Diagnostic value of homocysteine, C-reactive protein and bilirubin for coronary artery disease

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القيمة التشخيصية للهوموسيستئين، والبروتين المتفاعل سي، والبيليروبين، في أمراض الشرايين التاجية

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الخلاصة: أجرى الباحثون تقييماً لثلاث محدِّدات مميّزة جديدة خاصة بأمراض الشرايين التاجية (البيليروبين، والهوموسيستئين الكلي (t-Hcy)، والبروتين المتفاعل سي العالي الحساسية (hs-CRP)، على 319 مريضاً يعانون من آلام بالصدر، قُسِّموا إلى فئتَيْن وفقاً لنتائج تصوير الأوعية التاجية: فئة المصابين بأمراض الشرايين التاجية (262)، وفئة غير المصابين بهذه الأمراض (57) وكانت هناك فئة شاهدة قوامها 50 شخصاً من الأصحاء. وتبيَّن أن أعلى قيمة تشخيصية قد تحقَّقت لدى الفئة التي خضعت لاختبار الهوموسيستئين الكلي من بين المرضى الموثَّق مرضهم بصور الأوعية التاجية، في حين كانت أقل قيمة تشخيصية لدى الفئة التي خضعت لاختبار البيليروبين. وكانت معدلات الحساسية والنوعية للبيليروبين، والبروتين المتفاعل سي العالي الحساسية، والهوموسيستئين الكلي (وفقاً لمنحنيات الخاصية العاملة في المتلقّي): 70.09%، و50%، و76.8% على التوالي، و40.4%، و80.7%، و70.2% على التوالي. ويستنتج من ذلك أنه من غير الممكن التعرُّف على المرضى المُختَطَرين بأمراض الشرايين التاجية من خلال قياس مستويات البيليروبين المصلي، في حين قد تمثِّل اختبارات الهوموسيستئين الكلي، والبروتين المتفاعل سي العالي الحساسية، واسمات أفضل.

ABSTRACT We evaluated 3 new markers for coronary artery disease (CAD) [bilirubin, total homocysteine (t-Hcy) and high-sensitivity C-reactive protein (hs-CRP)] in 319 patients with chest pains divided into 2 groups based on coronary angiography: CAD group (*n* = 262) and non-CAD group (*n* = 57). A control group consisted of 50 healthy subjects. t-Hcy had the highest diagnostic value for diagnosis of angiographically documented patients; bilirubin had the lowest. The sensitivities and specificities (based on ROC curves) of bilirubin, hs-CRP and t-Hcy were 70.9%, 50% and 76.8% respectively, and 40.4%, 80.7% and 70.2% respectively. We conclude that serum bilirubin levels cannot identify people at risk of CAD and t-Hcy and hs-CRP may be stronger markers.

Valeur diagnostique de l'homocystéine, de la protéine C réactive et de la bilirubine dans la maladie coronarienne

RÉSUMÉ Nous avons évalué 3 nouveaux marqueurs de la maladie coronarienne (MC), à savoir la bilirubine, l'homocystéine totale (HcyT) et la protéine C réactive ultrasensible (CRPus) [ou hautement sensible (CRPhs)], chez 319 patients se plaignant de douleurs thoraciques répartis en 2 groupes en fonction des résultats de la coronarographie : le groupe avec MC (n = 262) et le groupe sans MC (n = 57). Un groupe témoin était constitué de 50 sujets sains. En présence d'une MC confirmée par l'angiographie, l'HcyT s'est avérée avoir la plus haute valeur diagnostique, la bilirubine ayant la plus faible. Les courbes ROC (pour *Receiver Operating Characteristic*) montrent pour chacun des trois marqueurs, bilirubine, CRPus et HcyT, respectivement une sensibilité de 70,9 %, 50 % et 76,8 % et une spécificité de 40,4 %, 80,7 % et 70,2 %. Nous en concluons que la bilirubinémie est dans l'incapacité d'identifier les sujets à risque de MC et que l'HcyT et la CRPus peuvent être des marqueurs plus puissants.

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Introduction

Coronary artery disease (CAD) often occurs in the absence of traditional risk factors. This study evaluated 3 promising new markers for cardiovascular risk: bilirubin, total homocysteine (t-Hcy) and high-sensitivity C-reactive protein (hs-CRP) [*1,2*]. Natural antioxidant defences have evolved to protect humans against deleterious effects of free radicals. The primary enzymatic defences are intracellular, but other antioxidant defences are largely extracellular, including antioxidative substrates such as uric acid and unconjugated bilirubin, the predominant bile pigment in the intravascular compartment. For many years, the bile pigment was considered as a toxic waste product formed during haem catabolism. However, more recent evidence suggests that bilirubin is a potent physiological antioxidant that may provide important protection against atherosclerosis, CAD and inflammation. In 1994, Schwertner et al. were the first to observe a significant inverse correlation between total bilirubin plasma concentrations and the prevalence of CAD [*3*]. Subsequently, Hopkins et al. noted that patients with early familial CAD had a mean total serum bilirubin of 8.9 (SD 6.1) μmol/L compared with 12.4 (SD 8.1) μmol/L in healthy control subjects [*4*]. Low serum bilirubin concentrations have been shown to be independently and inversely associated with an increased risk for CAD [*3*]. The strength of the association between bilirubin and CAD appears to be similar to that of high-density lipoprotein-cholesterol (HDL-C). The antioxidant capacity of bilirubin and its ability to provide potent scavenging of peroxyl radicals have led to suggestions that mildly increased circulatory bilirubin may have a physiological function to protect against disease processes that involve oxygen and peroxyl radicals [*5,6*]. Antioxidant activity and cardioprotective potential may be attributable to any of the bilirubin forms, including free unconjugated bilirubin, protein-bound unconjugated bilirubin, delta bilirubin or mono- or di-conjugated bilirubin. Under physiological conditions, the predominant circulatory form of bilirubin is the unconjugated, albumin-bound form [*7–9*].

In recent years, "non-traditional factors" such as hs-CRP, total homocysteine, as well as oxidative stress, have been proposed as risk factors for the development and progression of atherosclerosis and atherothrombotic cardiovascular disease [*10–13*]. The purpose of this study was to examine the relationship between traditional and non-traditional biomarkers of CAD in coronary angiography patients and in apparently healthy control subjects.

Methods

Subjects

All patients referred to the Department of Cardiology, University of Gaziantep between March 2003 and August 2003 for whom clinical data were available were included in our study. Thus 319 subjects were included who were admitted to hospital with chest pain and underwent coronary angiography. These patients were divided into 2 groups: the CAD group which consisted of 262 patients (63 females and 199 males) with stenosis of the coronary arteries and the non-CAD group (57 patients; 4 females and 53 males) which consisted of patients in whom CAD was excluded by coronary angiography (degree of stenosis < 20% indicating the absence of clinically relevant coronary stenosis). A third group was recruited which consisted of 50 apparently healthy control subjects (17 females and 23 males). The controls underwent physical

examination and routine laboratory investigation to verify their health status and that they were not taking any drugs. Age- and sex-matched individuals without any clearly evident chronic disease were recruited as control subjects.

Each angiogram was read jointly by at least 2 cardiologists. Coronary artery lesions were magnified, traced and measured with calipers to determine the percentage of diameter narrowing of the artery. All coronary angiographies were performed in the same centre. Patients with CAD were further divided into groups according to the maximum coronary stenosis at angiography: 0%–20% (no detectable CAD), 20%–49% (mild disease), 50%–70% (moderate disease, and 70%–100% (severe disease). Further classification of severity of the disease was done by counting the number of diseased vessels (0 to 3).

All participants were weighed and measured, gave blood samples and were questioned about established cardiovascular risk factors, including diabetes, smoking and hypertension. Although the healthy subjects did not undergo coronary angiograms they had a comprehensive physical examination by a physician, completed the World Health Organization standard Rose questionnaire on chest pain, and answered other questions about their past medical history [*14*]. None of the individuals in the healthy group had angina or a prior history of CAD. All of them had normal electrocardiograms according to the Minnesota Coding Criteria [*14*]. Obesity was defined as a body mass index (BMI) greater than 27.8 kg/m² as proposed by the National Institutes of Health Consensus Statement [*15*]. Diabetes mellitus was considered present in patients with a known history of diabetes and in patients with a fasting glucose $\geq$ 126 mg/dL (7.0 mmol/L) according to the American Diabetes Association criteria [*16*].

All 50 control subjects were monitored for somatic illness throughout the investigation period and were excluded if symptoms of infection or systemic illness were present (acute or chronic liver disease, cancer, renal disorder, rheumatic disease, etc.). Patients diagnosed with acute coronary syndrome 6 months prior to the study were excluded. Additional exclusion criteria included the use of aspirin, S-adenosyl-methionine, vitamin supplements, alcohol, anticonvulsants, estrogen, lipid-lowering therapy and other medications that might affect bilirubin, CRP and homocysteine metabolism. Thus 319 individuals were included in our patient group after these exclusions.

The study was approved by the Ethics Committee of Gaziantep University, and the individuals participating in the study gave their informed consent.

Laboratory methods

Blood samples of 319 patients and 50 controls (in EDTA tubes and tubes without additives) were taken at the time of admission between 08:00 and 10:00 after an overnight fast. Blood was centrifuged at 3000 g for 10 minutes at 4 ºC. After separation, the aliquots were frozen at –70 °C until analysis.

We measured serum total bilirubin by a diazo method with a detergent to accelerate azo-coupling and to prevent the precipitation of protein. The test was performed by means of an autoanalyser (Hitachi Modular DP Systems, Roche Diagnostics, and Mannheim, Germany). Total bilirubin levels below 1.1 mg/dL are normal for adults. Measurement is linear from 0.1 to 30 mg/dL. The intra-assay imprecision (coefficient of variation) was 1.3% and inter-assay imprecision (coefficient of variation) was 1.9% at a bilirubin concentration of 2.1 mg/dL.

Serum hs-CRP and t-Hcy concentrations were determined with the Immulite® one

analyser and Immulite® reagent (DPC, Los Angeles, USA) according to the manufacturer's instructions. The assays were linear from 2 to 50 μmol/L (t-Hcy) and 10 to 160 mg/L (hs-CRP); calibrators and controls were supplied by manufacturers. Specifications of intra-assay and inter-assay coefficients of variation of hs-CRP and t-Hcy assays were assessed from quality control data of the laboratory, which were 5.3% and 6.0% (intra-assay for hs-CRP), 9.1% and 9.9% (inter-assay for hs-CRP), 1.5% to 3.0% (intra-assay for t-Hcy) and 1.7% to 3.2% (inter-assay for t-Hcy).

Statistical analysis

Summary statistics were evaluated for all variables. Differences between the 3 groups were tested with a *t*-test for independent samples or Mann–Whitney test for continuous variables and a chi-squared test for categorical variables. The difference in current smoking frequency between the groups was tested by the Fisher exact test. Spearman correlation coefficients were obtained for biomarkers and each study variable for CAD patients. To determine independent predictors of the presence of CAD, multivariate logistic regression analysis was done using a model including all variables with a *P*-value < 0.15 on univariate analysis.

None of the results changed if the log of bilirubin and t-Hcy was used instead of the untransformed values. Because the distribution of hs-CRP is rightward skewed, values derived from log-transformed means were used as means for this variable throughout the study; these values virtually coincided with median values. Area under the curve (AUC) values in receiving operating characteristics (ROC) curve (as a measure of discriminating efficacy) were used for comparison of the diagnostic values of different analyses (including only the CAD and non-CAD groups, using angiography as the gold standard). Optimal cut-off levels, sensitivity and specificity of CRP were selected based on the ROC curves. Only 11 women were under age 50 years and there was no difference in bilirubin, hs-CRP and t-Hcy levels between pre- and postmenopausal women. Therefore, these variables were not further considered in the analysis. Two-tailed $P < 0.05$ values were considered. All statistical analyses and illustrations were obtained with *SPSS*, version 9.0 and MedCalc statistical software.

Results

Summary statistics are given for patients with CAD, without CAD (patients with normal angiogram) and for apparently healthy subjects in Table 1. There was no significant difference between the groups in BMI, waist/hip ratio and age. Mean [standard deviation (SD)] serum bilirubin levels were significantly higher in apparently healthy subjects [0.81 (SD 0.32) mg/dL] than patients without CAD [0.52 (SD 0.25) mg/dL] and the patients with CAD [0.55 (SD 0.39) mg/dL] who underwent coronary angiography ($P < 0.01$). However, t-Hcy [10.7 (SD 5.14) μmol/L] and hs-CRP [0.43 (SD 0.61) mg/dL] levels were significantly lower in individuals in the apparently healthy group and the groups undergoing coronary angiography; without CAD [13.0 (SD 8.61) μmol/L, 1.27 (SD 2.78) mg/dL, $P < 0.01$] and CAD [19.4 (SD 8.73) μmol/L, 1.54 (SD 0.87) mg/dL, $P < 0.01$] respectively. Additionally, serum t-Hcy and hs-CRP were significantly higher in patients with CAD compared to those without CAD ($P < 0.05$). Mean serum levels of hs-CRP [1.7 (SD 2.1) mg/dL] and t-Hcy [19.8 (SD 9.6) μmol/L] were highest in the patients who smoked (not shown in the table).

Table 1 **Summary statistics of the groups**

Variable	Healthy controls (*n* = 50)	Patients without CAD (*n* = 57)	Patients with CAD (*n* = 262)
Female (%)[a,b]	34	6	24
Age (years)	50 (8.74)	50 (8.54)	53 (8.48)
Smoking (%)[a,b]	16	19	42
Hypertension (No.)[d]	20	34	39
Diabetes (No.)[c]	0	2	18
Body mass index (kg/m^2)	27.8 (4.96)	28.1 (5.34)	27.8 (3.96)
Waist/hip ratio	0.91	0.92	0.95
Total bilirubina (mg/dL)	0.81 (0.32)	0.52 (0.25)	0.55 (0.39)
Total homocysteine (μmol/L)[a,b]	10.7 (5.14)	13.0 (8.61)	19.4 (8.73)
High-sensitivity C-reactive protein (mg/dL)[a,b]	0.43 (0.61)	1.27 (2.78)	1.54 (0.87)
Lipoprotein little A antigen (g/L)[a,b]	0.23 (0.24)	0.31 (0.32)	0.43 (0.33)
White blood cells (10^3/μL)[d]	7219 (1768)	7903 (1997)	8316 (2527)
Total cholesterol (mg/dL)[b]	174 (42.12)	170 (36.95)	188 (42.88)
Triglycerides (mg/dL)[b]	170 (87.13)	156 (69.25)	199 (113.33)
High-density lipoprotein cholesterol (mg/dL)[c,a]	43 (11.12)	34 (8.31)	31 (7.93)
Uric acid (mg/dL)[d]	5.5 (1.24)	6.1 (49.06)	6.4 (1.81)
Glucose (mg/dL)[d]	86 (13.27)	103 (20.21)	113 (58.50)
Aspartate aminotransferase (U/L)	32 (12.55)	34 (11.27)	39 (39.58)
Creatine kinase-MB (U/L)	26 (24.29)	26 (17.67)	32 (26.39)
Urea (mg/dL)	35 (9.78)	34 (11.27)	38 (17.81)
Platelets(10^3/μL)	263 (86.53)	249 (63.87)	268 (66.33)
Haemoglobin (g/dL)	13.9 (1.76)	13.5 (1.55)	13.9 (1.39)
Haematocrit (%)	40.7 (4.34)	39.9 (4.70)	40.8 (3.80)

[a]*Healthy control group versus patients without CAD and those with CAD;* P < *0.01.*
[b]*Patients without CAD versus patients with CAD;* P < *0.05.*
[c]*Healthy control group versus patients with CAD;* P < *0.001.*
[d]*Patients with CAD versus healthy control group;* P < *0.05.*
Values are means (standard deviations) except where indicated otherwise.
CAD = coronary artery disease.

Correlation coefficients of biomarkers of CAD in patients in whom CAD was angiographically documented are given in Table 2. There was a negative correlation between bilirubin and sex (male) (r = –0.183, P < 0.01 and r = –0.199, P < 0.01 respectively); in contrast, a significant positive correlation between bilirubin and serum t-Hcy concentrations was found (r = 0.330, P < 0.001). Bilirubin was also significantly correlated with triglycerides (r = –0.183, P < 0.01) and uric acid (r = 0.127, P < 0.05), but was not significantly correlated with any other study variable.

Table 2 **Correlation coefficients of biomarkers of coronary artery disease (CAD) in patients diagnosed by angiography (Spearman coefficients)**

Variable	Bilirubin	Total homocysteine	High-sensitivity C-reactive protein
Body mass index (kg/m^2)	NS	NS	NS
Sex (male/female)	r = –0.199**	r = 0.219***	r = 0.145**
Age (years)	NS	r = 0.158**	r = 0.267***
Smoking	NS	r =0.136*	NS
High-sensitivity C-reactive protein (mg/dL)	NS	r = 0.466***	–
Total homocysteine (μmol/L)	r = 0.330***	–	r = 0.466***
Bilirubin (mg/dL)	–	r = 0.330***	NS
Lipoprotein little A antigen (g/L)	NS	NS	r = 0.200***
White blood cells (10^3/μL)	NS	r = 0.138*	r = 0.250***
Total cholesterol (mg/dL)	NS	r = 0.129*	NS
Triglycerides (mg/dL)	r = –0.183**	NS	NS
High-density lipoprotein cholesterol (mg/dL)	NS	NS	r = 0.140**
Uric acid (mg/dL)	r = 0.127*	r = 0.150**	r = 0.150**
Stenotic vessels (No.)	NS	r = 0.450***	NS

**Significant at* P < *0.05; **Significant at* P < *0.01; ***Significant at* P < *0.001. NS = not significant.*

Total Hcy and hs-CRP concentrations showed many correlations with other study parameters (Table 2). There was a positive correlation between serum t-Hcy and sex, age, smoking, hs-CRP and number of stenotic vessels. In addition there was positive correlation between hs-CRP and sex, age and t-Hcy.

The predictor variables obtained by regression analysis for the number of stenotic vessels in CAD patients are given in Table 3. Sex (male, P = 0.001), age (P = 0.004) and t-Hcy (P = 0.0001) were strongly correlated with the number of stenotic vessels (severity of disease); hypertension (P = 0.019) was moderately associated, and HDL-C (P = 0.022), glucose (P = 0.028), total cholesterol (P = 0.047) were weakly associated. Bilirubin, hs-CRP and other parameters were not related to the number of diseased vessels and the degree of occlusion (P > 0.05).

Optimal cut-off levels and the associated diagnostic performances (sensitivity, specificity and diagnostic value) of serum bilirubin, hs-CRP, t-Hcy, based on ROC analysis, are given in Table 4. Optimal cut-off levels for bilirubin, hs-CRP and t-Hcy providing the maximum efficiency found in patients (n = 319) with CAD were 0.59 mg/dL, 1.09 mg/dL and 12.1 μmol/L respectively. ROC curve-based sensitivities

Table 3 **Regression analysis: predictor variables for the number of stenotic vessels in patients with coronary artery disease**

Variable	Beta	Standard error of the mean	*P*-value
Sex (male)	0.396	0.163	0.0001
Age (years)	0.166	0.008	0.004
Smoking	0.018	0.153	0.782
Body mass index (kg/m^2)	–0.007	0.015	0.894
Hypertension	0.141	0.142	0.019
Total bilirubin (mg/dL)	–0.057	0.177	0.336
Total homocysteine (μmol/L)	0.213	0.213	0.0001
High-sensitivity C-reactive protein (mg/dL)	0.028	0.032	0.631
Lipoprotein little A antigen (g/L)	0.083	0.200	0.152
Uric acid (mg/dL)	–0.033	0.038	0.587
Triglycerides (mg/dL)	0.097	0.001	0.130
Total cholesterol (mg/dL)	0.122	0.002	0.047
High-density lipoprotein cholesterol (mg/dL)	0.172	0.000	0.022
Glucose (mg/dL)	0.128	0.001	0.028
White blood cells (10^3/μL)	0.007	0.000	0.906

of bilirubin, hs-CRP and t-Hcy levels were 70.9%, 50.0%, 76.8% respectively. The specificities of bilirubin, hs-CRP and t-Hcy were 40.4%, 80.7% and 70.2% respectively (data of ROC curves are shown in Figures 1–3).

Discussion

To the best of our knowledge, the present study is the first to assess the diagnostic performance and relationship of bilirubin with hs-CRP and t-Hcy for cardiovascular disease in men and women in an angiographically documented design. The study demonstrated that patients with angiographically confirmed CAD had significantly higher serum hs-CRP and t-Hcy levels than non-stenotic patients (patients with normal angiogram) and the apparently healthy control group. These data strongly suggest that serum t-Hcy helps to identify individuals at risk of atherosclerosis (AUC value 0.781), especially among those with elevated hs-CRP and decreased bilirubin levels. t-Hcy showed the highest AUC value (0.781) compared to hs-CRP (0.648) and bilirubin (0.507).

In agreement with previous reports, we found that the bilirubin levels in serum were significantly lower in the patients with CAD than in age- and sex-matched controls [*3,17,18*]. We found that a serum bilirubin concentration of 10.0 μmol/L (0.58 mg/dL) discriminated between high and low cardiovascular risks. This association was

Table 4 **Optimal cut-off levels and associated specificity, sensitivity and diagnostic value of concentrations of biomarkers for the diagnosis of angiographically documented coronary artery disease**

Variable	Cut-off level	Sensitivity (%)	Specificity (%)	Diagnostic value (area under the curve)	+LR	–LR
Bilirubin	0.59 mg/dL	70.9	40.4	0.507	1.19	0.72
White blood cells	6700 $10^3/\mu L$	70.0	38.8	0.535	1.14	0.77
Uric acid	4.5 mg/dL	88.7	28.1	0.578	1.23	0.45
High-density lipoprotein cholesterol (female)	31 mg/dL	64.9	47.1	0.598	1.22	0.75
High-density lipoprotein cholesterol (male)	25 mg/dL	28.5	95.0	0.599	5.70	0.75
Total cholesterol	184 mg/dL	52.6	73.6	0.630	1.99	0.64
Lipoprotein little A antigen	0.24 g/L	59.5	64.9	0.630	1.70	0.62
Triglycerides	144 mg/dL	65.4	58.2	0.631	1.56	0.59
High-sensitivity C-reactive protein	1.09 mg/dL	50.0	80.7	0.648	2.59	0.62
Total homocysteine	12.1 μmol/L	76.8	70.2	0.781	2.67	0.29

+LR = positive likelihood ratio.
–LR = negative likelihood ratio.

independent of the extent of CAD, BMI, diabetes, hypertension and smoking. Individuals in the top quintile of serum bilirubin concentration had an 80% reduction of the CAD risk compared with individuals in the lowest quintile [*4*]. In 1995, Breimer et al. performed a prospective study of 7685 middle-aged men enrolled in the British Regional Heart Study and found that both low and high bilirubin concentrations were associated with an increased risk of CAD [*7*]. More recently, Vitek et al. reported on the prevalence of CAD in individuals with Gilbert syndrome who were found to have a CAD prevalence of 2% compared with 12.1% in the general population [*18*]. A meta-analysis of 11 studies has shown a negative relationship between serum bilirubin concentration and severity of atherosclerosis in men ($r = –0.31$, $P < 0.0001$) [*19*] but we did not find such an association in either men or women. However, we found that the number of stenotic coronary arteries was significantly associated with elevated serum t-Hcy and hs-CRP concentration. Several researchers have investigated the risk of myocardial infection in individuals with the *UGT1A1*28* allele [*20,21*]. According to the "oxidative modification hypothesis", which suggests atherogenesis is initiated by oxidization of low-density lipoprotein particles, it has been suggested that increased physiological concentrations of serum bilirubin may reduce atherogenic risk by reducing oxidation. An involvement of bilirubin in immune reactions and inflammatory processes has also been documented [*22–24*]. Smoking causes oxidative stress and production of acute phase reactants, such as CRP, temporary ischaemia, repeti-

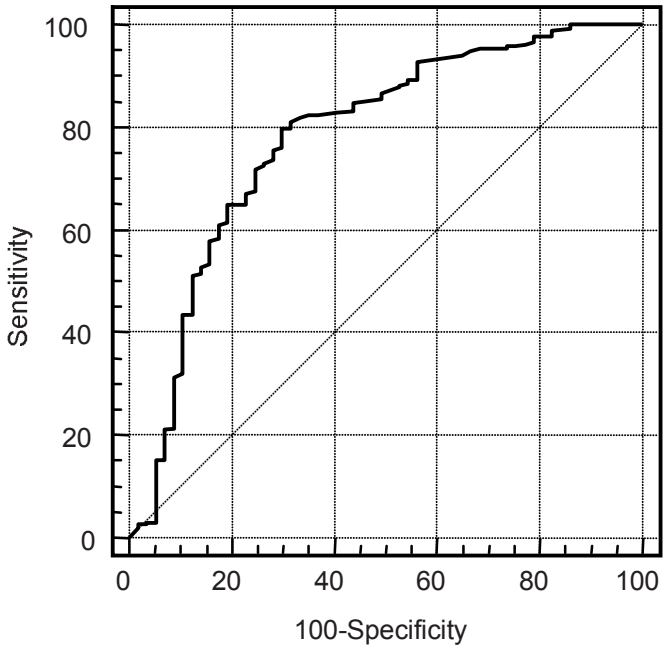

Figure 1 **ROC curve of total homocysteine**

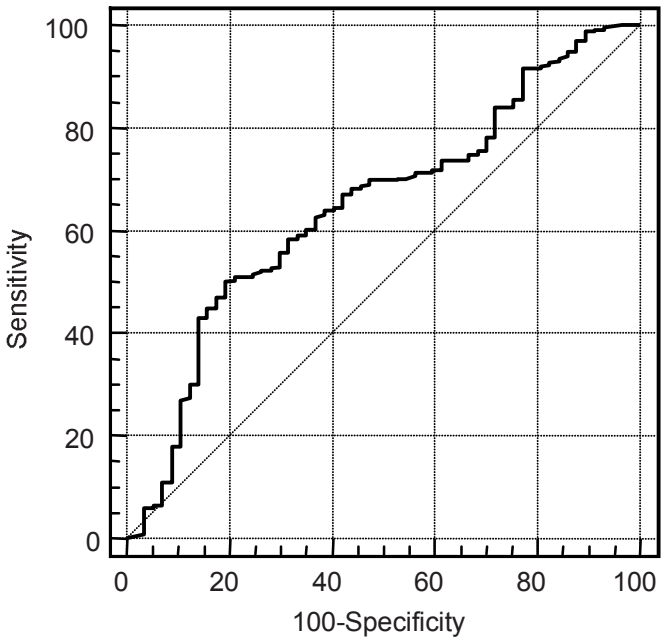

Figure 2 **ROC curve of high-sensitivity C-reactive protein**

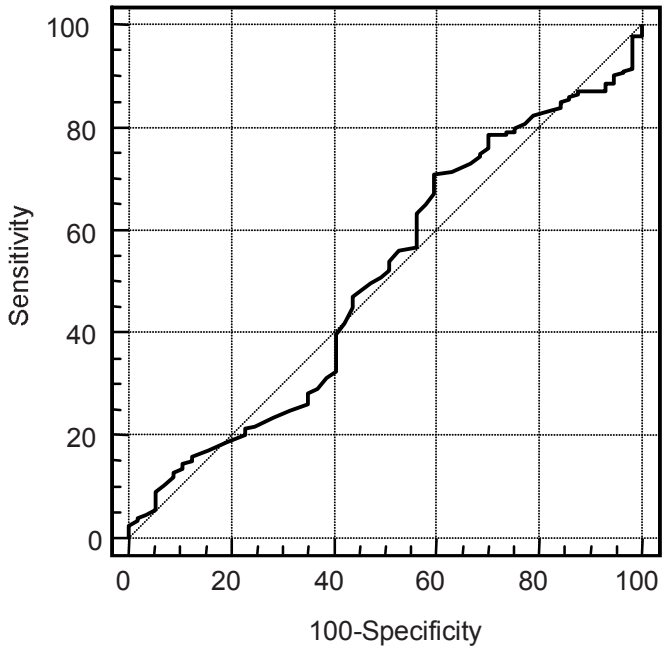

Figure 3 **ROC curve of total bilirubin**

tion of inflammation and reactive oxygen species. Thus, these factors constitute endothelial injury, which increases platelet aggregation, abnormalities of fibrinolysis and smooth muscle cell proliferation, and accelerate the development of thrombosis and atherosclerosis [*25,26*].

Earlier studies have reported differences in the levels of t-Hcy, ranging from 13.9–20.1 μmol/L in persons with CAD [*27,28*]. We found a mean t-Hcy level of 19.4 (SD 8.73) μmol/L in the CAD group, 10.7 (SD 5.14) μmol/L in the healthy group and 13.0 (SD 8.61) μmol/L in the non-CAD group. Some differences between reported serum t-Hcy levels may be related to analytical methods and ethnic differences. Bortolotto et al. grouped patients as hypertensive and hypertensive plus CAD. When compared, the plasma t-Hcy levels were significantly higher in the hypertensive plus CAD group [*29*]. Our study agrees with this study in that we obtained a similar association. The regression coefficient of hypertension with arterial blood pressure was 0.141 (SEM 0.142), $P = 0.019$. Also, homocysteine enhances oxidative stress. A study in 19 centres in Europe reported high homocysteine levels and increased risk of CAD in smokers [*30*]. We found that the t-Hcy levels tended to increase in the presence of more cardiovascular risk factors, i.e. male gender, older age, diabetes mellitus, hyperlipidaemia and certain chronic diseases. As expected, traditional coronary risk factors were more prevalent among those participants with elevated levels of t-Hcy and hs-CRP in our study, as in other studies [*31–33*]. More recently, McConnell et al. [*34*] and Lear et al. [*35*] have reported gender differences in C-reactive protein. The observed gender differences have important

implications for the establishment of cut-off points for cardiovascular risk stratification [*36*]. We found that lower total serum bilirubin was associated with a higher risk of CAD among men, but the pattern was much clearer in women (Table 2). Contrary to the findings of Djoussé et al. [*13*], our study provides only suggestive evidence for a lower risk for women. However, the relatively small number of CAD cases in women means that our study had less statistical power in women. Women may be more susceptible to low levels of bilirubin. The fact that the median age at baseline was 50 years in women indicates that most of the women in our study were postmenopausal. It is possible that in this older age group, the effects of bilirubin are not off-set by those of estrogen.

A plausible biological mechanism is necessary to support a causal association between serum bilirubin and CAD outcome. The levels of bilirubin may be related to an inflammatory condition in patients with CAD [*37,38*]. Another possibility is that low bilirubin concentration is not *per se* a major causative factor in the development of CAD, but rather a reflection of the presence of this ailment. According to this view, low bilirubin is a result of increased oxidative activity in CAD-prone individuals, leading to consumption of a natural antioxidant such as bilirubin [*39–41*]. Our data suggest that serum bilirubin concentration is more closely associated with the oxidative stress marker serum uric acid level [–0.033 (SEM 0.038), $P < 0.587$] than smoking. These findings conflict with those that have found subjects who smoke and have low serum bilirubin antioxidant concentrations [*20*]. Problems in risk assessment also arise from overlapping properties (shared pathophysiological pathway) of traditional risk factors such as hypertension, obesity, age, gender, smoking and diabetes [*42–49*].

To conclude, we found little evidence of an association between the serum concentration of bilirubin and atherosclerosis. In contrast, the concentration of novel (t-Hcy and hs-CRP) and traditional risk markers may be stronger markers for atherosclerosis in CAD patients.

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Regional consultation on establishing guidelines on management and care for acute coronary conditions

The World Health Organization Regional Office for the Eastern Mediterranean organized the above-mentioned regional consultation to establish guidelines on the management and care for acute coronary conditions, in Cairo, Egypt, from 27 to 29 March 2007.

The objectives of the consultation were:

- to review the progress made in the management and care of acute coronary conditions among countries of the Regions; and
- to set regional strategies for the management and care of acute coronary conditions.

Experts from Egypt, Islamic Republic of Iran, Lebanon, Pakistan, Qatar, Saudi Arabia, Syrian Arab Republic, Tunisia, United Arab Emirates, United Kingdom, as well as WHO concerned staff, participated in the consultation.