chem* 1994;40(1):18-23.
- Schwertner HA, Vitek L. Gilbert syndrome, UGT1A1*28 allele, and cardiovascular disease risk: possible protective effects and therapeutic applications of bilirubin. *Atherosclerosis* 2008;198(1):1-11.
- Vitek L, Schwertner HA. The heme catabolic pathway and its protective effects on oxidative stress-mediated diseases. *Adv Clin Chem* 2007;43:1-57.
- Stocker R, Perrella MA. Heme oxygenase-1: a novel drug target for atherosclerotic diseases? *Circulation* 2006;114(20):2178-89.
- Abraham NG, Asija A, Drummond G, Peterson S. Heme oxygenase-1 gene therapy: recent advances and therapeutic applications. *Curr Gene Ther* 2007;7(2):89-108.
- Perrella MA, Yet SF. Role of heme oxygenase-1 in cardiovascular function. *Curr Pharm Des* 2003;9(30):2479-87.
- 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):16712-9.
- Wu TW, Fung KP, Wu J, Yang CC, Weisel RD. Antioxidation of human low density lipoprotein by unconjugated and conjugated bilirubins. *Biochem Pharmacol* 1996;51(6):859-62.
- Schwertner HA. Association of smoking and low serum bilirubin antioxidant concentrations. *Atherosclerosis* 1998;136(2):383-7.
- Vitek L, Jirsa M, Brodanova M, Kalab M, Marecek Z, Danzig V, et al. Gilbert syndrome and ischemic heart disease: a protective effect of elevated bilirubin levels. *Atherosclerosis* 2002;160(2):449-56.
- Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. *Science* 1987;235(4792):1043-6.
- Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001;285(19):2486-97.
- Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med* 1999;340(2):115-26.
- Rodriguez-Portel M, Herrman J, Chade AR, Krier JD, Breen JF, Lerman A, et al. Long-term antioxidant intervention improves myocardial microvascular function in experimental hypertension. *Hypertension* 2004;43(2):493-8.
- Zhu XY, Daghini E, Chade AR, Rodriguez-Portel M, Napoli C, Lerman A, et al. Role of oxidative stress in remodeling of the myocardial microcirculation in hypertension. *Arterioscler Thromb Vasc Biol* 2006;26(8):1746-52.
- Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA* 2003;289(19):2560-72.
- American Diabetes Association. Standards of medical care in diabetes. *Diabetes Care* 2004;27 Suppl 1:S15-35.
- Horsfall LJ, Rait G, Walters K, Swallow DM, Pereira SP, Nazareth I, et al. Serum bilirubin and risk of respiratory disease and death. *JAMA* 2011;305(7):691-7.
- Clark JE, Foresti R, Sarathchandra P, Kaur H, Green CJ, Motterlini R. Heme oxygenase-1-derived bilirubin ameliorates postischemic myocardial dysfunction. *Am J Physiol Heart Circ Physiol* 2000;278(2):H643-51.
- Ollinger R, Bilban M, Erat A, Froio A, McDaid J, Tyagi S, et al. Bilirubin: a natural inhibitor of vascular smooth muscle cell proliferation. *Circulation* 2005;112(7):1030-9.
- Wu TW. Is serum bilirubin a risk factor for coronary artery disease? *Clin Chem* 1994;40(1):9-10.
- Siow RC, Sato H, Mann GE. Heme oxygenase-carbon monoxide signalling pathway in atherosclerosis: anti-atherogenic actions of bilirubin and carbon monoxide? *Cardiovasc Res* 1999;41(2):385-94.
- Platt JL, Nath KA. Heme oxygenase: protective gene or Trojan horse. *Nat Med* 1998;4(12):1364-5.
- Durante W, Schafer AI. Carbon monoxide and vascular cell function (review). *Int J Mol Med* 1998;2(3):255-62.
- Johnson RA, Kozma F, Colombi E. Carbon monoxide: from toxin to endogenous modulator of cardiovascular functions. *Braz J Med Biol Res* 1999;32(1):1-14.
- Salonen JT, Nyyssönen K, Korpela H, Tuomilehto J, Seppänen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. *Circulation* 1992;86(3):803-11.
- Nakagami T, Toyomura K, Kinoshita T, Morisawa S. A benefi-

cial role of bile pigments as an endogenous tissue protector: anti-complement effects of biliverdin and conjugated bilirubin. *Biochim Biophys Acta* 1993;1158(2):189-93.

28. Willis D, Moore AR, Frederick R, Willoughby DA. Heme oxygenase: a novel target for the modulation of the inflammatory response. *Nat Med* 1996;2(1):87-90.