Relationship Between Homocysteine Level and Acute Kidney Injury in Patients with Acute Ischemic Stroke: A Postprint

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Abstract

Background Acute ischemic stroke (AIS) is the second leading cause of death globally after coronary heart disease. Acute kidney injury (AKI) represents one of the more severe complications following AIS. Homocysteine (Hcy) may be an important factor contributing to renal injury and accelerating deterioration of renal function. However, current research on the relationship between Hcy and AKI remains limited, particularly among AIS patients. Objective To investigate the association between Hcy levels and AKI occurrence in AIS patients, and to provide novel insights for the prevention and treatment of AKI in this population. Methods A total of 1202 AIS patients admitted to the Department of Neurology at the Second Hospital of Tianjin Medical University between January 2018 and April 2021 were enrolled. Patients were stratified into normal Hcy, mild hyperhomocysteinemia (HHcy), and moderate-to-severe HHcy groups based on plasma Hcy levels. Multivariate logistic regression was employed to analyze the impact of Hcy as both a continuous and categorical variable on post-AIS AKI development. Subgroup analysis was conducted to explore the Hcy-AKI relationship across different subpopulations. Restricted cubic spline regression was utilized to investigate the non-linear relationship between Hcy and AKI. Results Among 1202 AIS patients, 150 (12.48%) developed AKI. Multivariate logistic regression demonstrated that after adjusting for confounding variables, each 1 mol/L increase in Hcy was associated with a 1.035-fold increase in the risk of AKI following AIS (95%CI: 1.019~1.052). Compared with the normal Hcy reference group, the risk of AKI increased by 1.770-fold (95%CI: 1.150~2.724) and 2.927-fold (95%CI: 1.671~5.126) for mild and moderate-to-severe HHcy patients, respectively. Subgroup analysis revealed that whether as a categorical or continuous variable, the effect of Hcy on AKI occurrence was more pronounced in patients with histories of hypertension, diabetes, and stroke

than in those without underlying comorbidities. Restricted cubic spline regression indicated a non-linear association between Hcy and AKI risk, manifesting as an upward convex curve. Conclusion In AIS patients, Hcy constitutes a risk factor for AKI both as a continuous and categorical variable. Monitoring Hcy levels facilitates early identification and prevention of AKI, thereby improving patient prognosis.

Full Text

Preamble

The Relationship Between Homocysteine Level and Acute Kidney Injury in Patients with Acute Ischemic Stroke

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Abstract

Background: Acute ischemic stroke (AIS) is the second leading cause of death worldwide after coronary heart disease. Acute kidney injury (AKI) represents one of the most serious complications following AIS, and homocysteine (Hcy) may be an important factor contributing to renal injury and accelerated deterioration of renal function. However, few studies have examined the relationship between Hcy and AKI, particularly in AIS patients.

Objective: To investigate the association between plasma Hcy levels and AKI occurrence in AIS patients, and to provide novel insights for AKI prevention and treatment in this population.

Methods: We enrolled 1,202 AIS patients admitted to the Department of Neurology at the Second Hospital of Tianjin Medical University between January 2018 and April 2021. Patients were stratified into normal Hcy, mild hyperhomocysteinemia (HHcy), and moderate-to-severe HHcy groups based on plasma Hcy levels. Multivariate logistic regression was used to analyze the effect of Hcy on post-AIS AKI both as a continuous and categorical variable. Subgroup analyses explored the Hcy-AKI relationship across different populations, and

restricted cubic spline regression was employed to examine potential non-linear associations.

Results: Among 1,202 AIS patients, 150 (12.48%) developed AKI. Multivariate logistic regression revealed that after adjusting for confounders, each 1 mol/L increase in Hcy was associated with a 1.035-fold increase in AKI risk (95% CI: 1.019–1.052). Compared with normal Hcy patients, those with mild and moderate-to-severe HHcy exhibited 1.770-fold (95% CI: 1.150–2.724) and 2.927-fold (95% CI: 1.671–5.126) increases in AKI risk, respectively. Subgroup analyses showed that Hcy exerted a more pronounced effect on AKI risk in patients with hypertension, diabetes, or stroke history than in those without these comorbidities, whether analyzed as a continuous or categorical variable. Restricted cubic spline regression demonstrated a non-linear, convex curve relationship between Hcy and AKI risk.

Conclusion: Hcy is a significant risk factor for AKI in AIS patients, irrespective of whether it is treated as a continuous or categorical variable. Monitoring Hcy levels may facilitate early identification and prevention of AKI, thereby improving patient outcomes.

Keywords: Acute ischemic stroke; Homocysteine; Acute kidney injury

Introduction

Acute ischemic stroke (AIS) is the second leading cause of death globally and a major source of disability and reduced life expectancy, imposing substantial health, economic, and social burdens on patients and society. The brain and kidneys share important physiological characteristics in terms of anatomy, vascular regulation, and hemodynamics, and interact through central autonomic neural networks, sympathetic nervous systems, and inflammatory immune responses. Acute kidney injury (AKI) is a serious yet often underrecognized complication of AIS, with approximately 11.60% of ischemic stroke patients developing AKI, which significantly increases both short- and long-term morbidity and mortality. Identifying and controlling risk factors for post-AIS AKI is therefore crucial for improving patient outcomes.

Homocysteine (Hcy) is a naturally occurring derivative of methionine and cysteine that plays important roles in various biochemical reactions. Elevated Hcy levels have been established as an independent risk factor for multiple diseases, including stroke, coronary heart disease, and peripheral vascular disease. Research indicates that Hcy elevation is associated with glomerulosclerosis and renal interstitial fibrosis, and population studies have observed an inverse correlation between plasma Hcy levels and estimated glomerular filtration rate. The prevalence of hyperhomocysteinemia (HHcy) is markedly higher in patients with chronic kidney disease than in healthy individuals, suggesting that Hcy may be a key contributor to renal injury and accelerated renal function decline. How-

ever, few studies have specifically examined the relationship between Hcy and AKI, particularly in the AIS population. This study aims to investigate the association between plasma Hcy levels and AKI risk in AIS patients to identify high-risk individuals and inform novel preventive strategies.

Methods

1.1 Study Population

We retrospectively enrolled AIS patients admitted to the Department of Neurology at the Second Hospital of Tianjin Medical University between January 2018 and April 2021. Inclusion criteria were: (1) age $\geq$ 18 years; (2) diagnosis of acute ischemic stroke according to the *Chinese Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke 2018* and confirmed by experienced neurologists via CT/MRI; (3) completion of plasma Hcy and renal function tests within 2 days of admission; and (4) at least one follow-up renal function test within 7 days of admission. Exclusion criteria included: (1) pre-existing chronic renal insufficiency, nephritis, or kidney disease; (2) malignancy; (3) psychiatric disorders or severe cognitive impairment; (4) stroke caused by trauma or iatrogenic factors; and (5) substantially incomplete medical records. Based on these criteria, 1,202 AIS patients were included in the final analysis. The study was approved by the hospital ethics committee, and informed consent was obtained from all participants or their families.

1.2 Data Collection and Definitions

1.2.1 Clinical Data Collection Clinical data were extracted from the hospital electronic medical record system, including demographic information (name, sex, age, contact information), medical history (hypertension, diabetes, stroke, coronary heart disease, atrial fibrillation), lifestyle factors (smoking, alcohol consumption), admission NIHSS score, TOAST classification of ischemic stroke, and laboratory results. After at least 6 hours of fasting, venous blood samples were collected to measure total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), blood urea nitrogen (BUN), serum creatinine (Cr), uric acid (UA), plasma Hcy, and follow-up Cr levels within 7 days of admission. Serum Cr was measured using the enzyme-coupled rate method, and Hcy was determined by enzyme immunoassay.

1.2.2 AKI Definition AKI was diagnosed according to the 2021 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines: (1) increase in serum Cr $>$ 26.5 mol/L (0.3 mg/dL) within 48 hours; (2) increase in serum Cr to $\geq$ 1.5 times baseline within 7 days; or (3) urine output $<$ 0.5 mL/kg/h for $>$ 6 hours. Patients meeting any of these criteria were classified into the AKI group; all others comprised the non-AKI group.

1.2.3 Statistical Analysis Data analysis was performed using SPSS 25.0, Stata 15.0, and GraphPad Prism 9.0. Normality of continuous variables was assessed using the Shapiro-Wilk test. Normally distributed data are presented as mean ± standard deviation (± s) and compared using t-tests; categorical data are expressed as frequencies and percentages (n, %) and compared using ^{2} tests. To examine the Hcy-AKI relationship, Hcy was analyzed both as a continuous and categorical variable. Based on the *Expert Consensus on the Diagnosis, Treatment, and Prevention of Hyperhomocysteinemia*, patients were stratified into three groups: normal Hcy (≤ 15 mol/L), mild HHcy (15–30 mol/L), and moderate-to-severe HHcy (> 30 mol/L). Variables with $P < 0.1$ in univariate analysis were included in multivariate models. Multivariate binary logistic regression assessed the independent effect of Hcy on AKI risk. Subgroup analyses were conducted by age (< 60, 60–< 75, ≥ 75 years), sex, hypertension, diabetes, coronary heart disease, stroke history, stroke type, and stroke severity (mild: NIHSS ≤ 4; moderate: NIHSS 5–< 15; moderate-to-severe: NIHSS 15–20; severe: NIHSS ≥ 21). Restricted cubic spline regression with four knots (0.25, 0.50, 0.75, 0.95) was used to explore non-linear relationships. Statistical significance was defined as two-sided $P < 0.05$.

Results

2.1 Baseline Clinical Characteristics

The study included 1,202 AIS patients (745 men [61.98%] and 457 women [38.02%]; mean age 71.04 ± 11.68 years). AKI occurred in 150 patients (12.48%). Compared with the non-AKI group, the AKI group was older ($P = 0.004$), had a higher proportion of women ($P = 0.031$), and higher admission NIHSS scores ($P < 0.001$). Regarding stroke subtypes, the AKI group had higher rates of large-artery atherosclerosis (36.67%) and cardioembolism (21.33%), while the non-AKI group had more small-artery occlusion (44.77%; $P < 0.001$). No significant differences were observed in medical history (hypertension, diabetes, coronary heart disease, atrial fibrillation, stroke history) between groups ($P > 0.05$) . Laboratory results showed that admission Hcy, BUN, and UA levels were significantly higher in the AKI group ($P < 0.001$), while other parameters did not differ significantly .

2.2 Logistic Regression Analysis of Baseline Hcy and AKI

Univariate logistic regression identified sex, age, alcohol consumption, stroke type, admission NIHSS score, Hcy, BUN, and UA as factors associated with post-AIS AKI ($P < 0.1$) .

Multivariate logistic regression revealed that in Model 1 (unadjusted), each 1 mol/L increase in Hcy as a continuous variable was associated with a 4.4% increase in AKI risk (OR = 1.044; 95% CI: 1.030–1.059). As a categorical variable, mild and moderate-to-severe HHcy were associated with 2.487-fold (95% CI: 1.681–3.679) and 4.021-fold (95% CI: 2.414–6.699) increases in AKI

risk, respectively, compared with normal Hcy. Model 2 (adjusted for age and sex) yielded similar results, confirming Hcy as an independent AKI risk factor both as continuous and categorical variables ($P < 0.05$). Model 3 (adjusted for all significant univariate factors) showed that each 1 mol/L increase in Hcy increased AKI risk by 1.035-fold (95% CI: 1.019–1.052). Compared with normal Hcy, mild and moderate-to-severe HHcy increased AKI risk by 1.770-fold (95% CI: 1.150–2.724) and 2.927-fold (95% CI: 1.671–5.126), respectively .

2.3 Subgroup Analysis Results

When Hcy was analyzed as a continuous variable, AKI risk increased with baseline Hcy levels in female patients, those aged ≥ 75 years, those with hypertension, diabetes, moderate-to-severe stroke at admission, and those with LAA, SAA, or CE stroke subtypes ($P < 0.05$). Hcy remained a significant AKI risk factor regardless of coronary heart disease or stroke history, with more pronounced effects in patients with these comorbidities [Figure 1: see original paper].

As a categorical variable, mild HHcy increased AKI risk compared with normal Hcy in male patients, those < 75 years, those with hypertension, diabetes, stroke history, without coronary heart disease, and with mild stroke ($P < 0.05$). This effect was most pronounced in patients < 60 years (OR = 4.615; 95% CI: 1.544-13.798; $P = 0.006$) [Figure 2: see original paper].

Moderate-to-severe HHcy increased AKI risk compared with normal Hcy in female patients, those with hypertension or diabetes, across all age groups, regardless of coronary heart disease or stroke history, and in those with moderate or moderate-to-severe stroke and LAA, SAA, or CE subtypes ($P < 0.05$). The highest risk increase was observed in patients with diabetes (OR = 10.501; 95% CI: 3.634–30.345; $P < 0.001$). The effect was more pronounced in patients with coronary heart disease or stroke history compared with those without [Figure 3: see original paper].

2.4 Non-Linear Relationship Between Baseline Hcy and AKI

Restricted cubic spline regression revealed a non-linear, convex relationship between Hcy and AKI risk. When admission Hcy was < 17 mmol/L, AKI risk increased rapidly with rising Hcy levels; when Hcy ≥ 17 mmol/L, the risk increase plateaued [Figure 4: see original paper].

Discussion

Hcy is a non-essential sulfur-containing α-amino acid formed from methionine demethylation. Under normal conditions, Hcy is metabolized back to methionine or cysteine via the methionine cycle, methylation, and transsulfuration pathways with the help of B vitamins, maintaining low physiological concentrations. Environmental factors, genetic variations, and lifestyle habits can cause Hcy accumulation, leading to HHcy and various adverse health effects.

Elevated Hcy is associated with increased cardiovascular and cerebrovascular disease risk. A meta-analysis of 10 studies including 10,103 healthy individuals found that Hcy is an independent risk factor for coronary heart disease, with each 5 mol/L increase in Hcy associated with a 22% increase in coronary disease risk. Wu et al. demonstrated a dose-response relationship between Hcy and stroke risk, with each 1 mol/L increase raising stroke and ischemic stroke risk by 1.06-fold and 1.05-fold, respectively. Additionally, Hcy plays an important role in acute and chronic kidney injury. Cohen et al. analyzed 17,010 Israeli subjects and found that individuals with Hcy $\geq$ 15 mol/L had 7.43-fold higher risk of chronic kidney injury than those with normal Hcy. Our study demonstrates that Hcy is a significant AKI risk factor in AIS patients, whether analyzed as a continuous or categorical variable. Each 1 mol/L increase in Hcy increased post-AIS AKI risk by 1.035-fold; mild and moderate-to-severe HHcy increased AKI risk by 1.770-fold and 2.927-fold, respectively, compared with normal Hcy. Early monitoring and targeted intervention of plasma Hcy levels may prevent AKI, improve outcomes, and reduce healthcare burden.

Our subgroup analyses revealed that Hcy had more pronounced effects on AKI risk in patients with hypertension, diabetes, or stroke history, suggesting that Hcy may synergistically amplify renal damage from underlying risk factors. This aligns with Gao et al., who established HHcy models in hypertensive and non-hypertensive rats and found that while both HHcy groups and normal-Hcy hypertensive rats exhibited reduced glomerular filtration rate and renal structural damage, the hypertensive HHcy group showed the most severe impairment. This indicates that Hcy and hypertension may act synergistically to exacerbate renal injury. Therefore, patients with chronic comorbidities require more vigilant Hcy monitoring and intervention to mitigate renal damage and improve survival.

The mechanisms linking Hcy elevation to renal injury remain incompletely understood but may involve several pathways: (1) Renal tubular epithelial cells, which have high oxygen consumption and abundant mitochondria, are susceptible to Hcy-induced mitochondrial damage, swelling, fragmentation, and dysfunction, promoting renal cell apoptosis and injury progression; (2) Hcy accumulation may impair autophagy-mediated renal protection. Zhang et al. found that HHcy reduced expression of transcription factor EB, a key regulator of autophagy-related genes, thereby inhibiting autophagic activation, reducing renal clearance of toxic substances, and causing renal injury and homeostatic imbalance; (3) HHcy may inactivate protein kinase C-related endothelial nitric oxide synthase, reducing nitric oxide production, causing endothelial dysfunction, and impairing renal vascular tone regulation, thereby promoting renal injury; (4) Inflammation and oxidative stress play crucial roles in Hcy-mediated renal injury. Elevated Hcy activates NF- B, upregulating pro-inflammatory cytokines and downregulating anti-inflammatory cytokines, causing endothelial dysfunction and renal injury. Additionally, HHcy increases reactive oxygen species production, disrupts redox balance, enhances renal oxidative stress, and promotes glomerulosclerosis and tubulointerstitial lesions, triggering AKI. Further research is needed to elucidate these mechanisms to better prevent and

manage acute and chronic kidney injury.

This study has several limitations. First, as a single-center retrospective study requiring ≥ 2 renal function tests, selection bias may exist. Second, the limited number of moderate and severe HHcy patients necessitated combining them into a single moderate-to-severe HHcy group, which may affect results. Multi-center, large-sample prospective cohort studies are needed to confirm our findings.

In conclusion, Hcy is a significant risk factor for AKI in AIS patients, whether analyzed as a continuous or categorical variable, with more pronounced effects in those with chronic comorbidities. Monitoring Hcy levels may facilitate early AKI identification and prevention, thereby improving patient prognosis.

Author Contributions

WANG Xiaowen, LI Xin, and XIA Xiaoshuang conceptualized and designed the study and reviewed and edited the manuscript. WANG Xiaowen and XIAO Tongling managed data, performed formal analysis, and wrote the original draft. WANG Xiaowen, WANG Yi, and YANG Ying conducted the investigation. LI Xin acquired funding and provided resources.

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Note: Figure translations are in progress. See original paper for figures.