Utility of Trimethylamine Oxide (TMAO) in Predicting Early Neurological Deterioration after Acute Ischemic Stroke

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ABSTRACT

Objective: To investigate whether plasma trimethylamine N-oxide (TMAO) levels might predict early neurological deterioration (END) in individuals with acute ischemic stroke.

Study Design: Cohort study.

Place and Duration of the Study: Jiulongpo District Hospital of Traditional Chinese Medicine, Chongqing City, China, from January 2020 to December 2021.

Methodology: Patients presenting with ischemic stroke were classified into the END group and the non-END group. The National Institutes of Health Stroke Scale (NIHSS) total increasing by 2 points or more within 72 hours of admission was the definition of the END. Plasma TMAO levels were determined by high-performance liquid chromatographic and tandem mass spectrometry.

Results: Twenty-six (25%) of the 104 patients, diagnosed with END exhibited higher TMAO levels after admission (median 1.438 vs. 0.449 nmol/mL, p=0.001). Elevated plasma TMAO levels were significant predictors of END in univariate logistic analysis. After controlling for age, gender, and cardiovascular risk factors in the multivariate conditional logistic regression model, the plasma TMAO levels in the END group remained significantly higher than those in the non-END group (OR=6.646, 95% CI 2.434-18.147, p<0.001). In receiver operator characteristic (ROC) analysis, the sensitivity and specificity of TMAO in distinguishing the END group and the non-END group at 0.564 nmol/mL cutoff value were 0.885 and 0.679, respectively.

Conclusion: According to this research, the development of END on admission in patients with acute ischemic stroke may be positively correlated with the elevation in plasma TMAO levels.

Key Words: Trimethylamine N-oxide level, Acute ischemic stroke, Early neurological deterioration, NIHSS score.

INTRODUCTION

Ischemic stroke is one of the most significant types of stroke, according to the 2019 Global Burden of Disease (GBD) survey, its incidence and prevalence rate account for 69.6% and 77.8% of all strokes,[1] respectively. In China, 'stroke is the leading cause of mortality and long-term disability'.[2] Early neurological deterioration (END) affects short-term neurological recovery in patients with ischemic stroke and is linked to in-hospital mortality and disability.[3] END accounts for 23%-41% of ischemic strokes.[4]

A complex clinical process involving hemodynamic alterations, hemorrhagic abnormalities, metabolic diseases, immune responses, and other unknown factors may be the cause of END.[5] Making judgments towards END prevention may be aided by further investigation of various processes. Through further study of various processes, the prevention of END can be judged. Trimethylamine oxide (TMAO), a biomarker of the intestinal microbiota, has been associated with an increased risk of cardiovascular disease.[6] TMAO is an intestinal microorganism metabolite formed by the conversion of trimethylamine to heparin monooxygenase.[7] Elevated plasma TMAO levels are related to atherosclerosis,[8] and are associated with platelet hyperreactivity and inflammation.[9] Two recent studies have shown that higher fasting TMAO levels in patients with acute ischemic stroke indicate higher functional prognosis adverse events and mortality,[10] and higher TMAO levels are associated with the pathogenesis of first ischemic stroke and continue to increase neurological deficits in Chinese patients.[11]

Despite the obvious links between TMAO and a number of chronic disorders, it is still unclear whether elevated TMAO

levels can play a role in the onset and progression of neurological deficits following ischemic stroke. Therefore, this study aimed to investigate the association between plasma TMAO levels and END in patients with acute ischemic stroke, and the potential of TMAO as a clinical predictor of END.

METHODOLOGY

A total of 104 patients with acute ischemic stroke (symptoms onset time less than 24 hours) who were hospitalised in Jiulongpo District Hospital of Traditional Chinese Medicine in Chongqing, from January 2020 to December 2021, were prospectively included. Based on the relevant literature, the sample size was 109 calculated by G-power software (effect size=0.3, αerr prob=0.05, 1-βerr prob=0.9). Excluding related screening, 104 patients were enrolled. The inclusion criteria were informed consent from all patients, admission within 24 hours after onset, and a CT or MRI diagnosis of acute ischemic stroke. The type of ischemic stroke was diagnosed using Trial of Org 10,172 in Acute Stroke Treatment (TOAST) criteria.[12] Age less than 18 years old, TOAST aetiology classification for cardiogenic cerebral embolism, NIHSS score greater than 18 points, intravenous thrombolysis, mechanical thrombectomy, cancer, inflammation or infectious disease, haematological disease, severe renal failure, severe liver failure, automatic discharge within 7 days of admission, and death as the exclusion criteria.

Data were acquired using standardised case report forms. Characteristics, risk factors, hypertension, neurological diseases, stroke pathogenesis, and neuroimaging data were included in the baseline data. By using a clinical electronic medical record system, baseline data were gathered. General information included age and gender, prior medication history, risk factors such as atrial fibrillation and / or cardiovascular disease, and physical examination information. The laboratory test results included white blood cell count (WBC), high-sensitivity C-reactive protein (Hs-CRP), serum creatinine (Cre), interleukin-2 (IL-2) and interleukin-6 (IL-6), low-density lipoprotein cholesterol (LDL-C). Other examinations included the carotid-ultrasonography (CDFI), electrocardiogram (ECG), magnetic resonance angiography (MRA) or computed tomography angiography (CTA), and echocardiogram.

A neurologist utilised National Institutes of Health Stroke Scale (NIHSS) scores to assess the clinical severity upon admission. The END was defined as a 'deterioration of neurological impairment' within 72 hours after admission, as shown by a rise in the NIHSS score of at least 2 following admission.[13] The NIHSS continuous assessment was done within 7 days of enrollment in this prospective Cohort study.[14] Patients who satisfied the enrollment criteria were then divided into the END group (n=26, NIHSS score increased ≥2 points) and the non-END group (n=78, NIHSS score increased <2 points) based on NIHSS changes. The hospital ethical review committee authorised the study plan, and each patient or their family member completed an informed consent form. The study protocol was authorised and approved by the Ethical Review Committee of Jiulongpo District Hospital of Traditional Chinese Medicine in Chongqing. Informed consent was taken.

Fasting blood samples of each individual were collected using an EDTA tube within 24 hours of admission. Fresh specimens were obtained and centrifuged at 4℃ and 3000 rpm for 10 minutes, and the remaining plasma was then collected and stored in a refrigerator at -80°C for later use.

The Thermal Ultra-high Performance Liquid Chromatography (UPLC-U3000) was linked to a SCIEX 5600 mass spectrometer, which was the equipment utilised in this experiment. An Acquity Uplc Hilic column was used, measuring 1.7μm internal diameter, 2.1mm×100mm. After making the standard application solution and standard curve, the specimen was processed, the chromatographic conditions were set, and the mass spectrometry parameters were set up. Multi Quant 3.0 was used to analyse and compute each chromatographic piece of information based on the peak area.

SPSS 26.0 for Mac was used for statistical analysis. A value of $p < 0.05$ showed that all double-tailed tests were statistically significant. The K-s test was used to determine if the data distribution was normal. The categorical variable were represented by n(%), whereas mean or median were used to represent the continuous variable (interval, IQR). Continuous data with normal distribution were presented as means ± standard deviation and compared by using t-test. Continuous data with a skewed distribution were expressed as the median with interquartile range and compared using the Mann-Whitney U test. The categorical data were presented as numbers with frequency and compared by χ^2 test. The area under the curve (AUC) was calculated and the receiver operating characteristic curve was used to evaluate the anticipated value of TMAO for END. The Yuden index determines the optimal TMAO cut-off point. The correlation between plasma TMAO level and END was assessed using the Spearman rank correlation test. In the following linear regression analysis, any appreciable variations between baseline clinical characteristics and any selected confounding variables were addressed. Additionally, multivariate and binary logistic regression models that have been modified for all other relevant variables were used. The results of regression were represented as the odds ratio (OR) and the 95% confidence interval (CI).

RESULTS

Finally, 104 patients with acute ischemic stroke (mean age 65.8±9.3 years, 58 (55.8%) patients were male) were included in this research. END occurred in 26 patients (25%) within the first 72 hours after admission. The END group significantly outperformed the non-END group in terms of confirmed type-2 diabetes (p=0.012), NIHSS at admission (p <0.001), TMAO (p <0.001), LDL-C (p=0.033), and hs-CRP (p <0.001). Table I provides a summary of the diagnostic characteristics of those who participated in the research.

The plasma TMAO levels in the END group were significantly higher than those in the non-END group (1.438 [IQR 1.074-2.540] nmol/mL vs. 0.449 [IQR 0.351-0.774] nmol/mL; p <0.001). Spearman correlation coefficient was used to analyse the correlation between the incidence of END and TMAO levels.

**Table I: Baseline clinical and demographic characteristics.**

	Non-END group (n=78)	END group (n=26)	Z/t	p
Age, year	65.86±9.02	65.88±10.62	-0.012	0.604
Male, n (%)	45 (57.7%)	13 (50%)	-0.681	0.496
Vascular risk factors,%				
Hypertension, n (%)	33 (42.3%)	16 (61.5%)	-0.926	0.354
Type-2 diabetes, n (%)	21 (26.9%)	13 (50%)	-2.504	0.012[*]
Cardiovascular disease, n (%)	10 (12.8%)	8 (30.8%)	-1.605	0.109
Atrial fibrillation, n (%)	3 (3.8%)	4 (15.4%)	-1.443	0.149
Carotid artery plaque, n (%)	25 (32.1%)	4 (15.4%)	-1.524	0.127
History of Stroke, n (%)	5 (6.40%)	2 (7.7%)	-0.225	0.822
Clinical data				
TMAO (nmol/mL)	0.449 (0.351~0.774)	1.438 (1.074~2.540)	-6.438	<0.001
WBC (*10^9/L)	7.91±1.96	9.63±2.54	-3.575	0.231
LDL-C (mmol/L)	2.94±0.84	3.62±1.05	-3.349	0.033[*]
Cre (umol/L)	75.38±19.20	78.70±19.47	-0.761	0.968
IL-2 (ng/L)	30.38±6.39	37.74±8.99	-4.57	0.104
IL-6 (ng/L)	12.15±4.72	20.47±6.28	-7.134	0.145
Hs-CRP (mg/L)	3.74 (2.1~4.72)	5.91 (5.23~7.23)	-5.387	<0.001
Large intracranial artery stenosis ≥59%, n (%)	5 (6.40%)	9 (34.6%)	-3.632	<0.001
NIHSS at admission score	5 (4~8)	8 (7~9.25)	-4.195	<0.001
Stroke etiology, %				
Atherosclerosis, n (%)	41 (52.6%)	19 (73.1%)	-2.242	0.025[*]
Cardioembolism, n (%)	9 (11.5%)	6 (23.1%)		
Small arterial obstruction, n(%)	22 (28.20%)	0 (0.00%)		
Other, n (%)	4 (5.1%)	1 (3.8%)		
Unknown, n (%)	2 (2.6%)	0 (0.00%)		

TMAO, Trimethylamine N-Oxide; WBC, white blood cell count (WBC); LDL-C, low-density lipoprotein-cholesterol; Cre, creatinine; IL-2,Interleukin-2; IL-6, Interleukin-6; Hs-CRP, Hyper-sensitive C-reactive protein; NIHSS, National Institutes of Health Stroke Scale.
Continuous data are presented as means±standard deviation. Categorical data are presented as numbers (%).

Table II: Relationship between TMAO levels and END.

Model	OR (95%CI)	p-value
Crude model	5.643 (2.358-13.503)	<0.001
Adjusted model[1]	5.892 (2.392-14.515)	<0.001
Adjusted model[2]	6.646 (2.434-18.147)	<0.001

[1]Model 1, adjusted for age, sex; [2]Model 2, adjusted for Hypertension, Type-2 Diabetes, and Cardiovascular disease additionally; CI=confidence interval; OR= odds ratio.
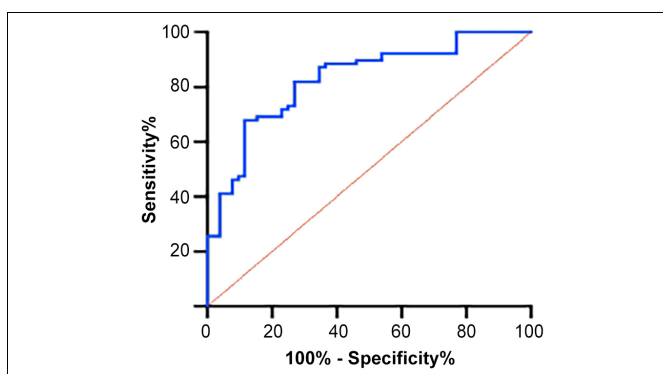
Figure 1: ROC curve of plasma TMAO level for END/non-END discrimination.

The results showed that the correlation between the incidence of END and TMAO value was 0.531 (p <0.01), indicating that there was a moderate positive correlation between the occurrence of END and TMAO levels. In the crude model, plasma TMAO levels were significantly increased in the END group (p<0.001). After adjusting for risk variables such as age and sex (adjusted Model 1, p<0.001), hypertension, type-2 diabetes, and cardiovascular disease (adjusted Model 2, p<0.001), in the multivariate logistic analysis, the elevated TMAO plasma levels still had a significant effect on the END-group (Table II).

ROC curve was used to analyse the diagnostic significance of TMAO in differentiating the END group from the non-END group. In order to maximise the sum of sensitivity and specificity to distinguish the END group and the non-END group, the beneficial cut-off plasma level of TMAO was 0.564nmol/mL.

The area under the ROC curve was 0.829 (95% confidence interval [CI]:74.1%-91.7%, sensitivity = 0.885, specificity = 0.679) (Figure 1). Compared with IL-2(AUC 0.763 95% CI:0.650-0.875), Cr(AUC 0.561 95% CI:0.434-0.688) and NIHSS score range (AUC 0.773 95% CI:0.684-0.862), it had stronger ability to differentiate, indicating that plasma TMAO concentration may be used to distinguish acute ischemia patients with END and non-END patients. However, it does not seem to be less accurate than hs-CRP(AUC 0.854 95% CI:0.784-0.924) and IL-6(AUC 0.869 95% CI:0.794-0.943).

DISCUSSION

In this clinical observational investigation that included a hospitalised population with the higher plasma TMAO concentration assessed within 24 hours of acute ischemic

infarction was linked to a higher risk of END. Other well-known predictors of clinical deterioration, such as gender, age, risk factors for cardiovascular disease, and the degree of neurological impairments, were not associated with this connection. There was no correlation between this relationship with other well-known clinical deterioration predictions such as age, cardiovascular risk factors, and severity of deficits in the nervous system.

In individuals with acute cerebral infarction, END itself is a risk factor for poor prognosis. In comparison to patients with non-END, those with END patients increased by 3.8 times, and the risk of future disability increased by 30 times.[15] END is a complicated pathological alteration that is impacted by a variety of mechanisms and circumstances.[16] According to epidemiological studies and related research, a National Institutes of Health nomogram made up of age, Hs-CRP, type-2 diabetes, atrial fibrillation, previous antiplatelet medication use, and the baseline stroke scale may accurately presume the risk of END in patients with acute ischemic infarction.[17]

The pathogenesis and clinical outcome of ischemic stroke is associated with inflammatory response. More and more studies have demonstrated that TMAO serves as both a prognostic indicator and a risk factor for stroke and cardiovascular disease.[18,19] Additionally, the amount of pro-inflammatory intermediates $CD14^+/CD16^+$ mononuclear cells was strongly correlated with the concentration of TMAO in the blood.[20] According to a multicenter study, the advanced plasma TMAO levels are still an independent predictor of new vascular lesions after adjusting for potential confounding factors. The plasma TMAO levels in patients with new lesions on diffusion-weighted images (DWI) after carotid artery stent implantation have obviously higher than in patients with no new lesions prior to the operation.[21] A recent study found that the levels of TMAO were significantly increased in END patients after acute ischemic stroke, with a mean average concentration of 4.8 mmol/L.[13] This study suggested that TMAO may predict END after acute ischemic stroke.

CONCLUSION

A rise in plasma TMAO levels in patients within 24 hours of acute ischemic stroke upon admission may be positively correlated with the occurrence of END. Plasma TMAO concentration may be used to distinguish acute ischemia patients with END and non-END patients. TMAO has the potential to serve as a promising biomarker of recurrent adverse vascular events in this patient population, which further questioned the potential of novel neuro-protective therapies based on the modulation of gut microbiota metabolites to prevent END in acute ischemic stroke patients. The limitations of this study include the fact that it only involved a small number of participants and was

implemented in a single centre, which raises the possibility of selection bias. In addition, if the dynamic changes in TMAO levels can be assessed, this will ultimately be useful. In order to thoroughly understand this correlation, it is necessary to further extend the research with a substantial sample size.

ETHICAL APPROVAL:
The study protocol was authorised and approved by the Ethical Review Committee of Jiulongpo District Hospital of Traditional Chinese Medicine in Chongqing.

PATIENTS' CONSENT:
All patients gave their informed permission.

COMPETING INTEREST:
The authors made it clear that they had no competing interest.

AUTHORS' CONTRIBUTION:
YK: Study concept, design, data maintenance, and first draft critical review.
JL, YS, YW: Data acquisition, checking, and statistics.
YS: Study design and compilation of results.
HC: Study design and statistical analysis.
DW: Interpretation of results and final approval of the manuscript.
All the authors have approved the final version of the manuscript to be published.

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