Impact of triglyceride playing on stroke severity correlated to bilirubin

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**Abstract**

Major lipids making effects on the occurrence of acute ischemic stroke (AIS) is well recognized, but their roles on stroke severity remain uncertain. To explore the exact roles of lipids playing on stroke severity and the possible mechanism, we conduct this observational study.

Data was collected from patients with AIS from February 2008 to May 2012. The level of major lipids was compared among AIS groups with different severity and investigated the correlation. Also, the relationship existed between major lipids and bilirubin. Mechanism of major lipids playing on stroke severity was researched to determine if oxidative stress reflected by bilirubin.

Lower triglyceride (TG) and higher high density lipoprotein cholesterol (HDL-C) were observed in severe stroke, and obvious correlation existed between TG and stroke severity or HDL-C and stroke severity. TG was associated negatively with direct bilirubin (DBIL) and total bilirubin (TBIL), and lower level of DBIL and TBIL were related to higher quartiles of TG. There was no obvious difference of DBIL and TBIL among the groups of quartiles of HDL-C. TG was the influence factor of stroke severity in severe stroke through multiple uninvariable logistic regression. But it was not the independent influence factor after multivariable logistic regression adjusted by DBIL or TBIL. However, HDL-C was the influence factor of stroke severity through both uninvariable and multivariable logistic regression.

Lower TG or higher HDL-C predicted severer stroke. The effect of TG on stroke severity was mediated by bilirubin, not HDL-C.

**Abbreviations:** AIS = acute ischemic stroke, DBIL = direct bilirubin, HDL-C = high density lipoprotein cholesterol, LDL = low density lipoprotein, LDL-C = low density lipoprotein cholesterol, NIHSS = National Institute of Health Stroke Scale, TBIL = total bilirubin, TC = total cholesterol, TG = triglyceride, UA = uric acid.

**Keywords:** bilirubin, lipids, oxidative stress, stroke severity

1. **Introduction**

Dyslipidemia increases the risk for acute ischemic stroke (AIS), which has been widely accepted. Elevated blood lipids could induce changes in the arterial and microcirculatory system by increasing the production of oxygen free radicals which causing oxidative stress.\(^{[1]}\) Guidelines suggest that controlling hyperlipidemia by statins is very important in the treatment and prevention of AIS. The most widely accepted view is that low density lipoprotein cholesterol (LDL-C) can increase cholesterol deposition in the arterial wall and lower triglyceride (TG) finally cause the atherosclerosis. It has been found that TG may be more important than LDL-C as a risk factor in cardiovascular disease.\(^{[2]}\) TG could inhibit the anti-inflammatory and anti-atherosclerotic activities of high density lipoprotein and finally damage endothelial cell.\(^{[3]}\)

Major lipids play an important role in the occurrence and development of AIS. Markaki et al reported that higher cholesterol levels were associated with improved long-term survival after AIS.\(^{[4]}\) Once the ischemic stroke occurred, lipids can also have the impact on stroke severity and prognosis. Some studies found that patients with lower level of TG were shown to suffer from severer stroke.\(^{[5–7]}\) Unlike others reported opposite result that higher level of TG suffered from severer stroke,\(^{[8]}\) some studies showed that there were no association between these two at all.\(^{[7,12]}\) Total cholesterol (TC) was same to the HDL-C\(^{[6,7,9,13]}\) and stroke severity,\(^{[9–11]}\) while some studies showed that there were no association between these two at all.\(^{[7,12]}\) Total cholesterol (TC) was same to the HDL-C\(^{[6,7,9,13]}\) and stroke severity,\(^{[9–11]}\) while some studies showed that there were no association between these two at all.\(^{[7,12]}\) In conclusion, the relation between lipids level and stroke severity is inconsistent.
What about its possible mechanism of major lipids playing on the stroke severity, there was none of studies involved in. Oxidative stress plays the important role through the whole course of atherosclerosis diseases induced by lipids, while bilirubin can partly reflect the level of oxidative stress. Bilirubin has been provided as an endogenous anti-oxidant for it has the ability to scavenge peroxys radicals and to inhibit low density lipoprotein (LDL) oxidation. Bilirubin is also involved in the pathogenesis of cardiometabolic disorders, during which oxidative-stress is considered to play an important role.

Dullaart et al found that higher level of bilirubin was related to lower risk of atherosclerosis diseases. Bilirubin has been provided as an endogenous anti-oxidant for it can partly reflect the level of oxidative stress. Oxidative stress plays the important role through the whole course of atherosclerosis diseases induced by lipids, while bilirubin can partly reflect the level of oxidative stress.

### 2. Material and methods

#### 2.1. Study subjects

Data for this study was collected from the hospitalized patients of department of neurology at the affiliated drum tower hospital of Nanjing university medical school from February 2008 to May 2012. The study was approved by the institutional committee of affiliated drum tower hospital of Nanjing university medical school (No. 2011051). At admission, plain CT scan of the head was done to rule out haemorrhage and MRI was done to identify the new infarction. Baseline clinical information and blood index (including lipids and bilirubin, et al) were collected and recorded.

#### 2.2. Definition of the stroke severity

Stroke severity was reflected by National Institute of Health Stroke Scale (NIHSS) scores on the day of admission. Subjects were divided into mild stroke (NIHSS < 8), moderate to severe stroke (8 ≤ NIHSS ≤ 15) and severe stroke (NIHSS > 15).

#### 2.3. Blood collection and analysis

Venous blood was collected following overnight fasting for at least 12 hours at the day of hospitalization, and analyzed by a solid-phase chemiluminescent immunometric assay on Immulite 2000 with the manufacturer’s reagents as directed to direct bilirubin (DBIL), total bilirubin (TBIL), uric acid, blood glucose, TG, TC, HDL-C, and LDL-C.

#### 2.4. Statistical analyses

Statistical analyses were performed with SPSS 17.0 software. The results were expressed as mean ± SD for the continuous variables (t-test) depending on their normal distribution. Logistic regression was used to determine the correlation. The level-risk relationship was denoted by OR, with a corresponding 95% CI, using logistic regression. Level of significance for statistical purposes was stated at P < .05.

### 3. Results

#### 3.1. Baseline characteristics

We enrolled AIS patients who signed informed consent were hospitalized in the department of neurology from February 2008 to May 2012. A total of 610 patients, including 385 males and 225 female, with an average age of 66.7 years old were enrolled. There were 434 patients who had hypertension and 202 patients who had DM and 81 patients who had AF coexistence with AIS. According to the NIHSS scores, 472 patients were allocated into mild stroke group, 108 were into moderate to severe stroke group and 30 were into severe stroke group. The mean ± SD of DBIL, TBIL, blood glucose, uric acid, TC, TG, HDL-C, LDL-C was 4.706 ± 2.541 umol/L, 18.313 ± 9.432 umol/L, 6.919 ± 2.832 mmol/L, 329.086 ± 102.708 mmol/L, 1.507 ± 0.987 mmol/L, 4.799 ± 1.031 mmol/L, 1.017 ± 0.747 mmol/L respectively.

#### 3.2. Correlation between major lipids and stroke severity

Lower level of TG and higher level of HDL-C were observed in the severe stroke group compared to the mild stroke group, and the difference was significant. Despite the difference of TC was also existed among 3 groups, but the highest level of it was in the moderate to severe stroke group. There was obvious correlation between TG and NIHSS scores (negative correlation), HDL-C and NIHSS scores (positive correlation) (Table 1).

#### 3.3. Correlation between major lipids and bilirubin

There was obvious correlation between TG and DBIL or TBIL (negative correlation), HDL-C and DBIL or TBIL (positive correlation). Then, TG was categorized into 4 groups according to the quartiles (Q1 < 0.88, 0.88 < Q2 < 1.27, 1.27 < Q3 < 1.8, Q4 > 1.8), we found that there was significant difference in DBIL and TBIL among groups with the change of TG. While HDL-C was categorized into 4 groups according to the quartiles (Q1 < 0.88, 0.88 < Q2 < 1.07, 1.27 = Q3 < 1.35, Q4 > 1.35), there was no obvious difference in DBIL and TBIL among 4 groups according to the level of HDL-C (Table 2).

#### 3.4. Influence factors of the stroke severity

TG was correlated to the stroke severity, so did the bilirubin. Also, HDL-C was correlated to the stroke severity, despite of there was no obvious difference of DBIL or TBIL among the quartiles of HDL-C. The relation among the lipids including TG

### Table 1

| Variables | Mild stroke (n = 472) | Moderate to severe stroke (n = 108) | Severe stroke (n = 30) | P |
|-----------|----------------------|-------------------------------------|-----------------------|---|
| TG        | 1.540 ± 1.022        | 1.440 ± 0.889                      | 1.089 ± 0.516         | .034 |
| TC        | 4.721 ± 1.017        | 5.092 ± 1.076                      | 4.968 ± 0.865         | .002 |
| HDL-C     | 1.105 ± 0.344        | 1.243 ± 0.380                      | 1.401 ± 0.420         | .001 |
| LDL-C     | 2.528 ± 0.743        | 2.717 ± 0.776                      | 2.551 ± 0.631         | .060 |

#### Table 2

Comparison of major lipids among different severe stroke groups and correlation between major lipids and NIHSS.

| Variables | R       | P   |
|-----------|---------|-----|
| TG and NIHSS | −0.121  | .003 |
| TC and NIHSS  | 0.067  | .032 |
| HDL-C and NIHSS | 0.194  | .000 |
| LDL-C and NIHSS | 0.030  | .484 |

HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, NIHSS = National Institute of Health Stroke Scale, TC = Total cholesterol, TG = triglyceride.

* P < .05 vs Mild stroke.

# P < .05 vs Moderate to severe stroke.
and HDL-C, stroke severity and bilirubin is unclear. To investigate the role of major lipids playing on the stroke severity, multiple logistic regression (mild stroke was set as reference) was performed between stroke severity and relative risk factors. It demonstrated that HDL-C, DBIL and TBIL were the influence factors of stroke severity, whatever univariable or multivariable logistic regression both in group of moderate to severe stroke and severe stroke. TG was also the influence factors of stroke severity in severe stroke subgroup through univariable logistic regression, with OR (95% CI, P) was 0.407 (0.205–0.808, 0.010), but the significance of it disappeared after multivariable logistic regression adjusted by DBIL or TBIL, the result was 0.601 (0.304–1.187, 0.142) or 0.586 (0.295–1.162, 0.126) (Table 3).

4. Discussion

Using the inpatients in our hospital with AIS, we confirmed that low level of TG or high level of HDL-C resulted in severe stroke. Both of TG and HDL-C have the impact on the stroke severity, but the mechanism behind it was not different, which was demonstrated with logistic regression that the role of TG playing on stroke severity was mediated by bilirubin partly, nor did the HDL-C.

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Table 2

| Variables | R | P  |
|-----------|---|----|
| TG and DBIL | −0.194 | .000 |
| HDL-C and DBIL | 0.086 | .033 |
| TG and TBIL | −0.160 | .000 |
| HDL-C and TBIL | 0.110 | .007 |

Table 3

| Variable | Beta estimate | OR | 95% CI | P |
|----------|--------------|----|--------|---|
| Multiple (moderate to severe stroke) | | | | |
| TG | −0.122 | 0.885 | 0.700–1.119 | .307 |
| HDL-C | 1.071 | 2.917 | 1.651–5.155 | .000 |
| Univariable | | | | |
| DBIL | 0.172 | 1.187 | 1.092–1.291 | .000 |
| TBIL | 0.048 | 1.049 | 1.028–1.072 | .000 |
| TG | 0.071 | 1.074 | 0.853–1.351 | .554 |
| Multivariable | | | | |
| HDL-C | 1.041 | 2.831 | 1.562–5.134 | .001 |
| DBIL | 0.165 | 1.180 | 1.083–1.285 | .000 |
| TG | 0.056 | 1.059 | 0.841–1.334 | .624 |
| Multivariable | | | | |
| HDL-C | 1.016 | 2.763 | 1.520–5.023 | .001 |
| Multiple (severe stroke) | | | | |
| TBIL | 0.047 | 1.048 | 1.026–1.070 | .000 |
| TG | −0.899 | 0.407 | 0.205–0.808 | .010 |
| HDL-C | 1.811 | 6.116 | 2.648–14.130 | .000 |
| Univariable | | | | |
| DBIL | 0.193 | 1.213 | 1.084–1.357 | .001 |
| TBIL | 0.056 | 1.058 | 1.026–1.090 | .000 |
| TG | −0.510 | 0.601 | 0.304–1.187 | .142 |
| Multivariable | | | | |
| HDL-C | 1.545 | 4.690 | 1.959–11.228 | .001 |
| DBIL | 0.165 | 1.180 | 1.048–1.328 | .006 |
| TG | −0.534 | 0.586 | 0.295–1.162 | .126 |
| Multivariable | | | | |
| HDL-C | 1.521 | 4.579 | 1.916–10.944 | .001 |
| TBIL | 0.049 | 1.051 | 1.018–1.085 | .002 |

Multiple logistic regression: mild stroke (NIHSS<8) was set as reference.

DBIL = direct bilirubin; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; TBIL = total bilirubin; TC = Total cholesterol; TG = triglyceride.
Atherothrombosis is the most common stroke etiology as we all know, which may be caused by lifestyle changes such as increased dietary fat and cholesterol intake. The relationship of major lipids to the risk of future ischemic stroke has been proved. Also, Yan’s study showed that a lower TG/HDL-C was independently associated with death and worse outcome at 3 months in AIS. But the correlation of lipids and stroke severity has long been controversial. Several studies reported patients with lower TG levels suffered from severe stroke, but another study demonstrated higher TG in patients with severe stroke. HDL-C was also reported that it had negative correlation or no correlation with stroke severity, so did the TC. While there was no correlation between LDL-C and stroke severity. In 1 word, there were few studies evaluated the relationship between all major lipids and stroke severity, none of which deal with the possible mechanism.

A principal discovery of our study was that patients with lower TG and higher HDL-C level were shown to suffer from severe stroke. This kind of negative correlation between TG and stroke severity was similar to that of Dziedzic et al. Bouziana and Tziomalos thought these associations might be explained by the relationship between low TG and malnutrition caused by stroke. Other studies have reported that severe stroke with swallowing disorder leading to malnutrition. While our study got the data of major lipids on the day of admission, so the dysphagia of AIS patients could be excluded. In addition, 2nd data in vitro suggested that TG might protect against fatty acid-induced lipotoxicity, which might explain the consequence we have achieved.

In the present study, higher HDL-C was associated with severe stroke, which was in contrary to Tziomalos and Bouziana and Tziomalos thought these associations might be explained by the relationship between low TG and malnutrition caused by stroke. Other studies have reported that severe stroke with swallowing disorder leading to malnutrition. While our study got the data of major lipids on the day of admission, so the dysphagia of AIS patients could be excluded. In addition, 2nd data in vitro suggested that TG might protect against fatty acid-induced lipotoxicity, which might explain the consequence we have achieved.

In contrast to the results of TG and HDL-C, neither TC nor LDL-C were related to the stroke severity in our research. There seldom was evidence that TC had the impact on stroke severity, besides that, patients with lower TC levels suffered from severe stroke reported by Koton et al. But in the research, the TG level was not evaluated, so researchers thought the association between lower TC and stroke severity might partly due to the lower TG. In our study, moderate to severe stroke group had the highest level TC and no correlation was between TC and NIHSS, so did the LDL-C, which was in line with Jain and Weir's studies.

In addition, we studied the correlation between bilirubin and major lipids above in patients of AIS, and we found that TG was associated negatively while HDL-C was associated positively with bilirubin, which was accorded with the previous report. Significant difference of DBIL or TBIL was also observed among the quartiles of TG, nor did HDL-C, demonstrated that TG was related to bilirubin more tightly. Bilirubin is not only a terminal metabolite of heme metabolism, but also an important endogenous antioxidant, which is correlated with the stroke severity and can partly reflect the intensity of oxidative stress of AIS. Anti-oxidative activities was one of the key functional properties of HDL, revealed that lipids had an impact on the oxidative stress. We want to know if the impact of lipids playing on stroke severity is related to bilirubin. To confirm our hypothesis, with univariable and multivariable logistic regression were performed, we found that TG had the correlation with stroke severity in univariable logistic regression, but had no association in multivariable logistic regression after adjusting DBIL or TBIL, which confirmed that the role of TG playing on stroke severity was partly mediated by bilirubin, served as the marker of oxidative stress. On the contrary, this kind of role was not found in HDL-C, demonstrated that the role of HDL-C playing on stroke severity was not through bilirubin.

Lower TG and higher HDL-C levels appeared to be associated with severer stroke. Besides, the correlation between TG and stroke severity was achieved through the effect of bilirubin, but the specific upstream and downstream signaling pathways was not clear. We look forward to the future, routine measurement such as TG, HDL-C, bilirubin may be useful in risk stratification in AIS. On the other hand, the specific mechanism remains unclear, more studies are needed to be explored about the molecular pathways behind it.

5. Limitations

Patients from a single neurological hospital in China is the main limitation of this study, and it will be easy to cause the deviation of grouping. Second, the number of severe stroke subtype was very small, which may have affected the statistical result of the study. Third, confounders like age, gender, DM, AF were not taken into consideration. In the future, we will expand the sample size, especially for the severe stroke subtype and take more confounders into consideration to make the study better.

6. Conclusion

Major lipids, such as TG and HDL-C, had an impact on the stroke severity. The role of TG playing on stroke severity was related to oxidative stress partly, nor did the HDL-C. Exact mechanisms behind it need to be further explored, which might be helpful to stroke treatment.

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Author contributions

Data curation: Yun Luo, Zheng Li, Jiahui Zhang. Formal analysis: Yun Luo. Methodology: Yun Luo. Writing – original draft: Zheng Li, Jiahui Zhang. Writing – review & editing: Yun Luo, Zheng Li.
References

[1] Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340:115–26.
[2] Lawler PR, Kottri G, Koh M, et al. Real-world risk of cardiovascular outcomes associated with hypertriglyceridaemia among individuals with atherosclerotic cardiovascular disease and potential eligibility for emerging therapies. Eur Heart J 2020;41:86–94.
[3] Miller M, Stone NJ, Ballantyne C, et al. Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. Circulation 2011;123:2292–333.
[4] Markaki I, Nilsson U, Kostulas K, et al. High cholesterol levels are associated with improved long-term survival after acute ischemic stroke. J Stroke Cerebrovasc Dis 2014;23:e47–53.
[5] Dziedzic T, Sadowski A, Gryz EA, et al. Lower serum triglyceride level is associated with increased stroke severity. Stroke 2004;35:e151–2.
[6] Weir CJ, Sattar N, Walters MR, et al. Low triglyceride, not low cholesterol concentration, independently predicts poor outcome following acute stroke. Cerebrovasc Dis 2003;16:76–82.
[7] Jain M, Jain A, Yerragundu N, et al. The triglyceride paradox in stroke survivors: a prospective study. Neurosci Lett 2013;503:179–83.
[8] Sinumnic AM, Nikolac N, Topic E, et al. Are serum lipids measured on stroke admission prognostic? Clin Chem Lab Med 2008;46:1163–7.
[9] Tziomalos K, Giampatzis V, Bouziana SD, et al. Prognostic significance of major lipids in patients with acute ischemic stroke. Metab Brain Dis 2017;32:395–400.
[10] Yeh PS, Yang CM, Lin SH, et al. Low levels of high-density lipoprotein cholesterol in patients with atherosclerotic stroke: a prospective cohort study. Atherosclerosis 2013;228:472–7.
[11] Putaala J, Sarbajna D, Mustanoja S, et al. Functional outcome in young adult ischemic stroke: impact of lipoproteins. Acta Neurol Scand 2013;127:61–9.
[12] Sanosson N, Saver JL, Kim D, et al. Do high-density lipoprotein cholesterol levels influence stroke severity? J Stroke Cerebrovasc Dis 2006;15:187–9.
[13] Koton S, Molshatzki N, Bornstein NM, et al. Low cholesterol, statins and outcomes in patients with first-ever acute ischemic stroke. Cerebrovasc Dis 2012;34:213–20.
[14] Luo Y, Li JW, Lu ZJ, et al. Serum bilirubin after acute ischemic stroke is associated with stroke severity. Curr Neurovasc Res 2012;9:128–32.
[15] Luo Y, Li J, Zhang J, et al. Elevated bilirubin after acute ischemic stroke linked to the stroke severity. Int J Dev Neurosci 2013;31:634–8.
[16] Vitek L. The role of bilirubin in diabetes, metabolic syndrome, and cardiovascular diseases. Front Pharmacol 2012;3:55.
[17] Dullaart RP, de Vries R, Lefrandt JD. Increased large VLDL and small LDL particles are related to lower bilirubin in Type 2 diabetes mellitus. Clin Biochem 2014;47:170–5.
[18] Dullaart RPF, Boersma J, Lefrandt JD, et al. The inverse association of incident cardiovascular disease with plasma bilirubin is unaffected by adiponectin. Atherosclerosis 2014;235:380–3.
[19] Tu WJ, Dong X, Zhao SJ, et al. Prognostic value of plasma neuroendocrine biomarkers in patients with acute ischaemic stroke. J Neuroendocrinol 2013;25:771–8.
[20] Stamler J, Neaton JD, FH. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). JAMA 1986;256:2823–8.
[21] Kooe R, Prizment AE, Blaes A, et al. Shared risk factors in cardiovascular disease and cancer. Circulation 2016;133:1104–14.
[22] Yang N, Lin M, Wang BG, et al. Low level of low-density lipoprotein cholesterol is related with increased hemorrhagic transformation after acute ischemic cerebral infarction. Eur Rev Med Pharmacol Sci 2016;20:673–8.
[23] Freiberg JG, Tyljaerg-Hansen A, Jensen JS, et al. Nonfasting triglycerides and risk of ischemic stroke in the general population. JAMA 2008;300:2142–52.
[24] Vauthey C, de Freitas GR, van Melle G, et al. Better outcome after stroke is associated with stroke severity. Curr Neurovasc Res 2012;9:128.
[25] Chapman MJ, Le Goff W, Guerin M, et al. Cholesteryl ester transfer protein: at the heart of the action of lipid-modulating therapy with fluvastatin and niacin, and cholesteryl ester transfer protein inhibitors. Proc Natl Acad Sci U S A 2003;100:3077–82.
[26] Malik KC, Guyton JR, Orringer CE, et al. Triglyceride-lowering therapies reduce cardiovascular disease event risk in subjects with hypertriglyceridaemia. J Clin Lipidol 2016;10:905–14.
[27] Chapman MJ, Le Goff W, Guerin M, et al. Cholesteryl ester transfer protein: at the heart of the action of lipid-modulating therapy with statins, fibrates, niacin, and cholesteryl ester transfer protein inhibitors. Eur Heart J 2010;31:149–64.
[28] Triolo M, Annema W, Dullaart RP, et al. Assessing the functional properties of high-density lipoproteins: an emerging concept in cardiovascular research. Biomark Med 2013;7:457–72.