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***Observational Study***

**Serum calcium, albumin, globulin and matrix metalloproteinase-9 levels in acute cerebral infarction patients**

Zhong TT *et al*. Serum calcium, albumin, globulin and MMP-9 for HT

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**Abstract**

BACKGROUND

Hemorrhagic transformation (HT) is a common complication in patients with cerebral infarction. However, its pathogenesis is poorly understood. The knowledge of factors that may increase risk for HT may help in improving the safety of thrombolytic therapy.

AIM

To investigate the predictive value of serum calcium, albumin, globulin and matrix metalloproteinase-9 (MMP-9) levels for HT after intravenous thrombolysis (IVT) in patients with acute cerebral infarction.

METHODS

Five hundred patients with acute cerebral infarction who received IVT with alteplase within 4.5 h after the onset of disease between January 2018 and January 2021 at our hospital were selected as the study subjects. They were divided into groups based on computed tomography scan results of the brain made within 36 h after thrombolysis. Forty patients with HT were enrolled in an observation group and 460 patients without HT were enrolled in a control group. Serum calcium, albumin, globulin and MMP-9 levels were compared between the two groups. Regression analysis was used to discuss the relationship between these indices and HT.

RESULTS

The previous history of hypertension, diabetes, atrial fibrillation, cerebrovascular diseases, smoking and alcohol intake were not associated with HT after IVT in patients with acute cerebral infarction (all *P* > 0.05). The National Institutes of Health stroke scale (NHISS) score was associated with HT after IVT in patients with acute cerebral infarction (*P* < 0.05). The serum calcium and albumin levels were lower in the observation group than in the control group (all *P* < 0.05). The levels of globulin and MMP-9 were significantly higher in the observation group than in the control group (all *P* < 0.05). Logistic regression analysis showed that NHISS score, serum calcium, albumin, globulins and MMP-9 were independent factors influencing the occurrence of HT following IVT in patients with cerebral infarction (*P* < 0.05).

CONCLUSION

Serum calcium, albumin, globulin and MMP-9 levels are risk factors for HT after IVT in patients with acute cerebral infarction. Moreover, NHISS score can be used as a predictor of post-thrombolytic HT.

**Key Words:** Stroke; Intravenous thrombolytic therapy; Intracranial hemorrhage; Blood calcium; Albumin; Globulin

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**Core Tip:** To better understand the pathogenesis of hemorrhagic transformation (HT) may help to avoid clinically relevant brain hemorrhages in patients with acute cerebral infarction treated with intravenous thrombolysis (IVT). The present study assessed the relationship between factors, including serum calcium, albumin, globulin and matrix metalloproteinase-9 levels (MMP-9), and the occurrence of HT following IVT in patients with acute cerebral infarction by comparing clinical and laboratory features between the HT and non-HT groups. National Institutes of Health stroke scale score, serum calcium, albumin, globulins and MMP-9 were independent factors influencing occurrence of HT in patients with cerebral infarction treated with IVT-recombinant tissue plasminogen activator.

**INTRODUCTION**

Acute cerebral infarction is one of the diseases that seriously threatens human health nowadays. Its high incidence, mortality, disability and recurrence cause heavy burden to families and society[1,2]. In China, the reported 1-mo case fatality rate after cerebral infarction was 2.3% to 3.2% and the 3-mo case fatality rate was 9% to 9.6%; the mortality/disability rate was 34.5% to 37.1% in inpatients with acute cerebral infarction[3,4]. Intravenous thrombolysis (IVT) remains the most convenient and acute therapy for cerebral infarction. However, thrombolysis is a two-edged sword and may cause a certain risk.

The primary complications of IVT include hemorrhagic transformation (HT), reperfusion injury and vascular occlusion. Among them, HT is the most dangerous as it directly affects the prognosis of patients with acute cerebral infarction[5,6]. Therefore, it is very important to predict the occurrence of HT and identify those patients who are prone to hemorrhage to improve the safety of thrombolysis and benefit patients as greatly as possible[7]. The present study discussed the relationship between serum calcium, albumin, globulin and matrix metalloproteinase-9 (MMP-9) levels and the occurrence of HT after IVT, and the association between these factors and the early prognosis to reduce the risk for HT after IVT under early intervention by predicting the factors influencing its occurrence and prognosis in patients with acute cerebral infarction.

**MATERIALS AND METHODS**

***General information***

A total of 500 patients who received IVT with alteplase within 4.5 h after the onset of acute cerebral infarction at our hospital were selected as the research subjects between January 2018 and January 2021. They were divided into groups based on computed tomography (CT) scan results of the brain within 36 h after thrombolysis. Forty patients with HT were enrolled in an observation group and 460 patients without HT were enrolled in a control group. Serum calcium, albumin, globulin and MMP-9 levels were compared between the two groups. All patients met the diagnostic criteria for acute cerebral infarction of Chinese Guidelines for Diagnosis and Treatment of Acute Ischemic Stroke 2018[1]. Inclusion criteria were as follows: (1) aged 18 to 80 years; (2) acute cerebral infarction onset time within 4.5 h; (3) intracranial hemorrhage confirmed by CT scan and acute large area cerebral infarction demonstrated by imaging; and (4) patients and patients’ family members having signed the informed consent form. Exclusion criteria were as follows[2]: (1) patients with cerebral hemorrhage confirmed by CT scan with symptoms suggesting the presence of subarachnoid hemorrhage; (2) patients who had previous history of brain arteriovenous malformation, intracranial tumor, brain aneurysm (ruptured or not ruptured); patients who had history of cerebral infarction, cerebral hemorrhage, traumatic brain injury or myocardial infarction except old lacunar infarct without symptoms of neurological deficit in the last 3 mo; patients with gastrointestinal hemorrhage or urinary hemorrhage in the last 3 mo; patients who underwent major surgery in the past 2 wk; patients who had vascular complications arising from hemostasis after arterial puncture; (3) patients with severe heart, liver and kidney dysfunction; (4) patients with active bleeding or trauma (fracture); (5) patients with systolic pressure > 180 mmHg or diastolic pressure > 100 mmHg before thrombolysis; (6) patients with platelet count below 100 × 109/L, blood glucose < 2.7 or > 22.2 mmol/L; (7) patients who received heparin therapy with prolonged activated partial thromboplastin time (APTT) or orally took anticoagulants with international normalized ratio (INR) > 1.5; (8) pregnant women; and (9) patients who refused to cooperate with the study.

***Treatment methods***

All patients received alteplase, an intravenously administered form of recombinant tissue plasminogen activator (rt-PA) (Cat. # S20160055, 20 or 50 mg powder/vial; Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany). For thrombolytic therapy, patients enrolled received rt-PA with the overall dose of 0.9 mg/kg (maximum dose of 90 mg) with 10% of total dose was administered *via* IV within 1 min and the remaining 90% of total dose administered *via* IV drip within 1 h. CT examination of the head was performed at 36 h after thrombolysis.

***Measures***

First, general information for patients were collected, including sex, age, history of hypertension, diabetes, atrial fibrillation, cerebrovascular disease, smoking and alcohol intake[8,9]. Second, neurological deficits were assessed using the National Institutes of Health stroke scale (NIHSS) score[10,11]. Serum calcium, albumin, globulin and MMP-9 levels were measured. An automatic biochemistry analyzer was used to measure serum calcium, albumin, globulin and MMP-9 levels before thrombolysis[12]. Enzyme-linked immunosorbent assay was used to test MMP-9 levels after thrombolysis.

***Statistical analysis***

SPSS18.0 software was used for all statistical analyses. Measurement data was expressed as mean ± standard deviation and inter-group difference was compared using Student’s *t* test. Statistical relationship between the two variables was determined using Spearman’s rank correlation coefficient. Enumeration data were expressed as percentages. Univariate analysis was constructed using *χ2* test. Logistic analysis was performed for measures with statistically significant difference, which were also used as independent variables. *P* < 0.05 represented a statistically significant difference.

**RESULTS**

On univariate analysis, there is no significant relationship between the previous history of hypertension, diabetes, atrial fibrillation, cerebrovascular diseases, smoking and alcohol consumption and the occurrence of HT after IVT in patients with acute cerebral infarction (all *P* > 0.05). However, NHISS score was significantly associated with hemorrhage after IVT in acute cerebral infarction (*P* < 0.05; Table 1).

Serum calcium and albumin levels were lower in the observation group than in the control group (*P* < 0.05) and globulin and MMP-9 levels were higher in the observation group than in the control group (*P* < 0.05; Table 2).

Multiple logistic regression analysis revealed that NHISS score, and serum albumin, globulin and MMP-9 levels were independent factors influencing the occurrence of HT after IVT in patients with acute cerebral infarction (*P* < 0.05; Table 3).

**DISCUSSION**

The incidence of cerebellar stroke is high in China. It is very important to study the therapies in the treatment of cerebellar stroke. Early IVT may improve prognosis and reduce disability and mortality[12]. However, IVT injection may cause severe complications and HT is one of the major complications to IVT. Therefore, to predict the risk of HT ahead of time is vital to ensure improvement in prognosis. Currently, studies on the prediction of HT following IVT in patients with acute cerebral infarction have focused on prediction models and imaging and biological markers but no specific marker had been identified[13-15]. In terms of participants, studies of prediction models for the prognosis of HT were mainly constructed with populations abroad and data on domestic population were limited. With regard to imaging factors, imaging characteristics of head susceptibility weighted imaging and CT scan can be used as predictive factors for the risk of HT. Nevertheless, the long duration of imaging tests and discrepancies among reading doctors may affect the safety of thrombolysis.

Fortunately, effective biologic markers could make up for the deficiency and may have potential to become rapid and accurate predictive factors for HT and prognosis. At present, some studies have found that S100B, fibronectin and MMP-9 were closely associated with HT after IVT but there has been some controversy about it; others have reported discovering that low serum calcium levels could become an independent factor for the prediction of the occurrence of HT after IVT, considering that serum calcium is an important factor involved in the coagulation process and low calcium levels may lead to vasoconstriction in ischemic zones followed by increased blood pressure induced by hemorrhage[16,17]. Moreover, another study hinted that elevation in globulins and reduction in albumin represented risk factors for HT after IVT based on the mechanism that these factors are involved in inflammation and oxidative stress[7].

Testing of the above-mentioned markers is easy and is widely used in the clinical practice and its domestic application needs to be further investigated. Towards this end, the present study surveyed serum calcium, albumin, globulin and MMP-9 levels and analyzed the predictive value of these factors for HT after IVT to explore the role in mechanisms of HT after IVT and the potential to become therapeutic targets for HT after IVT, ultimately to reduce the incidence of HT and improve the efficacy of thrombolysis.

In the studies of factors for the prediction of HT after IVT in patients with acute cerebral infarction, effective biologic markers may become rapid and widely used predictive markers. MMP-9 has been confirmed to be associated with destruction of the blood-brain barrier. In physiological states, the expression of MMP-9 was low in brain microvascular endothelial cells. When acute cerebral infarction occurred, the elevated MMP-9 level may further disrupt the blood-brain barrier and aggravate existing injuries. Investigation has established that MMP-9 was associated with acute cerebral infarction and subsequent HT. However, data on the association between HT after IVT and MMP-9 were limited[18]. After the increasing use of rt-PA for cerebral infarction in the clinical practice, various studies confirmed that rt-PA may induce expression of MMP-9, destruct blood-brain barrier and increase the risk for HT after IVT[19-21].

Serum albumin performs the majority of antioxidation in the body, inhibits apoptosis in endothelial cells and alleviates cellular oxidative stress. Decreased albumin levels may independently hint at the occurrence of HT after IVT. High globulin level was one of the risk factors for HT after IVT, which was mainly due to liver-derived pro-inflammatory cytokines and synthesized acute-phase response. The present study indicated that NHISS score, serum calcium, albumin, globulin and MMP-9 levels were independent factors influencing the occurrence of HT after IVT in patients with acute cerebral infarction.

**CONCLUSION**

To sum up, NHISS score, serum calcium, albumin, globulin and MMP-9 levels were identified as risk factors for HT after IVT, which can be used as predictive factors for HT after IVT. Functioning as a guide to clinical decision-making, these factors may help improve prognosis, reduce the length of hospital stay, decrease hospital expenses and lighten the burden of patients to a great extent.

**ARTICLE HIGHLIGHTS**

***Research background***

Intracranial hemorrhage is common after intravenous thrombolysis (IVT) in acute ischemic stroke. Increasing studies have focused on risk and predictive factors for hemorrhagic transformation (HT).

***Research motivation***

To understand the pathogenesis of HT and provide interventions that may help to increase the safety of IVT.

***Research objectives***

This study aimed to investigate the relationship of serum calcium, albumin, globulin and matrix metalloproteinase-9 (MMP-9) levels and HT after IVT.

***Research methods***

The study investigated patients receiving recombinant tissue plasminogen activator (rt-PA) for acute cerebral infarction. Demographic, clinical and laboratory information, HT and functional outcomes were compared between patients with HT and those without HT.

***Research results***

The study demonstrates that serum calcium and albumin levels were decreased in the HT group compared with the non-HT group and globulin and MMP-9 levels were increased in the HT group. This indicates that the low serum calcium and albumin levels and high globulin and MMP-9 levels are associated with the occurrence of HT following IVT in patients with cerebral infarction.

***Research conclusions***

National Institutes of Health stroke scale score, serum calcium, albumin, globulins and MMP-9 were independent factors influencing the occurrence of HT in patients with cerebral infarction treated with IVT-rt-PA.

***Research perspectives***

Further studies investigating additional potential factors are needed to confirm these results in patients treated with IVT-rt-PA to decrease the occurrence HT after IV rt-PA.

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**Footnotes**

**Institutional review board statement:** The study was reviewed and approved by the First Affiliated Hospital of Hebei North University Institutional Review Board.

**Informed consent statement:** All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

**Conflict-of-interest statement:** The authors declare having no conflicts of interest.

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**Table 1 Univariate analysis of hemorrhagic transformation after intravenous thrombolysis in patients with acute cerebral infarction**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Measure** | **Observation group, *n* = 40** | **Control group, *n* = 460** | ***t/χ2* value** | ***P* value** |
| Sex (Male/Female) | 23/17 | 287/173 | 1.230 | 0.154 |
| Age in yr (Mean ± SD) | 76.21 ± 10.33 | 75.87 ± 10.08 | 0.922 | 0.346 |
| History of hypertension (Yes/No) | 25/15 | 249/211 | 2.221 | 0.098 |
| History of diabetes (Yes/No) | 13/27 | 142/318 | 1.255 | 0.123 |
| History of atrial fibrillation (Yes/No) | 14/26 | 115/345 | 1.873 | 0.101 |
| History of cerebrovascular diseases (Yes/No) | 11/29 | 83/377 | 0.812 | 0.401 |
| History of smoking (Yes/No) | 21/19 | 253/207 | 2.437 | 0.086 |
| History of alcohol consumption (Yes/No) | 18/22 | 193/267 | 1.167 | 0.172 |
| NHISS score (Mean ± SD) | 15.21 ± 2.18 | 11.42 ± 2.65 | 17.321 | 0.001 |

NHISS: National Institutes of Health Stroke scale; SD: Standard deviation.

**Table 2 Comparison of serum calcium, albumin globulin and matrix metalloproteinase-9 between the two groups, mean ± SD**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Group** | ***n*** | **Calcium (mmol/L)** | **Albumin (g/L)** | **Globulin (g/L)** | **MMP-9 (ng/mL)** |
| Observation group | 40 | 2.09 ± 0.16 | 38.58 ± 5.11 | 29.32 ± 3.45 | 119.21 ± 12.65 |
| Control group | 460 | 2.29 ± 0.18 | 47.12 ± 6.43 | 25.61 ± 4.09 | 75.64 ± 10.05 |
| *t* value |  | 4.875 | 11.243 | 6.211 | 19.214 |
| *P* value |  | 0.043 | 0.001 | 0.033 | 0.001 |

MMP-9: Matrix metalloproteinase-9; SD: Standard deviation.

**Table 3 Multiple logistic regression analysis of hemorrhagic transformation after intravenous thrombolysis in patients with acute cerebral infarction**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameters** | **Regression coefficient** | **Wald test statistic** | **SD** | **OR value** | ***P* value** |
| NHISS score | 0.846 | 7.643 | 0.063 | 1.235 | 0.014 |
| Calcium | 0.758 | 6.557 | 0.054 | 1.322 | 0.035 |
| Albumin | 0.769 | 7.584 | 0.087 | 1.046 | 0.023 |
| Globulin | 0.772 | 6.810 | 0.071 | 1.135 | 0.033 |
| MMP-9 | 0.821 | 7.592 | 0.063 | 1.356 | 0.016 |

NHISS: National Institutes of Health stroke scale; MMP-9: Matrix metalloproteinase-9; OR: Odds ratio; SD: Standard deviation.



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