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Serum bilirubin improves the risk predictions of cardiovascular and total death in diabetic patients

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ABSTRACT

Background: Bilirubin is a potential endogenous inhibitor of atherosclerosis. We investigated the association of bilirubin and cardiovascular (CV) and all-cause mortality including potential improvements in bilirubin risk reclassification in asymptomatic diabetic patients.

Methods: We enrolled 2936 asymptomatic diabetic subjects. The serum bilirubin was measured, and future CV and all-cause death were the primary endpoints.

Results: The follow-up period was 5.4 ± 3.0 y. There were 218 deaths including 95 cardiovascular deaths. The occurrence of CV death and all-cause death were negatively correlated with increasing serum bilirubin quintiles and actual bilirubin values. Serum bilirubin was negatively associated with incident cardiovascular death (hazard ratio: 0.26, 95% CI, 0.11–0.61, $p = .01$) and all-cause death (hazard ratio: 0.30, 95% CI, 0.17–0.51, $p \leq .001$). The addition of bilirubin for cardiovascular death increased the C-statistic from 0.713 (95% CI, 0.664–0.762) to 0.729 (95% CI, 0.681–0.776) ($P = .008$) and showed an integrated discrimination improvement (IDI) of 0.012 ($P < .0171$) with 8.57% improvement in net reclassification analysis ($P = .0224$). These results suggest additional predictive value is possible via total bilirubin levels for future CV deaths in diabetic patients. In terms of all-death, the addition of bilirubin significantly increased the C-statistic (from 0.769 to 0.78, $P = .0064$)—a 3.52% net reclassification improvement ($P = .0307$). It did not improve the IDI ($p = .1505$).

Conclusions: Higher serum concentrations of bilirubin are associated with a decreased risk of developing CV and all-cause death in diabetic patients. Bilirubin improved the risk prediction of cardiovascular death but provided only a slightly better prediction of all-cause death than conventional risk factors.

1. Introduction

Diabetes is one of the most prevalent disorders worldwide and cardiovascular (CV) disease is the leading cause of mortality and morbidity in diabetic patients [1]. Therefore, improvements in risk prevention and adequate treatment are needed in these high-risk subjects. Recently, clinical studies have shown that low serum bilirubin values are significantly associated with a higher risk of cardiovascular disease [2–4]. In addition, the prospective Framingham Offspring Study showed that offspring with higher bilirubin levels had less cardiovascular disease development [5] suggesting a possible protective role of bilirubin against atherosclerosis [3]. Furthermore, patients with Gilbert

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disease—a hepatic disorder with abnormal accumulation of bilirubin—have a lower incidence of cardiovascular disease and fewer vascular complications [6,7]. Thus, serum bilirubin is negatively associated with cardiovascular disease risk, and low bilirubin levels might be a novel risk factor for the development of CV disease [4].

Although previous studies have shown that elevated serum bilirubin concentrations might protect against atherosclerosis, it remains unclear whether higher serum bilirubin concentrations work in favor of the cardiovascular system in patients with type 2 diabetes. Moreover, there are no studies investigating the effects of serum bilirubin in cardiovascular disease and total mortality—these are the most serious events among diabetic patients. Thus, it is unclear whether adding bilirubin could improve the risk stratification for diabetic patients.

2. Materials and methods

2.1. Study population

We enrolled subjects from patients undergoing a health examination at Taipei Veterans General Hospital; 59,089 subjects were screened for enrollment from November 17, 1997 to December 31, 2007, and subjects with diabetes were enrolled. Diabetes was defined if one of the following existed: current use of anti-diabetic agents, fasting glucose ≧7.0 mmol/l (126 mg/dl), or 2-h postprandial glucose ≧ 11.1 mmol/l (200 mg/dl). Metabolic syndrome (MS) was defined as fulfillment of at least 3 of the following 5 criteria: (1) blood pressure > 130/85 mmHg, (2) fasting plasma glucose level > 110 mg/dl, (3) hypertriglyceridemia with triglyceride level > 150 mg/dl, (4) high-density lipoprotein-cholesterol (HDL-C) concentration < 1.0 mmol/l in men or < 1.3 mmol/l in women, and (5) central obesity with a waist circumference > 90 cm in men or > 80 cm in women. The definition of central obesity was modified for an Asian population [8].

Our goal was to investigate the association between serum bilirubin and subsequent development of cardiovascular death among asymptomatic diabetic patients. Thus, subjects with hepatobiliary disorder including hepatitis B and C were excluded as were those with existing impaired liver function and abnormal aminotransferase (AST) and alanine aminotransferase (ALT). Because the study subjects were enrolled from those coming for a routine checkup and not the diabetes clinic, few subjects have major diabetic complications. Therefore, we focused on well-controlled or early-stage diabetic patients to evaluate the predictive value of serum bilirubin in risk stratification. Subjects with a history of coronary artery disease, stroke, peripheral artery disease, alcoholism, or chronic renal insufficiency (creatinine ≧ 1.5 mg/dl) were excluded. We also excluded those with valvular disease, congestive heart failure NYHA function II-VI, idiopathic cardiomyopathy, and evidence of abnormalities on resting electrocardiography (ECG) such as atrial fibrillation. Finally, 2936 diabetic individuals were enrolled from which we selected patients who were asymptomatic in terms of ischemic heart disease based on the criteria, but with potential event risks from diabetic complications as previously described [9]. The study was approved by the institutional review board at our institution.

2.2. Biochemical analysis

All subjects were enrolled after presenting for an annual checkup; biochemical studies were measured during this checkup. Blood samples were obtained from all patients after an overnight fast > 12 h. Total bilirubin was measured using a Hitachi 7600 chemistry analyzer with a vanadate oxidation method (Wako Pure Chemical Industries, Ltd.) in the central laboratory. The CVs for bilirubin were 1.9%, and the inter-assay CV was 1.3%. Similar measurements for total bilirubin have been used in our prior works [10–12]. Lipid profiles including triglycerides, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) cholesterol, serum total bilirubin, albumin, creatinine, AST, ALT, and glucose were also measured at the same time.

2.3. Determination of cardiovascular and all-cause death

Clinical events were CV death and all-cause death. The dates and causes of death among participants were obtained from the National Death Registry, which records all valid information on cause of death in Taiwan via the International Classification of Disease (ICD), Ninth Revision. The ICD-9 codes used for cardiovascular death were 390 to 459. The accuracy of cause-of-death coding in Taiwan's National Death Registry database has been validated [13,14].

2.4. Statistical analysis

Data were expressed as the mean ± SD for numeric variables and as the number (percent) for categorical variables. Baseline characteristics are represented by quintiles of serum total bilirubin levels. Comparisons of continuous variables between groups were performed by ANOVA. Subgroup comparisons for categorical variables were assessed by the χ^2 or Fisher's exact test. Multivariate Cox regression analysis estimated the association of bilirubin with risk of CV death and all-cause death after adjusting for clinical parameters (Model 1: crude; Model 2: age, gender, and status of hypertension and metabolic syndrome; and Model 3: age, gender, hypertension, metabolic syndrome, blood pressure, fasting glucose, 2-h postprandial glucose, and lipid profile including HDL, LDL and triglycerides).

We used three methods to evaluate the improvements achieved with including total bilirubin in predicting adverse outcomes [8]. First we plotted receiver operating characteristic (ROC) curves for baseline covariates with or without bilirubin to evaluate the ability of serum bilirubin to classify risk [2]. The incidences of cardiovascular or all-cause death were the primary outcomes. The C statistic—a measure of the area under the ROC curve—was calculated with and without bilirubin value. We also evaluated the value of adding bilirubin to reclassify risk beyond traditional cardiovascular risk factors. This was done by calculating the net reclassification improvement (NRI) and integrated discrimination improvement (IDI) for cardiovascular and all-cause death risk categories [14–17]. NRI is the difference between percentage of reclassifying those with events into high-risk categories and those without events into lower risk categories (indicating successful improvement in discrimination performance), and the percentage of reclassifying those with events into low risk categories and those without events into high-risk categories (indicating failure in improving discrimination performance [14–17]. The IDI calculation is a clinically less intuitive method to assess reclassification that does not rely on prespecified risk categories but represents a continuous measure [17]. All of these analyses were applied to estimate the improvement in risk reclassification after adding serum total bilirubin levels. These analyses were performed with SPSS software, ver 15.0; ROC curves and C statistics were generated with SAS 9.0.

3. Results

3.1. The association of serum bilirubin and cardiovascular death and all-cause death

A total of 2936 subjects with type 2 diabetes (1009 males and 1927 females; mean age of 62.7 ± 11 y) were enrolled in this study (see Fig. 1). Table 1 shows the baseline characteristics of the study subjects. We divided all subjects into 5 quintiles to demonstrate the baseline characteristic differences according to bilirubin values. With increasing quintiles of serum total bilirubin, subjects with higher bilirubin were more likely to be young, female, low BMI, non-hypotensive and with lower blood pressure, glucose, and TG and higher HDL ($p < .05$) (Table 1). This suggests that higher baseline bilirubin is associated with fewer cardiovascular risks.

There were 218 deaths during the follow-up period (mean, 5.4 ± 3.0 years) including 95 cardiovascular deaths. The occurrence of

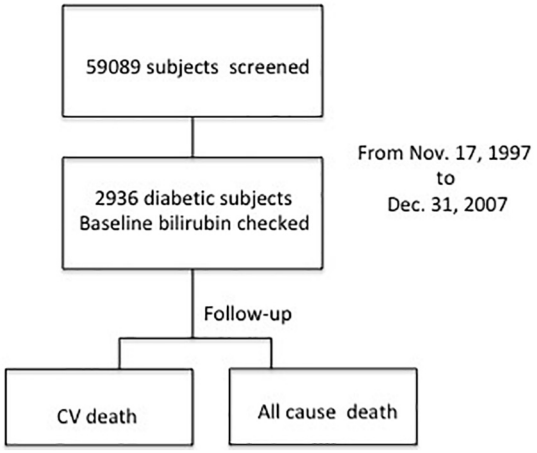

Fig. 1. Study flow.

CV death and all-cause death were also negatively correlated with increasing serum bilirubin quintiles and actual bilirubin values (Table 1). To determine the predictive value of total bilirubin for future CV death and all-cause death in diabetes, we used a hazard risk ratio via Cox regression analysis adjusted with comorbidities and other biochemical parameters. After adjusting for traditional risk factors and biochemical profiles including age, gender, hypertension, metabolic syndrome, blood pressure, fasting blood sugar, 2-h postprandial glucose, and lipid profiles (Table 2; Model 2 & Model 3), serum bilirubin concentration was negatively associated with increased risk of CV death (hazard ratio: 0.26, 95% CI, 0.11–0.61, $p = .01$) and all-cause death (hazard ration: 0.30, 95% CI, 0.17–0.51, $p \leq .001$) (Table 2). The trends toward decreased risks of CV and all-cause mortality were significant across the increasing quintiles of bilirubin values (p for trend $= 0.001$ and $p < .001$, respectively) (Table 2, quintile part). Both showed that higher baseline bilirubin is associated with fewer CV deaths/all-cause deaths.

3.2. Additional predictive values after considering adding total bilirubin in predicting future risk in diabetes

Next, receiver-operating-characteristic (ROC) curves for baseline covariates with or without bilirubin value were plotted to classify risk and evaluate whether serum bilirubin value would further improve prediction of CV and all-cause death. ROC analysis generates a C statistic (AUC) reflective of accuracy. This minimal yet significant improvement after the addition of bilirubin to the clinical risk factors model in CV death from 0.713 (95% CI, 0.664–0.762) to 0.729 (95% CI, 0.681–0.776) ($P = .008$) and for all-cause death from 0.769 (95% CI, 0.730–0.808) to 0.780 (95% CI, 0.742–0.818)($P = .0064$) (Table 3).

We further reclassified subjects into risk categories using the net reclassification-improvement (NRI). Among study subjects who died due to CV causes, the consideration of total bilirubin resulted in 11 individuals being reclassified into higher-risk categories (correct direction, green shaded cells); 5 were inappropriately classified into a lower-risk category (red-shaded cells). Conversely, among individuals who did not have CV death during follow-up, total bilirubin concentrations led to undesirable reclassification of risk in up to 176 individuals. The inclusion of bilirubin concentrations appropriately reclassified 240 individuals into lower-risk categories (Table 4). The use of bilirubin allowed us to reclassify 8.57% of cardiovascular death ($p = .0224$) (Table 3) with an integrated discrimination improvement (IDI) of 0.012 (0.001–0.023) ($p < .0171$). For all-cause death, the inclusion of bilirubin resulted in a 3.52% risk prediction improvement via NRI ($p = .0307$) but an insignificant IDI value of 0.003 (−0.003–0.009) ($p = .1505$) (Table 3).

4. Discussion

In this study, we demonstrated that serum levels of total bilirubin were negatively associated with an increased risk of CV death and all-cause death among diabetic patients suggesting that higher serum levels of bilirubin had a potential protective role in diabetic patients. Furthermore, the reclassification analysis evaluated the combined predictive value after adding bilirubin to the clinical risk factors. This showed that bilirubin measurements significantly improved risk stratification of CV death but only provided a slightly better prediction of all-cause death than conventional risk factors.

Bilirubin is an end product of heme catabolism and has been used as a marker of hepatobiliary and hematopoietic diseases in clinical practice [18]. Bilirubin is a natural product of heme catabolism by heme oxygenase (HO), and the induction of HO activity was recently shown to reduce metabolic and CV complications [19–21]. Bilirubin offers radical-scavenging and antioxidant activity that is more potent than even a-tocopherol [22]. Bilirubin also has a potent inhibitory effect on

Table 1
Baseline characteristics of the study population.

		Total Bilirubin					P for trend
	Total	Q1 (≦0.4)	Q2 (0.50–0.60)	Q3 (0.7)	Q4 (0.8–0.9)	Q5 (≧1.0)	
n	2936	521	606	523	670	616	
Age, y	62.7 ± 11.0	63.1 ± 11.2	63.0 ± 10.9	63.5 ± 11.1	62.6 ± 10.7	61.8 ± 11.1	0.034
Male, n (%)	1009 (34.4)	266 (51.1)	249 (41.1)	178 (34.0)	181 (27.0)	135 (21.9)	< 0.0001
BMI, kg/m^2	25.2 ± 3.6	25.4 ± 4.2	25.4 ± 3.7	25.2 ± 3.4	25.2 ± 3.4	25.0 ± 3.2	0.029
Hypertension, n (%)	1514 (51.6)	280 (53.7)	320 (52.8)	277 (53.0)	345 (51.5)	292 (47.4)	0.031
Smoking, n (%)	446 (15.2)	75 (14.4)	102 (16.8)	78 (15.0)	109 (16.3)	84 (13.6)	NS
ATP-defined metabolic syndrome, n (%)	1172 (39.9)	237 (45.5)	238 (39.3)	196 (37.5)	249 (37.2)	252 (40.9)	NS
Systolic BP, mmHg	136.1 ± 19.9	137.2 ± 21.1	136.6 ± 19.4	137.2 ± 19.7	135.7 ± 19.6	134.0 ± 20.0	0.005
Diastolic BP, mmHg	79.9 ± 11.7	78.4 ± 11.9	80.1 ± 12.1	80.0 ± 11.6	80.4 ± 11.2	80.4 ± 11.6	0.009
Fasting glucose, mg/dl	168.1 ± 62.1	162.4 ± 61.3	166.6 ± 61.9	165.1 ± 61.1	172.6 ± 61.4	172.2 ± 64.3	0.002
2-h plasma glucose, mg/dl	253.0 ± 103.1	245.2 ± 96.0	250.3 ± 100.2	246.4 ± 96.2	258.6 ± 111.5	261.8 ± 107.2	0.002
Triglycerides, mg/dl	187.7 ± 173.8	221.6 ± 226.8	189.1 ± 142.1	185.7 ± 223.0	178.4 ± 141.1	169.4 ± 124.8	< 0.0001
Cholesterol, mg/dl	202.1 ± 41.7	201.8 ± 47.3	201.6 ± 40.0	202.9 ± 42.3	202.9 ± 40.4	201.2 ± 39.0	NS
HDL-C, mg/dl	45.2 ± 11.8	44.0 ± 11.9	44.8 ± 11.5	45.2 ± 11.7	45.7 ± 11.9	45.9 ± 11.8	0.002
LDL-C, mg/dl	125.2 ± 34.0	121.7 ± 35.3	124.6 ± 33.7	126.2 ± 35.6	126.4 ± 32.3	126.6 ± 33.6	NS
Clinical outcomes							
Cardiovascular death	95 (3.2)	22 (4.2)	20 (3.3)	26 (4.9)	15 (2.2)	12 (1.9)	0.014
All-cause death	218 (7.4)	55 (10.5)	43 (7.1)	47 (8.9)	44 (6.5)	29 (4.7)	0.001

Data are mean ± SD; F test and X^2 test were used for continuous variables and categorical variables, respectively.

Table 2
Multivariate-adjusted associations of total bilirubin with cardiovascular and all-cause death.

	Total per1 mg/dl increase	*P*	Quintiles					P_{trend}
			Q1	Q2	Q3	Q4	Q5	
Cardiovascular death								
Model 1	0.31 (0.14–0.67)	0.003	Referent	0.91 (0.32–0.94)	0.95 (0.54–1.68)	0.42 (0.22–0.82)	0.39 (0.19–0.79)	0.004
Model 2	0.28 (0.13–0.62)	0.002	Referent	0.85 (0.30–0.90)	0.87 (0.49–1.54)	0.41 (0.21–0.79)	0.37 (0.18–0.74)	0.002
Model 3	0.26 (0.11–0.61)	0.010	Referent	0.71 (0.38–1.32)	0.95 (0.52–1.73)	0.39 (0.19–0.77)	0.35 (0.17–0.73)	0.001
All-cause death								
Model 1	0.37 (0.22–0.62)	< 0.001	Referent	0.80 (0.40–0.83)	0.71 (0.48–1.05)	0.52 (0.35–0.77)	0.39 (0.25–0.61)	< 0.001
Model 2	0.36 (0.21–0.60)	< 0.001	Referent	0.71 (0.38–0.76)	0.60 (0.41–0.90)	0.46 (0.30–0.69)	0.34 (0.21–0.54)	< 0.001
Model 3	0.30 (0.17–0.51)	< 0.001	Referent	0.56 (0.37–0.84)	0.62 (0.41–0.93)	0.44 (0.29–0.67)	0.32 (0.20–0.52)	< 0.001

Model1 was unadjusted.
Model2 was adjusted for age, sex, hypertension, metabolic syndrome status.
Model 3 was adjusted for variables in model 2 plus systolic blood pressure, diastolic blood pressure, 2-h plasma glucose, HDL cholesterol and triglyceride.

Table 3
Improvement in discrimination performance and calibration for risk prediction of cardiovascular death and all-cause death in the multivariate-adjusted model after including total bilirubin.

	C statistic	*P*	NRI (%)	*P*	IDI	*P*
Cardiovascular death						
Established risk factors*	0.713 (0.664–0.762)	0.0080	Referent	0.0224	Referent	0.0171
Established risk factors* + T.Bilirubin	0.729 (0.681–0.776)		8.57		0.012 (0.001–0.023)	
All-cause death						
Established risk factors*	0.769 (0.730–0.808)	0.0064	Referent	0.0307	Referent	NS
Established risk factors* + T.Bilirubin	0.780 (0.742–0.818)		3.52		0.003 (−0.003–0.009)	

NRI indicates net reclassification improvement; IRI, integrated discrimination improvement.
* Established risk factors includes smoking, age, gender, blood pressure, history of hypertension, hyperlipidemia, and lipid profiles (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol.

the activity of NAD(P)H oxidase. This plays an important role in determining ROS production [23,24]. In addition to scavenging oxygen radicals, bilirubin also has inhibitory effects against LDL oxidation [25] and is a potential endogenous inhibitor against atherosclerosis. An animal study using hyperbilirubinemic rats treated with biliverdin, the precursor of bilirubin, showed less balloon injury-induced neointima formation than controls [26]. Yamaguchi et al. also demonstrated that bilirubin is a physiological antioxidant in ischemia-reperfusion [27] further supporting the potential beneficial effect of bilirubin against vascular injury and atherosclerosis.

In addition to the possible molecular protective mechanism of bilirubin, clinical observations have shown that low concentrations of serum bilirubin were related to an increased risk of coronary artery disease [2,4,28,29], stroke [30–33], and diabetic vascular complications [34,35]. We found that serum concentrations of total bilirubin were negatively associated with an increased risk of CV death and all-cause death in diabetic patients suggesting that low serum bilirubin levels are an independent risk factor in diabetic patients and can be used as a marker of risk stratification in diabetic patients.

Ohnaka et al. demonstrated inverse associations of serum bilirubin and CRP to type 2 diabetes suggesting that higher concentrations of serum bilirubin probably confer protection against type 2 diabetes [18]. Higher concentrations of bilirubin correlated with a lower risk of diabetes and diabetic-related complications like diabetic nephropathy [34] and CAD in diabetic patients [39]. The beneficial effect of bilirubin may be mediated by its anti-oxidative [3,36] and anti-inflammatory effects [37–39].

Here, the addition of bilirubin to the clinical model not only improved the predictive power for CV death in diabetic patients (as assessed by the ROC curves) but also the reclassification of the subjects into different risk categories via the NRI. This suggests that low bilirubin is an independent marker of cardiovascular disease and increases the predictive value for CV death among diabetic patients.

Adding bilirubin also increased the AUC for all-cause death, but it did not have the same effect in reclassifying subjects' risk categories from the NRI and IDI results. One reasonable explanation is that bilirubin reduces oxidative stress—a key feature of atherosclerosis. CV deaths account for up to 15% of all-cause death according to the World Health Organization [14]. That is why adding the factor of low bilirubin significantly improves AUC in cardiovascular death and all-cause death but did not significantly change risk group categories of all-cause death.

One of the strengths of our study is that it is prospective, but there are still some limitations. First, although fasting and postprandial glucose concentrations were measured, the HbA1C value was not routinely measured. However, there is a linear and predictable relationship between HbA1c and glucose [40], and the HbA1C can be estimated accordingly. Second, all study subjects were enrolled during check-up—not the diabetic clinic; thus, information on the medication for diabetes control is unknown. Third, the validity of the present reclassification results and conclusions remains an arbitrary choice for cutoffs for risk categories. To date, no consensus-established risk-prediction scheme has been implemented. We used the same risk classes as in other reports on the risk algorithm [14,16]. In addition, a different definition of cut points may have resulted in changes to the net reclassification. We performed the integrated discrimination improvement analysis, which is not dependent on specified risk categories. The results of both analyses showed the same direction.

5. Conclusion

Serum bilirubin was negatively associated with increased risk of cardiovascular and all-cause death in diabetic patients independent of established CVD risk factors. Bilirubin improved the risk prediction of cardiovascular death but provided only a slightly better prediction of all-cause death than conventional risk factors.

Table 4
Reclassification of risk of cardiovascular and all-cause death after considering serum level of total bilirubin.

A) Cardiovascular death					
Without total Bilirubin	With total Bilirubin				
	<1%	1-3%	3-6%	≧6%	Total
	Participants who died from cardiovascular causes				
<1%	14 (0.15)	4 (0.04)	0 (0.00)	0 (0.00)	18
1-3%	1 (0.01)	22 (0.23)	2 (0.02)	0 (0.00)	25
3-6%	0 (0.00)	2 (0.02)	10 (0.11)	5 (0.05)	17
≧6%	0 (0.00)	0 (0.00)	2 (0.02)	33 (0.35)	35
Total	15	28	14	38	95
	Participants who did not die				
<1%	1359 (0.48)	63 (0.02)	0 (0.00)	0 (0.00)	1422
1-3%	101 (0.04)	482 (0.17)	66 (0.02)	0 (0.00)	649
3-6%	1 (0.00)	79 (0.03)	226 (0.08)	47 (0.02)	353
≧6%	0 (0.00)	7 (0.00)	52 (0.02)	358 (0.13)	417
Total	1461	631	344	405	2841

The net reclassification improvement of cardiovascular death was estimated at 8.57% (P=0.0224).

B) All-cause death					
Without total Bilirubin	With total Bilirubin				
	<10%	10-25%	25-35%	≧35%	Total
	Participants who died from all causes				
<10%	95 (1.00)	6 (0.06)	0 (0.00)	0 (0.00)	101
10-25%	2 (0.02)	33 (0.35)	2 (0.02)	1 (0.01)	38
25-35%	0 (0.00)	1 (0.01)	8 (0.08)	3 (0.03)	12
≧35%	0 (0.00)	0 (0.00)	1 (0.01)	66 (0.69)	67
Total	97	40	11	70	218
	Participants who did not die				
<10%	2372 (0.83)	43 (0.02)	0 (0.00)	0 (0.00)	2415
10-25%	50 (0.02)	193 (0.07)	14 (0.00)	1 (0.00)	258
25-35%	0 (0.00)	9 (0.00)	10 (0.00)	10 (0.00)	29
≧35%	1 (0.00)	1 (0.00)	3 (0.00)	11 (0.00)	16
Total	2423	246	27	22	2718

The net reclassification improvement of all-cause death was estimated at 3.52 % (P=0.0307)

References

[1] I. Martin-Timon, C. Sevillano-Collantes, A. Segura-Galindo, F.J. Del Canizo-Gomez, Type 2 diabetes and cardiovascular disease: have all risk factors the same strength? World J. Diabetes 5 (4) (2014) 444–470.

[2] H.A. Schwertner, W.G. Jackson, G. Tolan, Association of low serum concentration of bilirubin with increased risk of coronary artery disease, Clin. Chem. 40 (1) (1994) 18–23.

[3] G. Targher, C.D. Byrne, Circulating Markers of Liver Function and Cardiovascular Disease Risk, Arterioscler. Thromb. Vasc. Biol. 35 (11) (2015) 2290–2296.

[4] M.K. Akboga, U. Canpolat, A. Sahinarslan, Y. Alsancak, S. Nurkoc, D. Aras, S. Aydogdu, A. Abaci, Association of serum total bilirubin level with severity of coronary atherosclerosis is linked to systemic inflammation, Atherosclerosis 240 (1) (2015) 110–114.

[5] L. Djousse, D. Levy, L.A. Cupples, J.C. Evans, R.B. D'Agostino, R.C. Ellison, Total serum bilirubin and risk of cardiovascular disease in the Framingham offspring study, Am. J. Cardiol. 87(10) (2001) 1196–200; (a4, 7).

[6] L. Vitek, M. Jirsa, M. Brodanova, M. Kalab, Z. Marecek, V. Danzig, L. Novotny, P. Kotal, Gilbert syndrome and ischemic heart disease: a protective effect of elevated bilirubin levels, Atherosclerosis 160 (2) (2002) 449–456.

[7] T. Inoguchi, S. Sasaki, K. Kobayashi, R. Takayanagi, T. Yamada, Relationship between Gilbert syndrome and prevalence of vascular complications in patients with diabetes, JAMA 298 (12) (2007) 1398–1400.

[8] C.E. Tan, S. Ma, D. Wai, S.K. Chew, E.S. Tai, Can we apply the National Cholesterol Education Program Adult Treatment Panel definition of the metabolic syndrome to Asians? Diabetes Care 27 (5) (2004) 1182–1186.

[9] Y. Yamasaki, K. Nakajima, H. Kusuoka, T. Izumi, A. Kashiwagi, R. Kawamori, K. Shimamoto, N. Yamada, T. Nishimura, Prognostic value of gated myocardial perfusion imaging for asymptomatic patients with type 2 diabetes: the J-ACCESS 2 investigation, Diabetes Care 33 (11) (2010) 2320–2326.

[10] C.C. Chang, C.Y. Hsu, P.H. Huang, C.H. Chiang, H.B. Leu, C.C. Huang, J.W. Chen, S.J. Lin, Association of serum bilirubin with SYNTAX score and future cardiovascular events in patients undergoing coronary intervention, Acta Cardiol Sin. 32 (4) (2016) 412–419.

[11] S.S. Huang, W.L. Chen, H.B. Leu, P.H. Huang, S.J. Lin, J.W. Chen, Serum bilirubin levels predict future development of metabolic syndrome in healthy middle-aged nonsmoking men, Am. J. Med. 128 (10) (2015) e35–e41.

[12] S.S. Huang, P.H. Huang, H.B. Leu Ph, T.C. Wu, S.J. Lin, J.W. Chen, Serum bilirubin predicts long-term clinical outcomes in patients with cardiac syndrome X, Heart 96 (15) (2010) 1227–1232.

[13] K.L. Wang, H.M. Cheng, S.H. Sung, S.Y. Chuang, C.H. Li, H.A. Spurgeon, C.T. Ting, S.S. Najjar, E.G. Lakatta, F.C. Yin, P. Chou, C.H. Chen, Wave reflection and arterial stiffness in the prediction of 15-year all-cause and cardiovascular mortalities: a community-based study, Hypertension (Dallas Tex. : 1979) 55 (3) (2010) 799–805.

[14] H.J. Lin, B.C. Lee, Y.L. Ho, Y.H. Lin, C.Y. Chen, H.C. Hsu, M.S. Lin, K.L. Chien, M.F. Chen, Postprandial glucose improves the risk prediction of cardiovascular death beyond the metabolic syndrome in the nondiabetic population, Diabetes Care 32 (9) (2009) 1721–1726.

[15] M.J. Pencina, R.B. D'Agostino Sr., R.B. D'Agostino Jr., R.S. Vasan, Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond, Stat. Med. 27 (2) (2008) 157–172 (discussion 207-12).

[16] S. Kathiresan, O. Melander, D. Anevski, C. Guiducci, N.P. Burtt, C. Roos, J.N. Hirschhorn, G. Berglund, B. Hedblad, L. Groop, D.M. Altshuler, C. Newton-Cheh, M. Orho-Melander, Polymorphisms associated with cholesterol and risk of cardiovascular events, N. Engl. J. Med. 358 (12) (2008) 1240–1249.

[17] R.B. Schnabel, M.G. Larson, J.F. Yamamoto, L.M. Sullivan, M.J. Pencina, J.B. Meigs, G.H. Tofler, J. Selhub, P.F. Jacques, P.A. Wolf, J.W. Magnani, P.T. Ellinor, T.J. Wang, D. Levy, R.S. Vasan, E.J. Benjamin, Relations of biomarkers of distinct pathophysiological pathways and atrial fibrillation incidence in the community, Circulation 121 (2) (2010) 200–207.
[18] K. Ohnaka, S. Kono, T. Inoguchi, G. Yin, M. Morita, M. Adachi, H. Kawate, R. Takayanagi, Inverse associations of serum bilirubin with high sensitivity C-reactive protein, glycated hemoglobin, and prevalence of type 2 diabetes in middle-aged and elderly Japanese men and women, Diabetes Res. Clin. Pract. 88 (1) (2010) 103–110.
[19] L.Y. Lin, H.K. Kuo, J.J. Hwang, L.P. Lai, F.T. Chiang, C.D. Tseng, J.L. Lin, Serum bilirubin is inversely associated with insulin resistance and metabolic syndrome among children and adolescents, Atherosclerosis 203 (2) (2009) 563–568.
[20] A. Ayer, A. Zarjou, A. Agarwal, R. Stocker, Heme Oxygenases in Cardiovascular Health and Disease, Physiol. Rev. 96 (4) (2016) 1449–1508.
[21] L.E. Fredenburgh, A.A. Merz, S. Cheng, Haeme oxygenase signalling pathway: implications for cardiovascular disease, Eur. Heart J. 36 (24) (2015) 1512–1518.
[22] R. Stocker, Y. Yamamoto, A.F. McDonagh, A.N. Glazer, B.N. Ames, Bilirubin is an antioxidant of possible physiological importance, Science (New York, N.Y.) 235 (4792) (1987) 1043–1046.
[23] J.Y. Kwak, K. Takeshige, B.S. Cheung, S. Minakami, Bilirubin inhibits the activation of superoxide-producing NADPH oxidase in a neutrophil cell-free system, Biochim. Biophys. Acta 1076 (3) (1991) 369–373.
[24] S. Lanone, S. Bloc, R. Foresti, A. Almolki, C. Taille, J. Callebert, M. Conti, D. Goven, M. Aubier, B. Dureuil, J. El-Benna, R. Motterlini, J. Boczkowski, Bilirubin decreases nos2 expression via inhibition of NAD(P)H oxidase: implications for protection against endotoxic shock in rats, FASEB journal 19 (13) (2005) 1890–1892.
[25] J. Neuzil, R. Stocker, Free and albumin-bound bilirubin are efficient co-antioxidants for alpha-tocopherol, inhibiting plasma and low density lipoprotein lipid peroxidation, J. Biol. Chem. 269 (24) (1994) 16712–16719.
[26] R. Ollinger, M. Bilban, A. Erat, A. Froio, J. McDaid, S. Tyagi, E. Csizmadia, A.V. Graca-Souza, A. Liloia, M.P. Soares, L.E. Otterbein, A. Usheva, K. Yamashita, F.H. Bach, Bilirubin: a natural inhibitor of vascular smooth muscle cell proliferation, Circulation 112 (7) (2005) 1030–1039.
[27] T. Yamaguchi, M. Terakado, F. Horio, K. Aoki, M. Tanaka, H. Nakajima, Role of bilirubin as an antioxidant in an ischemia-reperfusion of rat liver and induction of heme oxygenase, Biochem. Biophys. Res. Commun. 223 (1) (1996) 129–135.
[28] L.H. Breimer, G. Wannamethee, S. Ebrahim, A.G. Shaper, Serum bilirubin and risk of ischemic heart disease in middle-aged British men, Clin. Chem. 41 (10) (1995) 1504–1508.
[29] P.N. Hopkins, L.L. Wu, S.C. Hunt, B.C. James, G.M. Vincent, R.R. Williams, Higher serum bilirubin is associated with decreased risk for early familial coronary artery disease, Arterioscler. Thromb. Vasc. Biol. 16 (2) (1996) 250–255.
[30] T.S. Perlstein, R.L. Pande, M.A. Creager, J. Weuve, J.A. Beckman, Serum total bilirubin level, prevalent stroke, and stroke outcomes: NHANES 1999-2004, Am. J. Med. 121 (9) (2008) 781–788.e1.
[31] H. Kimm, J.E. Yun, J. Jo, S.H. Jee, Low serum bilirubin level as an independent predictor of stroke incidence: a prospective study in Korean men and women, Stroke 40 (11) (2009) 3422–3427.
[32] E. Oda, R. Kawai, A possible cross-sectional association of serum total bilirubin with coronary heart disease and stroke in a Japanese health screening population, Heart Vessel. 27 (1) (2012) 29–36.
[33] R.Y. Li, Z.G. Cao, J.R. Zhang, Y. Li, R.T. Wang, Decreased serum bilirubin is associated with silent cerebral infarction, Arterioscler. Thromb. Vasc. Biol. 34 (4) (2014) 946–951.
[34] M. Fukui, M. Tanaka, E. Shiraishi, I. Harusato, H. Hosoda, M. Asano, G. Hasegawa, N. Nakamura, Relationship between serum bilirubin and albuminuria in patients with type 2 diabetes, Kidney Int. 74 (9) (2008) 1197–1201.
[35] S.S. Najam, J. Sun, J. Zhang, M. Xu, J. Lu, K. Sun, M. Li, T. Wang, Y. Bi, G. Ning, Serum total bilirubin levels and prevalence of diabetic retinopathy in a Chinese population, Journal of diabetes 6 (3) (2014) 221–227.
[36] A. Kontush, T. Spranger, A. Reich, S. Djahansouzi, B. Karten, J.H. Braesen, B. Finckh, A. Kohlschutter, U. Beisiegel, Whole plasma oxidation assay as a measure of lipoprotein oxidizability, BioFactors (Oxford, England) 6 (2) (1997) 99–109.
[37] S. Tapan, T. Dogru, I. Tasci, C.N. Ercin, T. Ozgurtas, M.K. Erbil, Soluble CD40 ligand and soluble P-selectin levels in Gilbert's syndrome: a link to protection against atherosclerosis? Clin. Biochem. 42 (9) (2009) 791–795.
[38] S. Yoshino, S. Hamasaki, S. Ishida, T. Kataoka, A. Yoshikawa, N. Oketani, K. Saihara, H. Okui, T. Shinsato, H. Ichiki, T. Kubozono, S. Kuwahata, S. Fujita, D. Kanda, M. Nakazaki, M. Miyata, C. Tei, Relationship between bilirubin concentration, coronary endothelial function, and inflammatory stress in overweight patients, J. Atheroscler. Thromb. 18 (5) (2011) 403–412.
[39] Y.H. Chen, L.Y. Chau, J.W. Chen, S.J. Lin, Serum bilirubin and ferritin levels link heme oxygenase-1 gene promoter polymorphism and susceptibility to coronary artery disease in diabetic patients, Diabetes Care 31 (8) (2008) 1615–1620.
[40] D.M. Nathan, J. Kuenen, R. Borg, H. Zheng, D. Schoenfeld, R.J. Heine, Translating the A1C assay into estimated average glucose values, Diabetes Care 31 (8) (2008) 1473–1478.