Ref: Ro J Neurol. 2023;22(3)
DOI: 10.37897/RJN.2023.3.1

Biliary markers of ischemic stroke and gender differences in China

Yang Xu[1], Wong Eng Hwa[1], Rusli Bin Nordin[2], Abdul Kareem Meera Mohaideen[1], Benjamin Samraj Prakash Earnest[1], Wong Yin How[1], Le Huahui[3]

[1]School of Medicine, Faculty of Health and Medical Sciences, Taylor's University (Lakeside Campus), Selangor, Malaysia
[2]Faculty of Medicine, Bioscience and Nursing, MAHSA University, Selangor, Malaysia
[3]Department of Neurology, Fuzhou First People's Hospital of Jiangxi Province, Fuzhou City, Jiangxi Province, China

ABSTRACT

Introduction. Stroke is one of the most important cause of disability and death worldwide. Ischemic stroke patients account for the majority of stroke patients, which takes large burden to patients' families. This study aimed to explore the association between cholyglycine (CG), total bilirubin (TBIL), indirect bilirubin (IDBIL), direct bilirubin (DBIL) and incidence of ischemic stroke, and to assess detect gender difference.

Methods. This was a case-control and retrospective study, all data was collected from medical records in Fuzhou First People's Hospital in China. Case group consists of 130 ischemic stroke patients at the Department of Neurology in Fuzhou First People's Hospital. Control group was 130 patients who didn't have ischemic stroke in the same period and in the same hospital. In our study, physical examination and clinical history of patients, biochemistry testing indexes of CG, TBIL, DBIL, IDBIL were recorded in medical records. Craniocerebral imaging was examined by magnetic resonance imaging (MRI) scan and/or computerized tomography (CT).

Results. The odds for ischemic stroke increased with CG (adjusted OR=3.028, 95% CI=2.065-4.440, p<0.001), TBIL (adjusted OR=1.110, 95%CI=1.031-1.194, p=0.005), SBP (adjusted OR=1.031, 95%CI=1.015-1.048, p<0.001) and FBS (adjusted OR=1.248, 95%CI=1.069-1.457, p=0.005). There was no gender difference in CG, (adjusted OR=3.898 95%CI=2.244-6.773, P<0.001) in males and (adjusted OR=1.901, 95%CI=1.231-2.935, p=0.004) in females. While there were gender differences in TBIL and DBIL, (adjusted OR=1.086, 95%CI=1.004-1.174, p=0.039), (adjusted OR=1.296, 95%CI=1.025-1.637, p=0.030).

Conclusion. CG, TBIL, SBP and FBS were independent predictors of ischemic stroke in China. CG seems more sensitive than TBIL as the predictor of ischemic stroke. TBIL and DBIL levels had gender difference with the incidence of ischemic stroke and no gender difference in CG levels.

Keywords: association, levels, incidence, ischemic stroke

INTRODUCTION

Stroke is the third main cause for disability and second main cause for death in the whole world [1]. Stroke takes patients lots of economic pressure and makes patients' families lost labor force, which brought patients and patients' families heavy blow. Worldwide, stroke cases were 12.2 million, prevalent cases were 101 million and death patients for stroke were 6.55 million in 2019, which were increased by 70% on stroke cases, increased by 85% on prevalent cases and increased by 43% for death cases of stroke compared to 1990 [2]. In China, morbidity due to ischemic stroke is also on the rise [3], the new cases of stroke were 3.94 million in 2019, the proportion was about 276.7/100000, the incidence of stroke was increased by 86.0% compared to 1990; The prevalent stroke cases were 28.76 million in 2019, which included 24.18 ischemic stroke cases, the prevalence was increased by 106.0% compared to 1990. The death cases of stroke were 2.19 million, which was increased by 32.3% compared to 1990 [4].

As high morbidity and mortality of stroke, the studies on biochemical predictors of ischemic stroke

Corresponding author:
Wong Eng Hwa
E-mail: EngHwa.Wong@taylors.edu.my

Article History:
Received: 14 September 2023
Accepted: 29 September 2023

were increasingly important recent years. Some references reported CG, TBIL [5] may have association with ischemic stroke. Because effective treating methods of stroke is still lacking now, taking prevention measures and screening sensitive predictors of stroke to reduce the morbidity and mortality is most important. Our study aimed to determine the association between CG, TBIL, IDBIL, DBIL and ischemic stroke, and to assess whether they were predictors of ischemic stroke and whether there were gender differences in their association.

METHODS

Study Design and Sample Size

This was a case-control (retrospective) and a single center research, all data collection was from the medical records of Fuzhou First People's Hospital of Jiangxi Province in China. Case group consists of 130 ischemic stroke patients at the Department of Neurology in Fuzhou First People's Hospital of Jiangxi Province, ranged from January 2017 to June 2023. Control group was 130 patients who didn't have ischemic stroke in the same period and in this hospital. In our study, physical examination and clinical history, biochemistry testing indexes of CG, TBIL, DBIL, IDBIL were obtained from the past medical records of patients. Craniocerebral imaging was done using CT and/or MRI scan. Plasma lipids and fasting blood sugars were also studied.

Calculate the minimum sample size for this study using G*Power 3.1.9.7 software. The calculations for sample size were based on the results of our preliminary study (20 case and 20 control) and previous literatures for these clinical parameters in this research. Significance level was set as 0.05 and power was 80% for this research. Based on the calculations, the minimum sample size for this study was 98 per group. Considering the missing data, the minimum sample size was 130 per group, which was considered sufficient for this study, the total sample size was 260.

Subject Recruitment

Inclusion criteria for case group: (1) Patients were diagnosed with ischemic stroke; (2) Diagnosis was confirmed by cerebral CT or MRI examination; (3) The patient age was above 18 years old. Exclusion criteria for case group: (1) Hemorrhagic stroke; (2) Sequelae of cerebral infarction, previous history of stroke; (3) liver cirrhosis; (4) intracranial infection and other severe craniocerebral diseases; (5) Similar diseases caused by non-vascular factors. Inclusion criteria for control group: (1) patients with no stroke history and no stroke; (2) the patient age was above 18 years old. Exclusion criteria for control group: (1) ischemic stroke, hemorrhagic stroke; (2) posterior circulation ischemia, lacunar infarction, cerebral infarction sequelae; (3) previous stroke history; (4) Liver cirrhosis; (5) serious craniocerebral diseases or intracranial infection; (6) Other diseases had resulted in the damage of nerve function.

Measures

All data was recorded in medical records between January 2017 to June 2023. (1) Physical examinations: systolic blood pressure (SBP), diastolic blood pressure (DBP). (2) Clinical history: Sociodemographic characteristics (age, gender); past medical history: stroke history; history of present illness: coronary heart disease (CHD), hypertension, diabetes mellitus (DM), dyslipidemia. (3) Biochemistry indexes measurement: CG, TBIL, DBIL, IDBIL, triglycerides (TG), total cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C), fasting blood sugar (FBS). Patients' blood samples were sent to the laboratory of this hospital to test and quality control. (4) Brain imaging examination: Patients in case and control group were all checked by CT or MRI scan to exclude or confirm ischemic stroke.

Statistical Analysis

Data collection stage began from January 2023 to June 2023. The missing data was filled by series mean method. Data was analyzed with IBM SPSS 26 software. Describe normal distribution data in quantitative variables by means and standard deviations, describe not normal distribution data in quantitative variables by medians and interquartile ranges. Describe data in qualitative variables with frequencies and percentages. Compare two groups in quantitative variables with using 2 independent samples t-test to analyze the data which was normally distributed as well as the variance was equal. Compare two groups in quantitative variables with using Mann-Whitney U test to analyze the data which was not normal distribution or the variance was not equal. Among qualitative variables compare two groups with Chi-square test. Take 17 clinical parameters of CG, TBIL, DBIL, IDBIL, age, gender, SBP, DBP, FBS, TG, TC, HDL-C, LDL-C, hypertension, CHD, DM, dyslipidemia as independent variables. Use univariate binary logistic regression and multivariate binary logistic regression to analyze the association between these predictors and ischemic stroke. The predictors whose p-value was under 0.05 in univariate binary logistic regression were entered into multivariate logistic regression analysis, select variables which were finally included in this model with forward stepwise method. Calculate the area under curve (AUC) of receiver operating characteristic (ROC) to access this model's discrimination. In all analysis, set the significance level as 0.05.

Ethical Statement

This research was approved by Fuzhou First People's Hospital Ethics Committee.

RESULTS

Characteristics of quantitative variables in this study were shown as Table 1. Compare case group and control group, the mean levels of CG were 2.19 mg/L (SD 1.18 mg/L) and 1.37 mg/L (SD 1.23 mg/L), TBIL were 13.00 μmol/L (SD 6.88 μmol/L) and 11.25 μmol/L (SD 6.07 μmol/L), DBIL were 4.10 μmol/L (SD 2.30 μmol/L) and 3.30 μmol/L (SD 1.93 μmol/L), IDBIL were 8.85 μmol/L (SD 4.88 μmol/L) and 8.10 μmol/L (SD 4.85 μmol/L/L), FBS were 5.40 mmol/L (SD 1.90 mmol/L) and 5.00 mmol/L (SD 1.40 mmol/L), TG were 1.53 mmol/L (SD 1.03 mmol/L) and 1.30 mmol/L (SD 0.90 mmol/L), HDL-C were 1.10 mmol/L (SD 0.30 mmol/L) and 1.17 mmol/L (SD 0.40 mmol/L), SBP were 147.00 mmHg (SD 26.00 mmHg) and 133.00 mmHg (SD 23.00 mmHg), DBP were 81.00 mmHg (SD 15.00 mmHg) and 80.00 mmHg (SD 13.00 mmHg), Age was 65.71 years old (SD 11.64 years old) and 61.88 years old (SD 12.75 years old). There were differences in CG, TBIL, DBIL, IDBIL, FBS, TG, SBP, DBP, Age between case and control group, the levels of cases were higher than controls while case was lower than control in HDL-C, the differences were statistically significant (p<0.05). There was no difference in LDL-C and, TC between case and control group. Characteristics of qualitative variables in this study were shown as table 2. Among case group, there were 91 (70.0%) males compared to 39 (30.0%) females in case group; there were 75 (57.7%) patients with hypertension compared to 55 (42.3%) patients without. There were 32 (72.7%) DM patients with ischemic stroke compared to 12 (27.3%) DM patients without. Their differences were all significant ((P<0.05). There was no difference in dyslipidemia and CHD between case and control group.

The results of univariate binary logistic regression indicated the significant predictors were CG, TBIL, DBIL, IDBIL, FBS, TG, HDL-C, SBP, DBP, hypertension, DM, age, gender (P<0.05), the results were shown as table 3. The variables whose p-value was under 0.05 in univariate analysis were included into multivariate binary logistic regression, select predictors in multivariate analysis with forward stepwise method. There was no difference in TC, LDL-C, CHD, dyslipidemia in univariate analysis (P>0.05), which was excluded in further multivariate analysis. In multivariate binary logistic analysis, after controlling for gender and age, the variables of CG, TBIL, SBP and FBS still had significant association with the incidence of ischemic stroke (P<0.05), the results were shown as table 4 and table 5. The probability of ischemic stroke increased with CG (adjusted OR=3.028, 95% CI=2.065-4.440, p<0.001), TBIL (adjusted OR=1.110, 95%CI=1.031-1.194, p=0.005), SBP (adjusted OR=1.031, 95%CI=1.015-1.048, p<0.001) and FBS (adjusted OR=1.248, 95%CI=1.069-1.457, p=0.005). The males had higher odds of being ischemic stroke compared with the females (OR= 4.904, 95% CI 2.550-9.433, P<0.001). For every unit increased in CG, TBIL, SBP, FBS levels, the probability of being ischemic stroke increased by 3.028, 1.110, 1.031 and 1.248, respectively; the odds of males being ischemic stroke were 4.904 times more compared to females. CG, TBIL, SBP and FBS were independent predictors for ischemic stroke (P<0.05). There was no multicollinearity among these four independent predictors.

CG, TBIL, SBP, FBS and gender were finally included in this model, drew the ROC curve, after calculations the AUC of this prediction model was 0.854 (Fig 1), indicating this model had an excellent discrimination (Table 6, Table 7).

Gender differences of variables characteristics in case and control group among males and females were shown as Table 8. CG and DBIL levels in case group were higher than control group both in males and females, the difference was significant (P<0.05). There was no difference in TBIL and IDBIL between case group and control group in males and females, separately. There was no gender difference in CG levels with the incidence of ischemic stroke (Table 9), (adjusted OR=3.898 95%CI=2.244-6.773, P<0.001) in males and (adjusted OR=1.901, 95%CI=1.231-2.935, p=0.004) in females. While there were gender differences in TBIL and DBIL levels with ischemic stroke, elevated levels of TBIL and DBIL had association with ischemic stroke in males but not females, (adjusted OR=1.086, 95%CI=1.004-1.174, p=0.039), (adjusted OR=1.296, 95%CI=1.025-1.637, p=0.030).

TABLE 1. Characteristics of quantitative variables: Mann-Whitney U test and 2 independent samples t-test

Variables	Group		p-value
	Case(n=130)	Control(n=130)	
CG	2.19(1.18)	1.37(1.23)	0.000
TBIL	13.00(6.88)	11.25(6.07)	0.003
DBIL	4.10(2.30)	3.30(1.93)	0.000
IDBIL	8.85(4.88)	8.10(4.85)	0.014
FBS	5.40(1.90)	5.00(1.40)	0.003
TC	4.49±0.93	4.49±0.90	0.954
TG	1.53(1.03)	1.30(0.90)	0.009
LDL-C	2.41±0.75	2.47±0.68	0.486
HDL-C	1.10(0.30)	1.17(0.40)	0.034
SBP	147.00(26.00)	133.00(23.00)	0.000
DBP	81.00(15.00)	80.00(13.00)	0.023
Age	65.71±11.64	61.88±12.75	0.012

TABLE 2. Characteristics of qualitative variables: Chi-Square test

Variables	Group		p-value
	Case (n=160)	Control (n=160)	
Gender Male Female	 91(65.0%) 39(32.5%)	 49(35.0%) 81(67.5%)	0.000
Hypertension Yes No	 75(65.8%) 55(37.7%)	 39(34.2%) 91(62.3%)	0.000
DM Yes No	 32(72.7%) 98(45.4%)	 12(27.3%) 118(54.6%)	0.001
CHD Yes No	 7(77.8%) 123(49.0%)	 2(22.2%) 128(51.0%)	0.090
Dyslipidemia Yes No	 9(64.3%) 121(49.2%)	 5(35.7%) 125(50.8%)	0.272

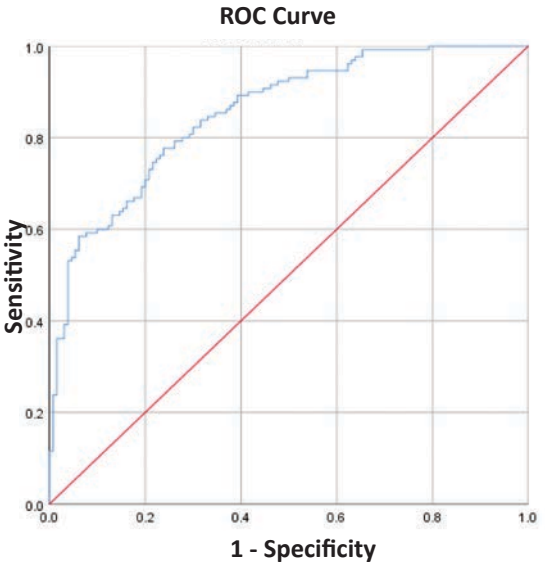

FIGURE 1. ROC curve

TABLE 6. AUC value for this study

AUC	p-value	95%CI
0.854	0.000	0.810-0.899

TABLE 7. Indices for good model fit

Hosmer-Lemeshow Test (p-value)	Classification table (Overall percentage)	Model Summary (-2 Loglikelihood)
0.566	76.2%	243.590[a]

TABLE 3. Results of the variables in univariate binary logistic regression (n=260)

Variables	B	SE	Wald	df	p-value	OR(95%CI)
CG	0.954	0.166	32.886	1	0.000	2.596(1.874, 3.596)
TBIL	0.094	0.028	11.538	1	0.001	1.099(1.041, 1.161)
DBIL	0.194	0.071	7.493	1	0.006	1.214(1.057, 1.395)
IDBIL	0.106	0.036	8.635	1	0.003	1.112(1.036, 1.194)
FBS	0.207	0.066	9.711	1	0.002	1.230(1.080, 1.401)
TC	-0.008	0.136	0.003	1	0.954	0.992(0.760, 1.296)
TG	0.290	0.143	4.125	1	0.042	1.336(1.010, 1.767)
LDL-C	-0.122	0.174	0.489	1	0.485	0.886(0.630, 1.245)
HDL-C	-1.201	0.484	6.159	1	0.013	0.301(0.117, 0.777)
SBP	0.034	0.007	21.493	1	0.000	1.034(1.020, 1.049)
DBP	0.028	0.011	6.388	1	0.011	1.028(1.006, 1.050)
Age	0.026	0.010	6.157	1	0.013	1.026(1.005, 1.047)
Gender Male Female	1.350 ref	0.263	26.264	1	0.000	3.857(2.302, 6.464)
Hypertension Yes No	1.157 ref	0.261	19.659	1	0.000	3.182(1.908, 5.307)
DM Yes No	1.167 ref	0.365	10.212	1	0.001	3.211(1.570, 6.567)
CHD Yes No	1.293 ref	0.812	2.536	1	0.111	3.642(0.742, 17.876)
Dyslipidemia Yes No	0.620 ref	0.572	1.175	1	0.278	1.860(0.606, 5.707)

TABLE 4. Results of variables in multivariate binary logistic regression (n=260)

Variables	B	SE	Wald	df	p-value	OR(95%CI)
CG	1.108	0.195	32.179	1	0.000	3.028(2.065, 4.440)
TBIL	0.104	0.037	7.793	1	0.005	1.110(1.031, 1.194)
SBP	0.031	0.008	14.241	1	0.000	1.031(1.015, 1.048)
FBS	0.221	0.079	7.844	1	0.005	1.248(1.069, 1.457)
Gender	1.590	0.334	22.700	1	0.000	4.904(2.550, 9.433)

TABLE 5. Results of variables in multivariate binary logistic regression with enter method, before and after controlled for Gender (n=260)

Variables	OR(95%CI)	p	AOR(95%CI)	p
CG	2.832(1.973, 4.065)	0.000	3.028(2.065, 4.440)	0.000
TBIL	1.134(1.057, 1.215)	0.000	1.110(1.031, 1.194)	0.005
SBP	1.031(1.015, 1.047)	0.000	1.031(1.015, 1.048)	0.000
FBS	1.179(1.028, 1.354)	0.019	1.248(1.069, 1.457)	0.005

TABLE 8. Gender difference for variables characteristics in male and female

Variables	Group		p-value
	Case	Control	
CG			
Male	2.19(1.33)	1.25(1.25)	0.000
Female	2.18(1.00)	1.51(1.06)	0.000
TBIL			
Male	13.80(7.60)	11.60(6.40)	0.083
Female	12.00(5.10)	11.10(6.10)	0.099
DBIL			
Male	4.20(2.30)	3.70(1.75)	0.042
Female	3.80(1.70)	3.10(2.10)	0.011
IDBIL			
Male	9.50(5.00)	8.60(5.10)	0.103
Female	8.60(4.40)	7.90(4.60)	0.247
FBS			
Male	5.20(1.40)	4.80(1.55)	0.037
Female	5.94(2.20)	5.10(1.42)	0.002
TC			
Male	4.43(1.13)	4.27(0.91)	0.645
Female	4.69±0.88	4.61±0.91	0.659
TG			
Male	1.45(1.06)	1.22(0.95)	0.078
Female	1.69(0.90)	1.38(0.87)	0.016
LDL-C			
Male	2.30(1.00)	2.40(1.05)	0.408
Female	2.53±0.71	2.51±0.68	0.859
HDL-C			
Male	1.10(0.40)	1.10(0.40)	0.944
Female	1.10(0.30)	1.20(0.42)	0.058
SBP			
Male	144.00(18.00)	139.00(20.00)	0.111
Female	151.00(32.00)	130.00(20.00)	0.000
DBP			
Male	80.00(15.00)	80.00(16.00)	0.554
Female	82.00(18.00)	80.00(13.00)	0.038
Age			
Male	64.18±11.34	64.55±13.09	0.860
Female	69.28±11.68	60.26±12.34	0.000
Hypertension			
Male (Yes)	48(72.7%)	18(27.3%)	0.070
Male (No)	43(58.1%)	31(41.9%)	
Female (Yes)	27(56.3%)	21(43.8%)	0.000
Female (No)	12(16.7%)	60(83.3%)	
DM			
Male (Yes)	16(76.2%)	5(23.8%)	0.244
Male (No)	75(63.0%)	44(37.0%)	
Female (Yes)	16(69.6%)	7(30.4%)	0.000
Female (No)	23(23.7%)	74(76.3%)	
CHD			
Male (Yes)	5(83.3%)	1(16.7%)	0.336
Male (No)	86(64.2%)	48(35.8%)	
Female (Yes)	2(66.7%)	1(33.3%)	0.201
Female (No)	37(31.6%)	80(68.4%)	
Dyslipidemia			
Male (Yes)	5(83.3%)	1(16.7%)	0.336
Male (No)	86(64.2%)	48(35.8%)	
Female (Yes)	4(50.0%)	4(50.0%)	0.274
Female (No)	35(31.3%)	77(68.8%)	

TABLE 9. Gender difference for multivariate binary logistic regression in male and female after controlled for age

Variables	Male		Female	
	AOR(95%CI)	p-value	AOR(95%CI)	p-value
CG	3.898(2.244, 6.773)	0.000	1.901(1.231, 2.935)	0.004
TBIL	1.086(1.004, 1.174)	0.039	1.082(0.999, 1.173)	0.053
DBIL	1.296(1.025, 1.637)	0.030	1.106(0.943, 1.298)	0.216
IDBIL	1.103(0.995, 1.222)	0.061	1.088(0.982, 1.206)	0.107

DISCUSSION

CG was a new biomarker of ischemic stroke. But there was almost no research that reported the association in CG levels with the morbidity of ischemic stroke till now. The results for our studies showed CG, TBIL, SBP and FBS levels had association with the morbidity of ischemic stroke. CG was a kind of conjugated cholic acid formed by the combination of glycine and cholic acid, it was also most important bile acid component in the serum of late pregnancy. When liver cells are damaged, the ability of liver cells to absorb CG decreases, resulting in an increase in the content of CG in blood. Previous studies on CG mainly focused on liver diseases [6] and the condition of pregnant women or fetuses during pregnancy [7], CG was found to be a biomarker for the diagnosis or prognosis of liver diseases or intrahepatic cholestasis of pregnancy (ICP) [8]. While there were almost no reports about the relationship between CG and ischemic stroke, currently, only one study published in 2023 by Ming-Hsiu Wu et al pointed that CG could be the predictor for the prognosis of the clinical outcomes of ischemic stroke [9]. Our study found CG was an independent predictor for ischemic stroke even after controlling gender and age. Increased CG levels also had association with the incidence for ischemic stroke, for every unit increased in CG, the probability of being ischemic stroke increased by 3.028. But it is worth mentioning that CG seemed more sensitive and reliable predictor for ischemic stroke than TBIL. Though TBIL and CG levels were both associated with the morbidity for ischemic stroke, TBIL tended to have two trends, TBIL levels were high or low in ischemic stroke patients, while CG levels tended to be consistently higher in patients with ischemic stroke compared to control group, which was different from TBIL. CG seemed to be more advantageous and stable than TBIL as a predictor of ischemic stroke. The researches on CG with ischemic stroke were too few, our study was new, there was no more study results to prove our results. Thus, researchers could note the association of CG and ischemic stroke, their relationship needs more researches to verify.

There were some studies [10] reported that TBIL levels had association with ischemic stroke, while the relationship between them remained a bit controversial, some researches showed higher TBIL levels suggested lower incidence of ischemic stroke, the morbidity for ischemic stroke decreased with increased TBIL levels [11], they were inverse association, considered TBIL to be a protective factor of ischemic stroke; On the contrary, other researches showed contrasting outcomes that higher TBIL levels didn't have relationship with the incidence for ischemic stroke [12] or they were shown an indefinite association between them [13]. More researches reported higher TBIL levels had positive association with the severity of ischemic stroke [14]. Our study results showed TBIL levels had significant association with ischemic stroke, elevated TBIL levels increased the incidence of ischemic stroke. Now in the limited studies on the association between TBIL levels and ischemic stroke, most studies tended to indicate TBIL levels had association with the morbidity for ischemic stroke [15], which were consistent to our results at this point, but for the specific association, more researches showed they had inverse association in TBIL levels with the morbidity of ischemic stroke [16] which were contrary to our result that it was a positive relationship between them. Thakkar et al pointed increased TBIL levels were related to lower incidence and death rate of ischemic stroke, they had protective function on stroke [17]. However, Kim Ekblom et al provided an opposite outcome and considered TBIL didn't have association with the morbidity of ischemic stroke [12]. The study by Kun Zhao et al also indicated their association was uncertain [13]. For different genders, our study showed there was a gender difference in TBIL, TBIL levels had association with ischemic stroke in males but not females, this was consistent with the study by Zhong et al that the TBIL levels in males had association with the incidence ischemic stroke [18], but there was a difference between us that our study showed a positive association between them compared with inverse association of their study. As the relevant reports were not many on TBIL levels and ischemic stroke, the specific effect of TBIL levels on the incidence of ischemic stroke was not very clear. This result in our study could only explain the association between TBIL levels within 48 hours of admission and ischemic stroke. However, the relationship between TBIL levels and the incidence of ischemic stroke may not be a simple linear, which was pointed by the study of Ying Liu et al that nonlinear association between TBIL levels and the morbidity of ischemic stroke [19]. Take a wild guess, the change of TBIL levels in ischemic stroke patients may be similar to uric acid which reduced first and then rose and finally it returned to a level and remained stable [20], it also may be U shaped [21], J shaped [22] or other shaped. But now there were no studies that repeatedly measured bilirubin levels at different times in ischemic stroke patients, most studies only measured TBIL levels one time at admission, we didn't know the specific changes of TBIL levels in the early phase for ischemic stroke. During our research, we found TBIL levels in ischemic stroke patients had two trends, too high or too low, some patients with ischemic stroke held high levels in TBIL while other ischemic stroke patients held low levels. In our opinion, so why the studies on the relationship of TBIL levels and ischemic stroke was shown to be a bit contradictory in current studies, according to different sample groups, we may get different results, so some researchers indicated TBIL levels and ischemic stroke were inverse association while others indicated indefinite association or no association between them, the selection of different sample groups may affect the results about their association, this need we try best to reduce the selection bias. So why our results were contrary to the specific association of some researches, this can't exclude the reason of the selection for sample groups. If we want to resolve this disagreement about the specific association between TBIL levels and the morbidity of ischemic stroke, we may need more better designed studies and more rigorous studies, reduce the selection bias to get more reliable results. We may also need repeatedly test TBIL levels at different times in acute ischemic stroke stage of patients to know their dynamic changes when ischemic stroke patients in the hospital, if possible even TBIL levels were measured one month or three months after discharge, also make comparison with combing mRS score and NIHSS score, even the dynamic TBIL levels combined with NIHSS score and mRS score and make a comparison with the cerebral infarction size at admission and at discharge to explore their association in the incidence, prognosis and clinical outcome of ischemic stroke. Meanwhile, attention should be paid to ensure sufficient sample size and adequate power, stroke patients in experiments as participants were preferably specialized ischemic stroke patients, with less comorbidity. It is necessary to conduct studies based on TBIL levels stratification, TBIL levels dynamic monitoring, gender difference, age stratification, ischemic stroke subtypes to explore their association. Together with vitro experiments are used to further explore the specific mechanism of the effect of TBIL levels on ischemic stroke. Then we may resolve this controversy and get more reliable results.

There were few studies on DBIL and IDBIL with the incidence of ischemic stroke [23]. Our study didn't observe DBIL and IDBIL levels at admission had association with ischemic stroke but only TBIL levels had association with the morbidity of ischemic stroke. Though DBIL and IDBIL had significance in the uni-

variate binary logistic regression, while they didn't have statistical significance in the multivariate analysis in our study. There were some studies which were consistent with our result that TBIL had association with the morbidity of ischemic stroke and no association shown in IDBIL [24]. While the study by Jiancheng Wang et al observed DBIL also had association with ischemic stroke [25] but we didn't. Our result was also a bit different with the study by Qiwei Peng et al [24], they pointed DBIL levels before ischemic stroke patients treated by thrombolysis had significant association with clinical outcome of ischemic stroke. Though our study and the study by Qiwei Peng et al. consistently indicated TBIL had association with ischemic stroke and no relationship in IDBIL with ischemic stroke, but we showed different result on DBIL with ischemic stroke. The difference is our result on DBIL was about DBIL with the morbidity for ischemic stroke while their result was about DBIL with the outcome for ischemic stroke. This difference can't be excluded to be one of the reason for different results on DBIL. They also made a comparison for DBIL, IDBIL and TBIL before or after thrombolysis in ischemic stroke patients while our participants were non-thrombolytic ischemic patients in our study. Another study by Honglian Duan et al indicated DBIL was the independent biomarker for the severity degree of ischemic stroke, increased DBIL at admission meant more severe degree of stroke [26]. A meta-analysis by Yumeng Song et al also held the similar view that TBIL and DBIL had positive relationship with the severity degree of ischemic stroke [23], DBIL levels had linear correlation with ischemic stroke severity. Elnaz Sagheb Asl et al also proved the levels of TBIL, DBIL and IDBIL had association with the death rate of ischemic stroke patients [27]. These studies on the relationship between bilirubin and ischemic stroke were more about bilirubin levels and stroke severity, outcome, and mortality, but less about their relationship with stroke incidence, this needs more researches to explore their association especially for TBIL, DBIL and ischemic stroke.

In our study, SBP was also shown to have relationship with the incidence of ischemic stroke. Our result was consistent to the study by Kazunori Toyoda et al [28] and other studies that patients with high SBP had higher possibility of having ischemic stroke [29, 30]. Our result suggested every unit increase in SBP levels, the probability of being ischemic stroke increased by 1.031. Our study showed increased FBS concentration had association with the morbidity of ischemic stroke. This result was in conformity with most studies on FBS levels and ischemic stroke [31]. The study by Tracy E Madsen et al also indicated higher FBS concentration had association with higher incidence for developing ischemic stroke compared with not high or normal FBS concentration [32]. The morbidity of ischemic stroke increased with higher concentration, for every unit increase in FBS concentration, the possibility of being ischemic stroke increased by 1.248. SBP and FBS were independent ischemic stroke predictors, it is very important to manage blood pressure and blood sugar well to reduce the incidence of ischemic stroke.

For further analysis of gender difference, our study showed there was no gender difference that elevated levels of CG were both related to the morbidity of ischemic stroke no matter in males or in females. While increased TBIL levels had association with the morbidity of ischemic stroke in males compared to no association in females. Though our result conformed to the study by Heejin Kimm et al. which also proved TBIL levels had association with the morbidity of ischemic stroke in males but not females [11], while their results were not completely similar to ours. They pointed that high TBIL levels suggested low incidence of ischemic stroke in males which contrasts with our study that high TBIL levels indicated high incidence of ischemic stroke. This need more better designed researches to further verify their association in gender difference. There was no association between DBIL and ischemic stroke in our study, DBIL levels were shown an association with ischemic stroke in males but not in females compared with no association in IDBIL with ischemic stroke no matter in males and females.

This research also had several limitations: As there were missing during the data collection, we had to use mean value filling method to fill up few incomplete data, this may have affected the result slightly. For the sample size, we had 17 independent variables, but the sample size can't cover the variables of TC, TG, LDL-C and HDL-C. The sample size is not sufficient to detect the gender difference for the variable of IDBIL. As this research is a retrospective study, it may have some selection bias, this study showed the result TBIL had gender difference with ischemic stroke, and TBIL levels had association in males not females, but another study of ours showed TBIL had association with the morbidity of ischemic stroke only in females though it also proved TBIL had gender difference. So, this result could only prove TBIL had gender difference in its level with ischemic stroke, but to which gender had association with ischemic stroke, it needs more studies to verify this. This is also a single center research, all data came from one center, this may lead the patients in our research may have some regional feature, in particular, CG is a relatively new biochemical indicator of stroke, results about CG lack references to compare, this may need multi-center and repeated researches to verify this result. The control group of this research was hospitalized patients, and had more comorbidities and more confounding factors which may affect

the study. It may increase the difficulty to detect the difference in case and control group. There were very few references about CG, DBIL, IDBIL and ischemic stroke to refer.

CONCLUSION

This study inferred increased levels of CG, TBIL, FBS and SBP levels had association with the incidence of ischemic stroke. CG, TBIL, FBS and SBP were independent predictors for ischemic stroke in China. CG seemed more sensitive than TBIL as the predictor of ischemic stroke. There was no gender difference between CG and the incidence of ischemic stroke. Increased TBIL, DBIL was significantly associated with ischemic stroke in males but not females. The association between theses biliary markers and ischemic stroke needs more better designed studies and more rigorous studies to further verify. Screening more sensitive predictors of ischemic stroke could better guide clinical work and give better treatment strategy, which is also beneficial to reduce the incidence and severity of ischemic stroke.

Conflict of interest: none declared
Financial support: none declared

REFERENCES

1. Zhu HY, Zhao SZ, Zhang ML, Wang Y, Pan ZM, Cheng HR, et al. Elevated Serum Uric Acid Increases the Risk of Ischemic Stroke Recurrence and Its Inflammatory Mechanism in Older Adults. *Front Aging Neurosci.* 2022; 14:822350. doi: 10.3389/fnagi.2022.822350. PMID: 35350634.
2. GBD 2019 Stroke Collaborators. Global, regional, and national burden of stroke and its risk factors, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Neurol.* 2021;20(10):795-820. doi: 10.1016/S1474-4422(21)00252-0. PMID: 34487721.
3. Wang W, Jiang B, Sun H, Ru X, Sun D, Wang L, et al. Prevalence, Incidence, and Mortality of Stroke in China: Results from a Nationwide Population-Based Survey of 480 687 Adults. *Circulation*. 2017;135(8):759-771. doi: 10.1161/CIRCULATIONAHA.116.025250. PMID: 28052979.
4. Ma Q, Li R, Wang L, Yin P, Wang Y, Yan C, et al. Temporal trend and attributable risk factors of stroke burden in China, 1990-2019: an analysis for the Global Burden of Disease Study 2019. *Lancet Public Health.* 2021;6(12): e897-e906. doi: 10.1016/S2468-2667(21)00228-0. PMID: 34838196.
5. Ouyang Q, Wang A, Tian X, Zuo Y, Liu Z, Xu Q, et al. Serum bilirubin levels are associated with poor functional outcomes in patients with acute ischemic stroke or transient ischemic attack. *BMC Neurol.* 2021;21(1):373. doi: 10.1186/s12883-021-02398-z. PMID: 34610800.
6. Zhang Wang, Zhou Yujun, Xu Xiuli, Tian Yaping, Zhang Chunyan. A Time-Resolved-Fluorescence Lateral Flow Assay for Rapid Detection of Cholyglycine Acid for the Diagnosis of Liver Diseases. *Journal of Biomaterials and Tissue Engineering.* 2021;2023-2029. doi: 10.1166/jbt.2021.2781.
7. Antonelli G, Visentin S, Tasinato A, Rocco G, Zemin F, Volentiera E, et al. Cholylglycine determination by an automated chemiluminescence immunoassay: preliminary results in the intrahepatic cholestasis of pregnancy. *J Lab Precis Med.* 2019; 4:35. doi: 10.21037/jlpm.2019.09.01.
8. He LF, Zhao Y, Wang ZP. Prediction model of fetal meconium-stained amniotic fluid in re-pregnant women with intrahepatic cholestasis of pregnancy. *Zhejiang Da Xue Xue Bao Yi Xue Ban.* 2015;44(3):264-8. doi: 10.3785/j.issn.1008-9292.2015.05.05. PMID: 26350006.
9. Wu MH, Chang CT, Lin YN, Chen CJ. Identification of a potential prognostic plasma biomarker of acute ischemic stroke via untargeted LC-MS metabolomics. *Proteomics Clin Appl.* 2023 Jun 27:e2200081. doi: 10.1002/prca.202200081. PMID: 37376802.
10. Wang G, Qiao L, Tang Z, Zhou S, Min J, Li M. Association between bilirubin levels and risk of stroke: a systematic review and meta-analysis. *BMJ Open*. 2023;13(5): e064433. doi: 10.1136/bmjopen-2022-064433. PMID: 37164466.
11. Kimm H, Yun JE, Jo J, Jee SH. Low serum bilirubin level as an independent predictor of stroke incidence: A prospective study in Korean men and women. *Stroke*. 2009;40(11):3422-7. doi: 10.1161/STROKEAHA.109.560649. PMID: 19713538.
12. Ekblom K, Marklund SL, Johansson L, Osterman P, Hallmans G, Weinehall L, et al. Bilirubin and UGT1A1*28 are not associated with lower risk for ischemic stroke in a prospective nested case-referent setting. *Cerebrovasc Dis.* 2010;30(6):590-6. doi: 10.1159/000319778. PMID: 20948202.
13. Zhao K, Wang R, Chen R, Liu J, Ye Q, Wang K, et al. Association between bilirubin levels with incidence and prognosis of stroke: A meta-analysis. *Front Neurosci.* 2023; 17:1122235. doi: 10.3389/fnins.2023.1122235. PMID: 36866331.
14. Luo Y, Li JW, Lu ZJ, Wang C, Guan DN, Xu Y. Serum bilirubin after acute ischemic stroke is associated with stroke severity. *Curr Neurovasc Res.* 2012;9(2):128-32. doi: 10.2174/156720212800410876. PMID: 22475397.
15. Sheng X, Du H, Tang Y. Decreased Serum Total Bilirubin Level Predicts Early Neurological Deterioration in Patients with Acute Ischemic Stroke. *Neuropsychiatr Dis Treat.* 2021; 17:1977-82. doi: 10.2147/NDT.S315330. PMID: 34168455.
16. Choi Y, Lee SJ, Spiller W, Jung KJ, Lee JY, Kimm H, et al. Causal Associations Between Serum Bilirubin Levels and Decreased Stroke Risk: A Two-Sample Mendelian Randomization Study. *Arterioscler Thromb Vasc Biol.* 2020;40(2): 437-45. doi: 10.1161/ATVBAHA.119.313055. PMID: 31801373.
17. Thakkar M, Edelenbos J, Doré S. Bilirubin and Ischemic Stroke: Rendering the Current Paradigm to Better Understand the Protective Effects of Bilirubin. *Mol Neurobiol.* 2019;56(8):5483-96. doi: 10.1007/s12035-018-1440-y. PMID: 30612336.
18. Zhong P, Wu D, Ye X, Wang X, Zhou Y, Zhu X, et al. Association of circulating total bilirubin level with ischemic stroke: a systemic review and meta-analysis of observational evidence. *Ann Transl Med.* 2019;7(14):335. doi: 10.21037/atm.2019.06.71. PMID: 31475205.
19. Liu Y, Wang J, Zeng WZ, Lyu QS. Nonlinear relationship between serum total bilirubin levels and initial ischemic stroke in patients with non-valvular atrial fibrillation. *J Int Med Res.* 2020;48(10):300060520962347. doi: 10.1177/0300060520962347. PMID: 33100092.
20. Brouns R, Wauters A, Van De Vijver G, et al. Decrease in uric acid in acute ischemic stroke correlates with stroke severity, evolution and outcome. *Clin Chem Lab Med.* 2010;48(3):383-90. doi: 10.1515/CCLM.2010.065. PMID: 20020821.
21. Yang Y, Zhang Y, Li Y, Ding L, et al. U-Shaped relationship between functional outcome and serum uric acid in ischemic stroke. *Cell Physiol Biochem.* 2018; 47(6): 2369–79.
22. Hu G, Li J, Wang Q, Wang C, Wang Y, Gong T, Liu D. J-shaped relationship between serum uric acid levels and the risk of ischemic stroke in high-risk individuals: A hospital-based observational study. *Clin Neurol Neurosurg.* 2020 Aug;195:105906. doi: 10.1016/j.clineuro.2020.105906. PMID: 32447150.
23. Song Y, Zhang X, Li C, Xu S, Zhou B, Wu X. Is Bilirubin Associated with the Severity of Ischemic Stroke? A Dose Response Meta-Analysis. *J Clin Med.* 2022;11(12):3262. doi: 10.3390/jcm11123262. PMID: 35743332.
24. Peng Q, Bi R, Chen S, Chen J, Li Z, Li J, et al. Predictive value of different bilirubin subtypes for clinical outcomes in patients with acute ischemic stroke receiving thrombolysis therapy. *CNS Neurosci Ther.* 2022;28(2):226-36. doi: 10.1111/cns.13759. PMID: 34779141.

25. Wang J, Zhang X, Zhang Z, Zhang Y, Zhang J, Li H, et al. Baseline Serum Bilirubin and Risk of First Stroke in Hypertensive Patients. *J Am Heart Assoc.* 2020;9(12): e015799. doi: 10.1161/JAHA.119.015799. PMID: 32486877.
26. Duan H, Cheng Z, Yun HJ, Cai L, Tong Y, Han Z, et al. Serum Bilirubin Associated with Stroke Severity and Prognosis: Preliminary Findings on Liver Function after Acute Ischemic Stroke. *Neurol Res.* 2023;45(1):62-69. doi: 10.1080/01616412.2022.2119724. PMID: 36165803.
27. Sagheb Asl E, Taheraghdam A, Rahmani F, Javadrashid R, Golzari SEJ, Ghaemian N, et al. Determination of the Predictive Value of Serum Bilirubin in Patients with Ischemic Stroke: A Prospective Descriptive Analytical Study. *Adv Pharm Bull.* 2018 Nov;8(4):715-19. doi: 10.15171/apb.2018.080. PMID: 30607344.
28. Toyoda K, Yamagami H, Kitagawa K, Kitazono T, Nagao T, Minematsu K, et al. Blood Pressure Level and Variability During Long-Term Prasugrel or Clopidogrel Medication After Stroke: PRASTRO-I. *Stroke*. 2021;52(4):1234-43. doi: 10.1161/STROKEAHA.120.032824. PMID: 33563017.
29. Hägg-Holmberg S, Dahlström EH, Forsblom CM, Harjutsalo V, Liebkind R, Putaala J, et al. The role of blood pressure in risk of ischemic and hemorrhagic stroke in type 1 diabetes. *Cardiovasc Diabetol.* 2019;18(1):88. doi: 10.1186/s12933-019-0891-4. PMID: 31288813.
30. Yu Y, Liu L, Huang J, Shen G, Chen C, Huang Y, et al. Association between systolic blood pressure and first ischemic stroke in the Chinese older hypertensive population. *J Int Med Res*. 2020;48(4):300060520920091. doi: 10.1177/0300060520920091. PMID: 32319338.
31. Denorme F, Portier I, Kosaka Y, Campbell RA. Hyperglycemia exacerbates ischemic stroke outcome independent of platelet glucose uptake. *J Thromb Haemost.* 2021 Feb;19(2):536-46. doi: 10.1111/jth.15154. PMID: 33118271; PMCID: PMC7902465.
32. Madsen TE, Long DL, Carson AP, Howard G, Kleindorfer DO, Furie KL, et al. Sex and Race Differences in the Risk of Ischemic Stroke Associated With Fasting Blood Glucose in REGARDS. *Neurology*. 2021;97(7): e684-e694. doi: 10.1212/WNL.0000000000012296. PMID: 34045272.