Decreased Serum Bilirubin Is Associated With Silent Cerebral Infarction

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Objective—The presence of silent cerebral infarction (SCI) increases the risk of transient ischemia attack, symptomatic stroke, cardiovascular disease, and dementia. Total bilirubin (TB) levels were demonstrated to be decreased in carotid intima–media thickness, cardiovascular disease, stroke, and peripheral arterial disease. However, little information is available concerning the correlation between TB and SCI.

Approach and Results—A cross-sectional study was conducted to evaluate the association between TB and SCI in 2865 subjects (1831 men and 1034 women) undergoing medical checkup. The participants with SCI had lower TB levels than those without SCI. The subjects with a low TB had a higher prevalence of SCI. Moreover, partial correlation showed that TB levels were tightly correlated with brachial-ankle pulse wave velocity after adjusting for confounding covariates (r=–0.149; P<0.001). Multivariate logistic regression analysis revealed that higher TB was associated with a lower risk of SCI (odds ratio, 0.925; 95% confidence interval, 0.897–0.954; P<0.001).

Conclusions—TB is a novel biochemical indicator for SCI regardless of classical cardiovascular risk factors. Early measurement of TB may be useful to assess the risk of SCI. **(*Arterioscler Thromb Vasc Biol*. 2014;34:946-951.)**

Key Words: atherosclerosis ■ bilirubin ■ cerebral infarction

Silent cerebral infarction (SCI) is a cerebral infarction that is evident on brain imaging but is not associated with a clinical symptom. In most cases, SCI is found as a lacunar infarction, that is, a small, deep cerebral infarct caused by occlusion of small penetrating cerebral arteries. Recent studies demonstrated that the presence of SCI predicts transient ischemia attack, clinically overt stroke, cardiovascular disease, and dementia.[1–3]

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A large body of evidence has highlighted a role for oxidative stress in atherosclerosis. Oxidative stress is induced by increased production of reactive oxygen species or decreased antioxidant capacity of endogenous antioxidant systems. Bilirubin is an effective antioxidant molecule that suppresses the oxidation of lipids and prevents the formation of plaque.[4] In several cross-sectional studies, bilirubin levels were found to be decreased in carotid intima–media thickness, cardiovascular disease, stroke, and peripheral arterial disease.[5–8] A meta-analysis further confirmed that bilirubin levels were reduced in coronary artery disease.[9]

To the best of our knowledge, the relationship between bilirubin levels and SCI has not yet been reported. We, therefore, conducted this study to assess the bilirubin levels in general population.

Materials and Methods

Materials and Methods are available in the online-only Supplement.

Results

Clinical and laboratory data of participants with and without SCI are shown in Table 1. Of the 2865 participants enrolled, 1831 (63.91%) were men and 1034 (36.09%) were women. Median (interquartile range) of total bilirubin (TB) concentration was 10.5 (7.8–13.8; range, 2.1–33.4) μmol/L in the whole cohort of individuals. Three hundred forty-three participants (11.97%; 274 men and 69 women) presented SCI. The patients with SCI were older and had higher body mass index (BMI), systolic blood pressure, diastolic blood pressure (DBP), total cholesterol (TC), triglyceride, low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose (FPG), and brachial-ankle pulse wave velocity (baPWV) levels and reduced TB, indirect bilirubin, direct bilirubin, estimated glomerular filtration rate (eGFR), and high-density lipoprotein cholesterol levels compared with the subjects without SCI. However, the levels of aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, and current use of statins and calcium channel blocker drugs in the 2 groups had no difference. Male sex, smoking, alcohol consumption, type 2 diabetes mellitus (DM), hypertension, and current use of angiotensin-converting enzyme inhibitor/angiotensin II receptor antagonist, aspirin, and hypoglycemic drugs had a higher prevalence in SCI group.

The demographic and biochemical characteristics of the study population according to TB quartiles are shown in Table 2. Mean age, BMI, systolic blood pressure, DBP,

Received on: August 21, 2013; final version accepted on: December 16, 2013.

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The online-only Data Supplement is available with this article at http://atvb.ahajournals.org/lookup/suppl/doi:10.1161/ATVBAHA.113.303003/-/DC1.

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Nonstandard Abbreviations and Acronyms

baPWV	brachial-ankle pulse wave velocity
BMI	body mass index
DBP	diastolic blood pressure
DM	type 2 diabetes mellitus
eGFR	estimated glomerular filtration rate
FPG	fasting plasma glucose
LDL-C	low density lipoprotein cholesterol
SCI	silent cerebral infarction
TB	total bilirubin
TC	total cholesterol

FPG, triglyceride, LDL-C, FPG, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, and baPWV decreased gradually as TB increased. Also, the percentage of alcohol consumption, DM and hypertension, and current use of statins, angiotensin-converting enzyme inhibitor/angiotensin II receptor antagonist, and hypoglycemic drugs reduced as TB quartiles increased. However, eGFR, high-density lipoprotein cholesterol, and the percentage of male sex and smoker increased as TB quartiles increased. TC and the percentage of use of calcium channel blocker and aspirin had no difference as TB quartiles increased.

The partial correlation between baPWV and TB levels was calculated. BaPWV statistically correlated with TB levels after adjustment for age, sex, BMI, smoking status, alcohol consumption, systolic blood pressure, DBP, FPG, TC, triglyceride, high-density lipoprotein cholesterol, LDL-C, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, eGFR, DM, hypertension, and current use of statins, aspirin, angiotensin-converting enzyme inhibitor/angiotensin II receptor antagonist, calcium channel blocker, and hypoglycemic drugs (r=–0.149; P<0.001).

The prevalence of SCI was calculated by the quartiles of serum TB levels (Figure). The prevalence rate of SCI in Q1, Q2, Q3, and Q4 was 21.33% (157/736), 11.57% (82/709), 9.36% (66/705), and 5.39% (38/715), respectively. The results indicated that the prevalence of SCI decreased as TB quartiles increased (P<0.001).

Stepwise multiple regression analysis was performed to evaluate risk factors for SCI using logistic regression model in Table 3. Twenty-one variables, including age, sex, BMI, smoking status, alcohol consumption, systolic blood pressure, DBP, TC, triglyceride, high-density lipoprotein cholesterol, LDL-C, FPG, aspartate aminotransferase, baPWV, TB, eGFR, DM, hypertension, and the use of angiotensin-converting enzyme inhibitor/angiotensin II receptor antagonist, aspirin, and hypoglycemic drugs, were entered into the original equation. The risk factors found to be significantly associated with SCI in the logistic regression analysis were sex, BMI, smoking status, DBP, LDL-C, FPG, eGFR, TB, DM, and baPWV. Notably, TB was found to be a newly observed independent risk factor for SCI (odds ratio, 0.925; 95% confidence interval, 0.897–0.954; P<0.001).

Table 1. Baseline Characteristics of the Analyzed Participants According to SCI Status

Variables	With SCI	Without SCI	*P* Value
n	343	2522	
Age, y	54.3 (9.4)	49.8 (9.9)	<0.001
Range	35–68	30–69	
Sex (male, %)	274 (79.9)	1557 (61.7)	<0.001
BMI, kg/m^2	25.9 (2.7)	24.7 (3.2)	<0.001
Smoker (%)	188 (54.8)	934 (37.0)	0.007
Alcohol consumption			
Never drinker (%)	40 (11.7)	511 (20.3)	<0.001
Light drinker (%)	196 (57.1)	1361 (54.0)	0.268
Moderate drinker (%)	67 (19.5)	469 (18.6)	0.676
Heavy drinker (%)	40 (11.7)	181 (7.2)	0.003
SBP, mm Hg	135.9 (12.3)	128.8 (12.3)	<0.001
DBP, mm Hg	78.8 (8.0)	74.4 (7.9)	<0.001
TC, mmol/L	5.25 (4.73–5.74)	5.12 (4.53–5.64)	0.009
TG, mmol/L	1.98 (1.40–2.58)	1.77 (1.24–2.41)	0.001
HDL-C, mmol/L	1.42 (1.21–1.62)	1.52 (1.31–1.68)	<0.001
LDL-C, mmol/L	2.78 (0.77)	2.64 (0.79)	0.004
FPG, mmol/L	6.10 (5.56–7.05)	5.60 (5.19–6.08)	<0.001
AST, U/L	22.0 (18.0–31.0)	21.0 (18.0–28.0)	0.078
ALT, U/L	19.0 (15.0–30.0)	19.0 (15.0–33.0)	0.431
GGT, U/L	28.0 (15.0–45.0)	28.0 (15.0–45.0)	0.649
baPWV, cm/s	1441.3 (156.3)	1277.9 (148.5)	<0.001
TB, μmol/L	8.3 (6.8–12.4)	10.7 (8.0–14.1)	<0.001
DB, μmol/L	3.4 (2.7–5.2)	3.9 (2.7–5.4)	0.001
IB, μmol/L,	5.0 (3.9–7.1)	6.6 (4.9–9.4)	<0.001
eGFR, mL/min per 1.73 m^2	70.2 (16.7)	78.5 (15.2)	<0.001
Hypertension (%)	172 (50.1)	584 (23.2)	<0.001
DM (%)	100 (29.2)	199 (7.9)	<0.001
Medication			
Statin (%)	10 (2.9)	70 (2.8)	0.883
ACEIs/ARBs (%)	31 (9.0)	147 (5.8)	0.021
CCBs (%)	25 (7.3)	144 (5.7)	0.244
Hypoglycemic agents (%)	36 (10.5)	97 (3.8)	<0.001
Aspirin (%)	53 (15.1)	244 (9.7)	0.001

Values are shown as mean (SD) or median (interquartile range) or percentage. *P* value was calculated using the Student *t* test or Mann–Whitney *U* test or χ^2 test. ACEI indicates angiotensin-converting enzyme inhibitor; ALT, alanine aminotransferase; ARB, angiotensin II receptor antagonist; AST, aspartate aminotransferase; baPWV, brachial-ankle pulse wave velocity; BMI, body mass index; CCB, calcium channel blocker; DBP, diastolic blood pressure; DB, direct bilirubin; DM, type 2 diabetes mellitus; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; GGT, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; IB, indirect bilirubin; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; SCI, silent cerebral infarction; TB, total bilirubin; TC, total cholesterol; and TG, triglyceride.

Discussion

In this cross-sectional study, we showed that the subjects with a low TB had a higher prevalence of SCI. Moreover,

Table 2. Clinical and Biochemical Characteristics of Subjects According to Total Bilirubin Quartiles

	Quartiles of Total Bilirubin				
	Q1	Q2	Q3	Q4	*P* Value
n	736	709	705	715	
Age, y	52.9 (9.1)	51.0 (9.4)	49.3 (10.6)	48.1 (10.1)	<0.001
Sex (male, %)	361 (49.0)	393 (55.4)	483 (68.5)	594 (83.1)	<0.001
BMI, kg/m^2	25.2 (3.2)	25.2 (2.9)	24.6 (3.2)	24.4 (3.3)	<0.001
Smoker (%)	225 (30.6)	250 (35.3)	276 (39.1)	371 (51.9)	<0.001
Alcohol consumption					
Never drinker (%)	189 (25.7)	376 (53.0)	117 (16.6)	54 (7.6)	<0.001
Light drinker (%)	162 (22.0)	387 (54.6)	113 (16.0)	47 (6.6)	<0.001
Moderate drinker (%)	116 (15.8)	395 (55.7)	139 (19.7)	55 (7.7)	<0.001
Heavy drinker (%)	84 (11.4)	399 (56.3)	167 (23.7)	65 (9.1)	<0.001
SBP, mm Hg	133.3 (12.4)	129.3 (12.9)	127.2 (12.4)	128.7 (11.6)	<0.001
DBP, mm Hg	75.6 (8.0)	75.4 (8.0)	75.2 (7.9)	73.4 (8.2)	<0.001
FPG, mmol/L	5.87 (5.45–6.38)	5.61 (5.10–6.13)	5.59 (5.16–6.06)	5.60 (5.21–6.04)	<0.001
TC, mmol/L	5.09 (4.64–5.58)	5.24 (4.52–5.76)	5.09 (4.54–5.64)	5.14 (4.52–5.64)	0.151
TG, mmol/L	1.93 (1.43–2.51)	1.80 (1.08–2.35)	1.69 (1.31–2.40)	1.73 (1.26–2.45)	<0.001
HDL-C, mmol/L	1.44 (1.17–1.64)	1.49 (1.31–1.66)	1.49 (1.30–1.75)	1.54 (1.35–1.71)	<0.001
LDL-C, mmol/L	2.73 (0.83)	2.60 (0.76)	2.60 (0.66)	2.72 (0.89)	0.001
AST, U/L	23.0 (19.0–28.0)	21.0 (17.5–28.0)	21.0 (17.0–28.0)	21.0 (18.0–28.0)	<0.001
ALT, U/L	21.0 (15.0–34.0)	19.0 (15.0–31.0)	19.0 (15.0–34.0)	20.0 (15.0–33.0)	0.001
GGT, U/L	31.0 (15.0–51.0)	29.0 (16.0–50.0)	26.0 (14.0–44.0)	23.0 (13.0–42.0)	<0.001
baPWV, cm/s	1342.9 (158.6)	1321.2 (159.7)	1279.2 (145.3)	1245.0 (152.0)	<0.001
eGFR, mL/min per 1.73 m^2	74.7 (16.3)	80.0 (16.7)	78.9 (13.6)	76.5 (15.2)	<0.001
Hypertension (%)	276 (37.5)	165 (23.3)	157 (22.3)	158 (22.1)	<0.001
DM (%)	160 (21.7)	48 (6.8)	58 (8.2)	33 (4.6)	<0.001
Medication					
Statin (%)	18 (2.4)	29 (4.1)	12 (1.7)	21 (2.9)	0.049
ACEIs/ARBs (%)	57 (7.7)	29 (4.1)	47 (6.7)	45 (6.3)	0.034
CCBs (%)	49 (6.7)	37 (5.2)	43 (6.1)	40 (5.6)	0.677
Hypoglycemic agents (%)	75 (10.2)	21 (3.0)	24 (3.4)	13 (1.8)	<0.001
Aspirin (%)	90 (12.2)	68 (9.6)	71 (10.1)	68 (9.5)	0.280

Data are expressed as mean (SD) or median (interquartile range) or percentage. *P* value was calculated using 1-way ANOVA test or Kruskal–Wallis or χ^2 test. ACEI indicates angiotensin-converting enzyme inhibitor; ALT, alanine aminotransferase; ARB, angiotensin II receptor antagonist; AST, aspartate aminotransferase; baPWV, brachial-ankle pulse wave velocity; BMI, body mass index; CCB, calcium channel blocker; DBP, diastolic blood pressure; DM, type 2 diabetes mellitus; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; GGT, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; and TG, triglyceride.

partial correlation found that TB levels were tightly correlated with baPWV after adjusting for covariates. Logistic regression analysis revealed a significant association of TB levels with the risk of SCI after adjustment for conventional vascular risk factors.

SCI has high prevalence in the elderly population. Some studies have demonstrated that SCI is linked to cognitive impairment, depression, and increased risk for stroke.[1–3] The majority of SCIs reflect penetrating artery disease and have shared pathogenesis with lacunar infarcts. Recent research has reported that endothelial dysfunction is involved in lacunar infarction and SCI.[10]

Serum bilirubin (a heme catabolic product) is among the most powerful endogenous antioxidative substances and is positively correlated with the total antioxidant capacity in Gilbert syndrome.[11] A lower prevalence of cardiovascular disease is found in Gilbert disease.[12] However, TB exerts a toxic effect in certain situations. Gut microflora greatly affects intravascular metabolism of bilirubin.[13] On the contrary, L-carnitine in red meat is metabolized by gut microbiota and generates proatherogenic metabolites.[14,15] Recent studies showed that the metabolites are associated with an elevated risk for cardiovascular disease.[16] In addition, vitamin B12 deficiency, which increases bilirubin levels, adds to the risk of stroke by increasing levels of homocysteine.[17] In contrast, higher plasma homocysteine level and decreased TB level are found in patients with diabetic retinopathy.[18] The apparent paradox between toxic and protective effects stresses again the double-edged sword character of bilirubin.

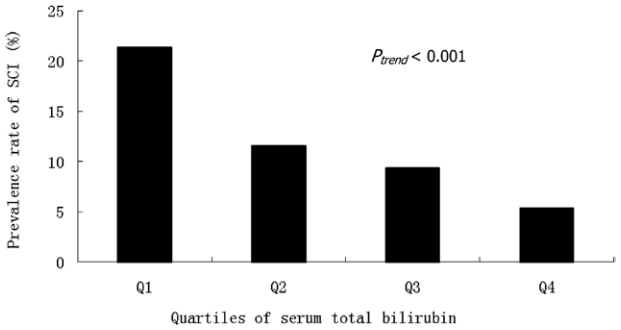

Figure. The association between serum total bilirubin levels and prevalence rate of silent cerebral infarction (SCI; %). Participants were stratified into quartiles according to their total bilirubin levels.

Our study reported that serum bilirubin has a tight correlation with baPWV and a significant association with the risk of SCI. Multiple mechanisms are probably involved. First, a body of evidence indicates that complement activation plays a key role in the development of atherosclerosis. Recent studies have demonstrated that unconjugated bilirubin inhibits complement activation via interfering with the interaction between C1 component of complement and immunoglobulins.[19] Moreover, heme oxygenase 1 and heme degradation products could attenuate complement-mediated acute inflammation and protect human vascular endothelial cells against complement-mediated injury.[20] In addition, our previous studies have observed that serum bilirubin has a tight correlation with baPWV. Furthermore, baPWV has been used as an index of subclinical atherosclerosis. Previous studies documented that increased baPWV is associated with metabolic syndrome, cardiovascular diseases, stroke, and renal disease, as well as elevated total mortality.[21–24] Second, mild unconjugated hyperbilirubinemia exerts a protective role in the development of atherosclerosis. Low-grade chronic inflammation induces vascular damage. Some inflammatory markers, such as C-reactive protein and interleukin-6, are reported to have a positive correlation with SCI.[25,26] Also, diminished TB is linked with diabetes mellitus, cardiovascular disease, peripheral artery disease, and stroke, which all are relevant to chronic inflammation.[6,7,27,28]

Table 3. Adjusted ORs and 95% CIs for the Presence of SCI, Based on Logistic Regression With Backward Stepwise Selection

Variables	β	OR (95% CI)	*P* Value
Sex (male/female)	0.956	2.600 (1.772–3.816)	<0.001
BMI, kg/m^2	0.046	1.047 (1.001–1.096)	0.047
Smoking (yes/no)	0.476	1.610 (1.172–2.212)	0.003
DBP, mm Hg	0.039	1.039 (1.019–1.060)	<0.001
baPWV, cm/s	0.006	1.006 (1.005–1.007)	<0.001
TB, μmol/L	−0.078	0.925 (0.897–0.954)	<0.001
LDL-C, mmol/L	0.269	1.309 (1.106–1.550)	0.002
FPG, mmol/L	0.434	1.543 (1.230–1.934)	<0.001
eGFR, mL/min per 1.73 m^2	−0.034	0.967 (0.958–0.976)	<0.001
DM (yes/no)	0.712	2.037 (1.169–3.551)	0.012

Adjusted ORs were derived from the final logistic regression model which includes all significant factors. baPWV indicates brachial-ankle pulse wave velocity; BMI, body mass index; CI, confidence interval; DBP, diastolic blood pressure; DM, type 2 diabetes mellitus; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; SCI, silent cerebral infarction; TB, total bilirubin; and β, partial regression coefficient.

Our results revealed that dyslipidemia is associated with SCI. The results are in line with those of other groups.[29,30] Furthermore, LDL-C subfraction LDL-C3 has been found in association with SCI in subjects with hypertension.[31] These findings are consistent with the idea that TB may play a protective role in the atherosclerotic process by preventing formation of oxidized LDL-C.[8,32]

A study reported that there was a higher prevalence of SCI in women than in men, whereas this higher prevalence was not found in the younger cohorts.[33–35] These conflicting data may be attributable to the differences in age and race.[36] In addition, a report has confirmed that there were significant racial differences in TB levels in different populations.[37] However, our study showed that sex was an important factor associated with SCI. Estrogen levels may be responsible for sex disparity. uridine 5'-diphospho-glucuronosyltransferase 1A1–mediated glucuronidation was an essential step for bilirubin elimination.[38] Estrogen could influence the production of bilirubin by inhibiting the uridine 5'-diphospho-glucuronosyltransferase 1A1–catalyzed bilirubin glucuronidation.[39,40] In addition, a recent study confirmed that there was an association between the uridine 5'-diphospho-glucuronosyltransferase 1A1 and TC and LDL-C levels in women with different hormonal status.[41] Similarly, a negative correlation between TB and blood pressure was only found in women.[42] Further research is warranted to clarify the sex difference of the effects of bilirubin.

The interpretation of this study has some limitations. First, the study was cross-sectional, and it is difficult to study the direction of causality. A prospective study is needed to clarify this point. Second, we could not get any information on waist circumference, insulin resistance, and C-reactive protein, which are important risk factors. Third, the study is lacking information about the genetic contributions to hyperbilirubinemia. Recent studies indicated that different genetic background plays a key role in controlling serum bilirubin.[43]

Conclusions

In summary, our study showed that TB is a novel biochemical indicator for SCI regardless of classical cardiovascular risk factors. Early measurement of TB may be useful to assess the risk of SCI.

Acknowledgments

We thank the staff of International Physical Examination and Healthy Center for their help with data collection.

Sources of Funding

This study was supported by the Heilongjiang provincial government postdoctoral science foundation (LBH-Z11060) and Natural Science Foundation of Heilongjiang Province of China (No. LC201005).

Disclosures

None.

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Significance

Bilirubin is an effective antioxidant molecule. The presence of silent cerebral infarction predicts transient ischemia attack, clinically overt stroke, cardiovascular disease, and dementia. Our study showed that total bilirubin is a novel biochemical indicator for silent cerebral infarction regardless of classical cardiovascular risk factors. Moreover, total bilirubin levels are tightly correlated with brachial-ankle pulse wave velocity after adjusting for confounding covariates. Early measurement of total bilirubin may be useful to assess the risk of stroke.