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ORIGINAL RESEARCH

Plasma Homocysteine (Hcy) Concentration Functions as a Predictive Biomarker of SPECT-Evaluated Post-Ischemic Hyperperfusion in Acute Ischemic Stroke

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Introduction: Homocysteine (Hcy) concentration has been reported to be associated with ischemic stroke. In this study, we aimed to investigate the potential of plasma Hcy in the prediction of post-ischemic hyperperfusion in AIS patients, which was diagnosed with the single-photon emission computed tomography (SPECT) method.

Methods: A total of 112 ischemic stroke patients were recruited in this study. According to whether the patients were subjected to post-ischemic hyperperfusion, all recruited subjects were divided into a post-ischemic hyperperfusion (+) group (N=48) and post-ischemic hyperperfusion (-) group (N=64). The basic demographical data, clinicopathological data and laboratory biochemical data were collected and compared. Level of homocysteine (Hcy) and cystatin-C (Cys-C) and their potential as predictive biomarker are also investigated.

Results: No significant differences were spotted between the post-ischemic hyperperfusion group (+) and post-ischemic hyperperfusion (-) group in respect to the basic demographical and clinicopathological data. And the serum Hcy levels were lower in the post-ischemic hyperperfusion (+) group. Moreover, ROC analysis indicated significant relationships between Hcy levels and the onset of post-ischemic hyperperfusion.

Conclusion: In conclusion, we validated that the plasma Hcy concentration can be used as a predictive biomarker of SPECT-evaluated post-ischemic hyperperfusion in patients suffering from acute ischemic stroke.

Keywords: homocysteine, cystatin-C, SPECT, ischemic, hyperperfusion

Introduction

In a previous report which investigated the prevalence of stroke in China, an evident increase from 2.28% in 2013 to 2.58% in 2019 was found, presenting higher percentage of male patient than the female patients.[1] Moreover, when investigating the data in a world wide scale, it was found that there were 12.2 million incident stroke cases and 101 million prevalent stroke cases in 2019, among which 6.55 million patients died of stroke.[2] Apart from the high death rate of 11.6% which contributed to its seconding ranking among other causes of mortality, stroke is also the second leading cause of disability.[2,3] And it is noteworthy that, among the incident stroke cases worldwide, over 60% cases were reported to be ischemic stroke cases.[2] Moreover, as Muhammad et al stated in their investigations, the risk factors associated with ischemic stroke may include age, sex, waist circumference, smoking habit, diabetes mellitus, body-mass index (BIM), systolic blood pressure, high fasting plasma glucose, total leukocyte count and neutrophil count.[4]

Transient ischemic attack (TIA) is defined as a transient and unexpected neurologic disorder induced by focal brain hypoperfusion or ischemia with no acute infarction spotted by brain imaging.[5–7] In contrast, acute ischemic stroke (AIS), commonly defined as ischemia with irreversible cerebral infarction, is a result of the absence of prompt arterial flow restoration to

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brain tissues.[5,8] Moreover, the consequences of AIS are divergent among different individuals, ie, AIS may either exhibit no effect on cerebral tissues, or results in a complete infarction.[9] Previous investigations acknowledged that these varied consequences are mainly associated with several key factors including the timing of recanalization, the efficiency of tissue reperfusion and the depth of hypoperfusion after middle cerebral artery occlusion.[10,11]

In previous clinical reports, the percentage of patients with post-ischemic hyperperfusion may vary from 10% to 15% in respect to the differences in the timing of analysis and the method of analysis.[12–15] Moreover, the efficiency of post-ischemic hyperperfusion detection is also influenced by pathological factors. For example, not all patients can have recanalization, which is partial or absent in some patients. And hyperperfusion is also transient, which indicated highly possible failure of detection in one single examination.[13]

In in previous report which studied the association between high homocysteine (Hcy) level and the risk of stroke, Tu et al found that plasma Hcy levels higher than 15.0μmol/L were identified in approximately 26% residents in China, and the levels of Hcy were found to be associated with age, sex, smoking, and even diabetes status.[16] Moreover, high Hcy levels were identified to be associated with higher incidence of ischemic stroke.[17] Also, the increased level of Hcy may exert a deleterious effect in the control of ubiquitin-containing proteinaceous deposits accumulation and modulation within the ischemic injury,[18] which accordingly lead to the impaired circulation in the brain and hypoperfusion/transient ischemia, potentially acting as a triggering factor for dementia and Alzheimer's disease,[19] thus implying the potential relationship between high Hcy level and hypoperfusion.[20]

To detect the presence of cerebral infarction, various brain imaging methods are applied, which included techniques such as arterial spin labeling (ASL) perfusion, computed tomography (CT) perfusion, positron emission tomography (PET) and single photon emission computed tomography (SPECT).[21–24] It has been suggested brain perfusion SPECT could differentiate ischemic from peri-ictal psychoses, and could also help to predict the incidence of early stroke after a transient ischemic attack.[25] SPECT was also suggested as an evaluation method for the status of perfusion after reperfusion therapy,[26,27] and hyperperfusion may be visualized in 123I-IMP brain perfusion SPECT with the potential of overestimation.[28] In this study, we aimed to identify a predictive biomarker of post-ischemic hyperperfusion in AIS patients, especially post-ischemic hyperperfusion diagnosed with the SPECT.

Materials and Methods

Patient Enrollment and Data Collection

In this prospective study, a total of 112 patients suffering from ischemic stroke were enrolled. The evaluation by brain perfusion SPECT was performed by two neurologists with no further information about the individuals taking the assay. Patients who also suffered from renal failure, cirrhosis, coronary heart disease, malignancy, or other brain disease including brain tumor, Alzheimer's disease were excluded from this study. And the patients were divided into a post-ischemic hyperperfusion (+) group (N=48) and post-ischemic hyperperfusion (-) group (N=64). The onset of post-ischemic hyperperfusion was evaluated via brain perfusion SPECT which was performed using N-isopropyl-4-[^{123}I]iodoamphetamine (123I-IMP) as the radioisotope tracer for the brain perfusion within 10 days starting from the onset of brain infarction. Basic patient demographic and clinicopathologic data were collected and studied. Peripheral blood samples were collected from each patients before the treatment for subsequent analysis, and the status of ischemic hyperperfusion was recorded as well with 14 days following the treatment. The institutional ethical committee of Yangpu Hospital has approved this study (Approval ID: LL-2021-SCI-008). All protocols of this study were performed according to the latest version of Declaration of Helsinki. Written informed consent was obtained from the study participants prior to study commencement.

Enzyme-Linked Immunosorbent Assay (ELISA) Assay of Hcy and Cys-C Level

Concentrations of Hcy and Cys in plasma samples were measured using ELISA assay kits. Plasma samples were collected and centrifuged for the collection of supernatant to analyze the Hcy and Cys-C level. The assay kits used were Homocysteine Assay Kit (MAK354-1KT, Sigma-Aldrich, MI, US) and Human Cystatin C ELISA Kit (ab119589, Abcam, Cambridge, UK). All procedures were performed according to the instruction provided by the kit manufactures.

Statistical Analysis

Student's *t*-test was performed to compare the differences of the participants' basic demographical and clinicopathological data between different patient groups. Univariate analysis of baseline characteristics and clinical outcome were carried out to assess the association between the included parameters and the incidence of post-ischemic hyperperfusion. The receiver operating characteristic (ROC) analysis was performed to analyze the predictive value of Hcy and Cys-C concentrations by calculating the area under the curve (AUC). All analysis were performed with the SPSS 22.0. P value less than 0.005 was set us the level of statistical significance.

Results

Basic Demographical and Clinicopathological Data of All Participants

The basic demographical and clinicopathological data of all participants were collected and compared. As shown in Table 1, when comparing basic characteristics such as age, sex, medical histories, medication histories, smoking habit and drinking habit, no

Table 1 Basic Demographical and Clinicopathological Data of All Participants

Characteristics	Post-Ischemic Hyperperfusion (+) (N=48)	Post-Ischemic Hyperperfusion (-) (N=64)	*P* value
Age, years	64.5 ± 5.8	68.1 ± 13.2	0.0846
Male sex, n(%)	12 (25.0)	15 (23.4)	0.8453
Current smoking, n(%)	15 (31.3)	23 (35.9)	0.6125
Current alcoholism, n(%)	16 (33.3)	21 (32.8)	0.9558
Medical history			
Hypertension, n(%)	28 (58.3)	32 (50.0)	0.3856
Diabetes mellitus, n(%)	12 (25.0)	21 (32.8)	0.3723
Coronary artery disease, n(%)	6 (12.5)	11 (17.2)	0.4947
Atrial fibrillation, n(%)	8 (16.7)	13 (20.3)	0.6306
Previous stroke, n(%)	12 (25.0)	18 (28.1)	0.7151
Medication history			
Antihypertensive therapy before enrollment, n(%)	15 (31.3)	22 (34.4)	0.7312
Antidiabetic therapy before enrollment, n(%)	8 (16.7)	11 (17.2)	0.9447
Antiplatelet therapy before enrollment, n(%)	3 (6.3)	5 (7.8)	0.7616
Anticoagulant therapy before enrollment, n(%)	1 (2.1)	3 (4.7)	0.4660
Treatment in extent time-window 3–4.5 h, n(%)	16 (33.3)	26 (40.6)	0.4317
High homocysteine level ≥15 μmol/L, n(%)	12 (25.0)	33 (51.6)	P<0.005
Blood pressure before rt-PA administration			
Systolic blood pressure, mmHg	145.2 ± 15.1	149.3 ± 11.3	0.1030
Diastolic blood pressure, mmHg	85.6 ± 9.5	88.1 ± 10.5	0.4078
Stroke severity			
NIHSS before rt-PA administration	9.5 ± 3.2	8.9 ± 2.5	0.2676
NIHSS at 24 h after rt-PA administration	8.6 ± 2.3	8.8 ± 3.2	0.7140
DNT (min)	33.2 ± 5.4	34.1 ± 3.8	0.3028
ONT (min)	63.5 ± 8.4	65.1 ± 7.2	0.2811
Massive cerebral infarction, n(%)	6 (12.5)	12 (18.8)	0.3715
Stroke subtype			
TOAST, n(%)			
LAA	26 (54.2)	32 (50.0)	0.6612
SAO	2 (4.2)	5 (7.8)	0.4383
CE	13 (27.1)	13 (20.3)	0.4011
SOE	1 (2.1)	3 (4.7)	0.4660
SUE	8 (16.7)	11 (17.2)	0.9447

(*Continued*)

Table 1 (Continued).

Characteristics	Post-Ischemic Hyperperfusion (+) (N=48)	Post-Ischemic Hyperperfusion (-) (N=64)	*P* value
OCSP, n(%)			
TACI	3 (6.3)	3 (4.7)	0.7117
PACI	32 (66.6)	38 (59.4)	0.4381
POCI	8 (16.7)	11 (17.2)	0.9447
LACI	5 (10.4)	12 (18.7)	0.2273

significant differences were spotted between the post-ischemic hyperperfusion group (+) and post-ischemic hyperperfusion (-) group. However, the numbers of patients with high plasma Hcy level were significantly different in different patient groups ($P < 0.005$), thus suggesting high Hcy level being associated with the incidence of hyperperfusion. Therefore, most parameters except for the Hcy level listed in Table 1 can be excluded from the list of interfering factors which affected the incidence and prognosis of post-ischemic hyperperfusion.

Laboratory Biochemical Measurement and Hcy Level in All Participants

We also collected the plasma samples from all participants and performed laboratory biochemical measurements. As shown in Table 2, hematologic parameters such as blood glucose level, albumin level, ALT level, AST level, creatinine level, eGFR level, triglyceride level, total cholesterol level, LDL-C level, HDL-C level, hemoglobin level, platelet count, fibrinogen level, PT and APTT level were all comparable between the post-ischemic hyperperfusion group (+) and post-ischemic hyperperfusion (-) group.

Homocysteine Level Functions as a Predictor of Post-Ischemic Hyperperfusion

As shown in Figure 1A, the level of plasma Hcy was significantly higher in the post-ischemic hyperperfusion (-) group compared with the post-ischemic hyperperfusion (+) group. Meanwhile, the Cys-C concentration is also evaluated, presenting no evident differences between the two patient groups (Figure 1B). Therefore, we suggested that Hcy level could function as a predictive biomarker of SPECT-evaluated post-ischemic hyperperfusion in AIS patients.

Table 2 Laboratory Biochemical Measurement Results of All Participants

Characteristics	Post-Ischemic Hyperperfusion (+) (N=48)	Post-Ischemic Hyperperfusion (-) (N=64)	*P* value
Homocysteine level (μmol/L)	12.34 ± 3.1	14.57 ± 3.14	0.0003
Blood glucose (mmol/L)	6.6 ± 0.7	6.5 ± 1.3	0.6303
Albumin (g/L)	42.5 ± 5.1	44.3 ± 7.2	0.1429
ALT (U/L)	16.3 ± 2.5	15.7 ± 3.7	0.3346
AST (U/L)	21.5 ± 3.3	22.5 ± 4.1	0.1686
Creatinine (μmol/L)	84.2 ± 8.2	81.4 ± 10.2	0.1215
eGFR (mL/min/1.73 m2)	73.9 ± 6.5	75.3 ± 8.2	0.3317
Triglyceride (mmol/L)	1.2 ± 0.3	1.3 ± 0.4	0.1493
Total cholesterol (mmol/L)	4.7 ± 0.7	4.4 ± 0.9	0.0518
LDL-C (mmol/L)	2.7 ± 0.6	2.8 ± 0.4	0.2928
HDL-C (mmol/L)	1.3 ± 0.2	1.2 ± 0.4	0.1151
Hemoglobin (g/L)	148.3 ± 18.2	144.5 ± 12.8	0.1973
Platelet count (*10^9/L)	193.5 ± 21.5	197.5 ± 22.5	0.3448
Fibrinogen level (g/L)	3.1 ± 0.8	2.9 ± 0.6	0.1333
PT (s)	12.3 ± 0.7	12.1 ± 0.8	0.1703
APTT (s)	30.8 ± 2.6	31.2 ± 3.8	0.5319

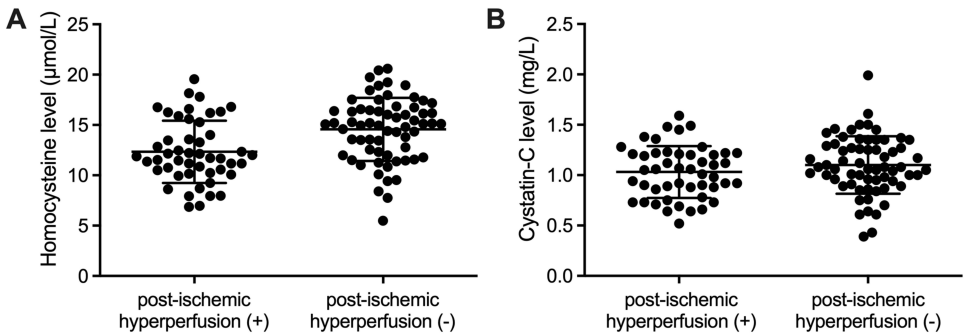

Figure 1 The level of Hyc and Cys-C in the post-ischemic hyperperfusion (+) group and the post-ischemic hyperperfusion (-) group; (**A**) The level of Hyc was lower in the post-ischemic hyperperfusion (+) group compared with the post-ischemic hyperperfusion (-) group; (**B**) The level of Cys-C was similar between the post-ischemic hyperperfusion (+) group and the post-ischemic hyperperfusion (-) group.

The ROC Analysis of Hcy and Cys-C Concentrations

Moreover, we also plotted the receiver operating characteristic (ROC) curve to evaluate the predictive ability of Hcy or Cys-C concentration for post-ischemic hyperperfusion. As shown in Figure 2A, the risk of post-ischemic hyperperfusion increased the level of Hcy elevated (AUC = 0.8358). In contrast, The AUC of Cys-C was only 0.5645, indicating the insignificant correlation between the onset of post-ischemic hyperperfusion and Cys-C concentrations (Figure 2B). Therefore, it can be suggested that the concentration of homocysteine functions as a predictive biomarker for post-ischemic hyperperfusion in acute ischemic stroke.

Univariate Logistic Regression Analysis of Participants Characteristics

To validate the potential role of Hcy in the prediction of post-ischemic hyperperfusion and screen out other possible hematological parameters for the prediction of post-ischemic hyperperfusion, univariate logistic regression analysis was performed for parameters which may influence the onset of post-ischemic hyperperfusion. As shown in Table 3, compared with other listed parameters, plasma Hcy level was demonstrated to be significantly associated with the incidence of post-ischemic hyperperfusion. Therefore, by studying a group of 112 AIS patients, we came to the conclusion that the level of plasma Hcy could function as a predictive biomarker of SPECT-evaluated hyperperfusion following the onset of AIS.

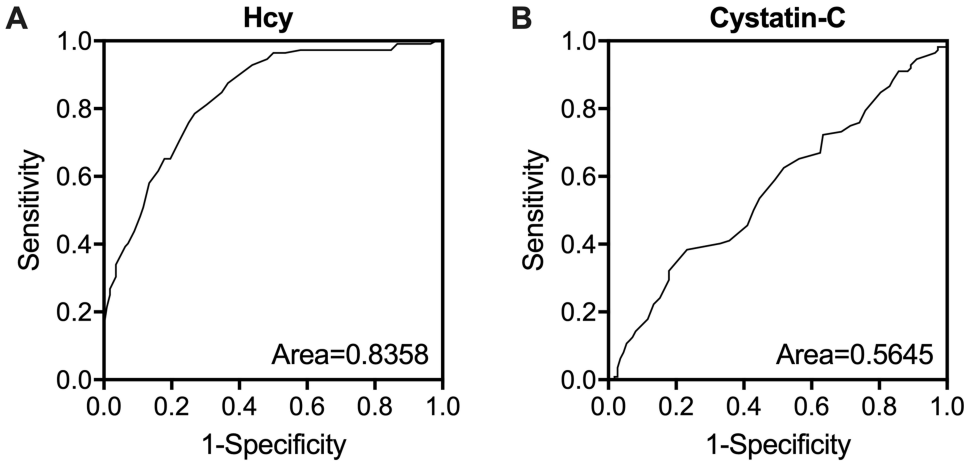

Figure 2 ROC curves of Hyc and Cys-C were plotted for patients in the post-ischemic hyperperfusion (+) group and the post-ischemic hyperperfusion (-) group; (**A**) AUC of Hyc was reduced in the post-ischemic hyperperfusion (+) group compared with the post-ischemic hyperperfusion (-) group; (**B**) AUC of Cys-C was similar between the post-ischemic hyperperfusion (+) group and the post-ischemic hyperperfusion (-) group.

Table 3 Univariate Analysis of Characteristics of All Participants

Exposure	OR 95% CI	*P* value
Homocysteine, μmol/L	1.07 (0.88, 1.28)	0.829
Gender		
Male	1.12 (0.54, 2.28)	
Female	Reference	0.414
Age, year	1.03 (0.95, 1.25)	0.080
Onset to treatment, min	1.02 (0.89, 1.51)	0.491

Discussion

Homocysteine (Hcy) has been reported to participate in various pathological mechanisms. For example, the up-regulation of Hcy level may induce consequences such as neurotoxicity,[29] endothelial dysfunction[30,31] and thrombosis formation.[32,33] In a retrospective cohort study by Feng et al, the increased Hcy concentrations were found to be correlated with higher risk of stroke and cardiovascular diseases.[34,35] And several previous investigations also reported that the elevated Hcy level often indicated poor prognosis in AIS individuals, with higher incidence of AIS.[36–38] Besides, during the process AIS, the level cysteine (Cys) was also increased,[39,40] and Cys was reported to interact with Hcy in the "one carbon folate cycle".[41] The increased plasma total Hcy level was spotted after the onset of AIS, and total Hcy level has been recognized as an independent risk factor of ischemic stroke.[42] Moreover, some reports also made a statement that the high serum Hcy level is associated with higher hematoma volume.[43] In our study, we found that the low plasma Hcy level was associated with higher risk of post-ischemic hyperperfusion in acute ischemic stroke, which is consistent with previously acknowledged reports.

The cystatin family has been reported is as competitive inhibitors of cysteine proteinases, and cystatin C is recognized as an inhibitor with broader spectrum which is secreted into the extracellular fluid.[44] And cysteine could protect cells from oxidative damage by eliminating the hydrogen peroxide.[45] Moreover, a catabolic process in which Hcy could be converted into cysteine was reported.[46] Previous studies also suspected cysteine or cystatin could be involved in the pathological processes of cardiovascular diseases, although controversial conclusions were made, some researchers suggested no evident correlation were found between cysteine and cardiovascular death,[47] while other insisted that concentration of cysteine could function as a potential biomarker in some cardiovascular diseases.[48–50] Therefore, in this study, we also investigated the correlation between Cys-C concentration and the onset of ischemic hyperperfusion. However, unlike the plasma Hcy level which could function as a predictive indicator of ischemic hyperperfusion in AIS, no evidences were found to validate Cys-C concentration as a biomarker as well.

Meanwhile, although our study identified plasma Hcy concentration as a predictive biomarker of SPECT-evaluated post-ischemic hyperperfusion in AIS, some randomized controlled trials came to a controversial conclusion that Hcy may not be significantly associated with the risk of cardiovascular diseases.[51–54] This controversial results could be contributed to the possibility that high plasma Hcy level may lead to impaired vascular wall integrity and brain vascular permeability dysfunction, and these consequences further cause damage to elastic structures, brain arteriole basal layer and microvessels.[55]

Therefore, these controversial publications suggested restrictions of our study. The sample size of our study is relatively limited, and randomized study is lacking in our reports to consolidate our findings. Moreover, as all participants in this study were enrolled from the same institution, a selection bias may present to influence the accuracy of our study. Also, this study evaluated post-ischemic hyperperfusion based on the findings of SPECT, which may not effectively identified all positive results or resulted in false positives.

Identifying a plasma biomarker can help in the diagnosis and prognosis of hyperperfusion in acute ischemic stroke. And further study on this biomarker may help to identify the relationship between the biomarker and the extent and duration of hyperperfusion. Also, newly identified biomarkers can help to instruct the disease treatments and interventions, and monitor the status of patients after treatment.

Conclusions

In this study, we found that the concentration of homocysteine functions as a predictive biomarker of SPECT-evaluated post-ischemic hyperperfusion in acute ischemic stroke.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Disclosure

The authors declare that they have no competing interests.

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