Int J Clin Exp Med 2020;13(7):5499-5505
www.ijcem.com /ISSN:1940-5901/IJCEM0110616

Original Article

Correlation of Hcy, inflammation level and cerebral infarction in hypertensive patients

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Received March 10, 2020; Accepted April 24, 2020; Epub July 15, 2020; Published July 30, 2020

Abstract: Objective: Our study aimed to investigate the correlation between serum inflammatory factors and Hcy levels with the occurrence of acute cerebral infarction in hypertensive patients. Methods: A total of 82 patients with acute cerebral infarction and hypertension in Xuzhou Central Hospital were enrolled as the study group, while 54 patients with acute single cerebral infarction were selected as the infarct group, and 30 healthy individuals were included as the normal group. The contents of serum Hcy level and inflammatory factors (IL-6, IL-8, TNF-α) at the 1st day after admission were detected. Pearson's correlation analysis was employed to assess the relations of the IL-6, IL-8, TNF-α and Hcy levels in patients with acute cerebral infarction and hypertension. Results: The levels of IL-6, IL-8, TNF-α and Hcy in the study group were significantly higher than those in the infarct and normal groups, while the IL-6, IL-8, TNF-α and Hcy levels in the infarct group were also significantly higher than those in the normal group (P<0.05). Among the 80 patients in the study group, 76 patients were identified with high levels of Hcy, with an average value of 17.4±6.1 μmol/L, among which, 63 cases were diagnosed with H-type hypertension, with an average Hcy content of 20.1±5.4 μmol/L. While 6 patients with hypertension were measured with an average Hcy content of 9.1±5.4 μmol/L. In the study group, the contents of IL-6, IL-8 and TNF-α were significantly correlated with Hcy level, while those levels in patients with different volumes of infarction were higher than those in the normal group (P<0.05). Conclusion: Our data indicate that IL-6, IL-8 and TNF-α play an important role in the formation of arterial plaque and have impact on the infarct volume.

Keywords: Hypertension, acute cerebral infarction, Hcy, inflammatory factors, C-reactive protein

Introduction

Hypertension is an important risk factor for cardio-cerebrovascular events. The main complications include left ventricular hypertrophy, left ventricular remodeling and carotid arteriosclerosis. Carotid atherosclerosis is the pathophysiological basis of acute cerebral infarction [1, 2]. Therefore, the treatment of hypertension in clinical prevention of cerebrovascular disease is a pivotal link, but the existing antihypertensive program can only reduce the incidence of acute cerebral infarction by about 38%; the effect of which is not satisfactory. Homocysteine (Hcy) is a sulfur-containing amino acid that can be obtained from methionine methylation, which is considered as a risk factor for cardiovascular diseases such as hypertension, heart failure, stroke and coronary heart disease [3]. Studies have shown that atherosclerosis is a chronic inflammatory disease, and various inflammatory factors in the serum play an important role in the inflammatory response and cerebral ischemia-reperfusion injury [4-6]. Numerous evidence reveals that pro-inflammation, which is associated with interleukin-1β (IL-1β), IL-6, IL-33 and tumor necrosis factor-α (TNF-α), is the leading cause of ischemic brain injury, thus anti-inflammatory therapy is an attractive candidate for ischemic brain damage [7-9]. Hypertension, on the other hand, can lead to vascular inflammation through promotion of perivascular macrophages and proinflammatory cytokine secretion in the arterial wall [10]. However, the changes in inflammation in the development of stroke due to high blood pressure remain to be determined. In this study, we explored the potential correlation among

Table 1. Comparison of clinical baseline data in each group

groups	cases	Age (year)	Male/female	BMI (Kg/m^2)	Smoking index (branch/year)	Drinking index (g/year)
Normal group	30	64.3±7.2	17/13	10.11±4.19	13.69±7.85	19.57±3.19
Infarct group	54	65.1±6.9	30/24	1.35±0.61	2.02±1.01	3.03±0.79
Study group	82	64.9±7.1	49/33	45.81±11.90	55.71±13.98	64.39±14.47
F		0.491	0.109	0.021	0.003	0.009
P		0.615	0.894	0.998	0.983	0.898

serum inflammatory factors and HCY levels in patients with acute cerebral infarction and hypertension, in order to provide clues for the further predictive evaluation of complications of hypertension.

Subject and methods

Subjects

Eighty-two patients with acute cerebral infarction and Hypertension from January 2017 to December 2017 in Xuzhou Central Hospital were enrolled in the study group, while 54 patients with acute single cerebral infarction were selected as the infarct group, and 30 healthy individuals were included as normal group. In the study group, 17 patients were identified with small volume cerebral infarction (<4 cm^3), 40 patients with middle volume cerebral infarction (4-10 cm^3), and 25 patients with mass cerebral infarction (>10 cm^3). The clinical data between the groups are shown in **Table 1**.

Inclusion and exclusion criteria

The subjects conform to the diagnostic criteria of hypertension and the diagnostic criteria of cerebral infarction; cases of cerebral infarction were the first onset and the duration of treatment was not more than 24 hours; all subjects did not have severe heart and lung insufficiency, liver and kidney dysfunction, tumor or other diseases; all subjects were determined to be without infection, blood pressure issues, lipid-regulation disorders, any other special treatment, or traumatic surgery history; all subjects signed an informed consent; any subjects with a history of mental illness were excluded.

Methods

The levels of serum HCY and inflammatory factors (interleukin-6, interleukin-8, tumor necrosis factor alpha) at the first day after admission were recorded. At the same time, the levels of these indicators in the cerebral infarction group and the normal group were detected. Three ml fasting venous blood was collected, centrifuged at 3000 g for 5 minutes. The serum was separated and stored at -20°C. Levels of IL-6, IL-8 and TNF-α were measured by enzyme-linked immunosorbent assay according to the instructions. HCY in the plasma of fasting venous blood was determined by photochemical methods.

Statistical treatment

Statistical analysis of the data was performed by SPSS19.0 statistical software. The measurement data are expressed as the mean ± standard deviation. Chi-square test was used for enumeration data. Continuous data from multiple groups were analyzed by using one-way ANOVA, with the Tukey's post hoc test. Pearson's correlation analysis was used to assess the relations of IL-6, IL-8, TNF-α and Hcy levels with acute cerebral infarction and hypertension. The results are statistically significant when $P< 0.05$.

Results

Comparison of IL-6, IL-8, TNF-α and Hcy levels in each group

The results showed that the average values of IL-6, IL-8, TNF-α and Hcy in the study group was significantly higher than that in the cerebral infarct group and normal group, and the IL-6, IL-8, TNF-α and Hcy levels in the infarct group were also significantly higher than those in normal group ($P<0.05$) (**Table 2**). These data suggested that IL-6, IL-8, TNF-α and Hcy may present as essential indicators in the development of acute cerebral infarction and hypertension.

Table 2. Comparison of IL-6, IL-8, TNF-α and Hcy levels in each group

groups	cases	IL-6 (pg/mL)	IL-8 (ng/mL)	TNF-α (ng/mL)	Hcy (μmol/L)
Normal group	30	7.19±1.43	0.52±0.09	18.35±6.71	4.1±1.7
Infarct group	54	12.81±6.79	1.67±0.62	46.59±12.98	12.1±3.5
Study group	82	16.81±5.43	2.49±1.01	60.13±18.61	18.7±8.3
F		5.831	10.893	18.753	13.215
P		0.016	0.001	0.000	0.000

Note: Any 2 groups compare, P<0.05.

Table 3. Pearson correlations among inflammatory factors and Hcy in patients with acute cerebral infarction and hypertension (r value)

	IL-6	IL-8	TNF-α	Hcy
IL-6		0.69*	0.65*	0.68*
IL-8	0.69*		0.49*	0.46*
TNF-α	0.65*	0.49*		0.49*
Hcy	0.68*	0.46*	0.49*	

Note: * = P<0.05.

High level of Hcy in the majority of patients with acute cerebral infarction and hypertension

We further analyzed the Hcy content in of patients with acute cerebral infarction and hypertension. Among the 80 patients in the study group, 76 patients were identified with high level of Hcy, with an average content of 17.4±6.1 μmol/L; among whom, 63 cases were associated with H-type hypertension, with an average Hcy content of 20.1±5.4 μmol/L; while 6 patients with hypertension were measured with an average Hcy content of 9.1±5.4 μmol/L. Our results showed that 92.7% patients with acute cerebral infarction and hypertension are accompanied by high level of homocysteine and patients with H-type hypertension accounted for 76.8%.

Correlations among inflammatory factors and Hcy in patients with acute cerebral infarction and hypertension

Pearson correlation analysis was performed and a significant positive correlation was found between IL-6 and IL-8; IL-8 and TNF-α; IL-6 and TNF-α; TNF-α and Hcy; IL-6 and Hcy; IL-8 and Hcy, respectively (P<0.05). This suggests that a relevant biological association exists among inflammatory factors and Hcy levels in patients with acute cerebral infarction and hypertension (**Table 3**; **Figure 1**).

Relationship between inflammatory factors and the volume of acute cerebral infarction in hypertensive patients

The results showed that the levels of IL-6, IL-8, TNF-α in the serum gradually elevated as the infarct volume in patients in the study group increased, and the levels of these inflammatory factors were significantly higher than those in normal group (P<0.05) (**Table 4**). These data together suggested that IL-6, IL-8 and TNF-α levels were positively correlated with infarct volume.

Discussion

Acute cerebral infarction is a common cardiovascular and cerebrovascular disease. Current epidemiological data show that hypertension and HCY are risk factors for acute cerebral infarction [7, 8]. The analysis of Hcy baseline levels in a hypertensive population showed that, with Hcy level at 10 μmol/L, about three-fourths of hypertensive patients had high levels of Hcy [9]. Another study also found that high Hcy level and hypertension have a certain synergic association, and can cause an increased risk of vascular disease [10]. In addition to the influence of Hcy, the role of inflammatory factors in the occurrence of acute cerebral infarction has attracted extensive attention recently. A number of studies [11-13] demonstrate that there are many inflammatory factors involved in the process of acute cerebral infarction ischemia/reperfusion injury. The increasing secretion of several inflammatory factors frequently induces the activation of signaling cascades and promotes the release of other inflammatory factors, which affects the neural-endocrine network system and results in brain tissue injury. It also suggests that a series of chain reactions mediated by cytokines play an important role in the development of acute cerebral infarction. However, whether inflammatory factors and hypertension can act together to promote the occurrence of acute cerebral infarction remains to be further discussed. This study therefore examined the changes of serum inflammatory factors and HCY levels in patients with acute cerebral infarction and hypertension, and analyzed its relationship with cerebral infarction.

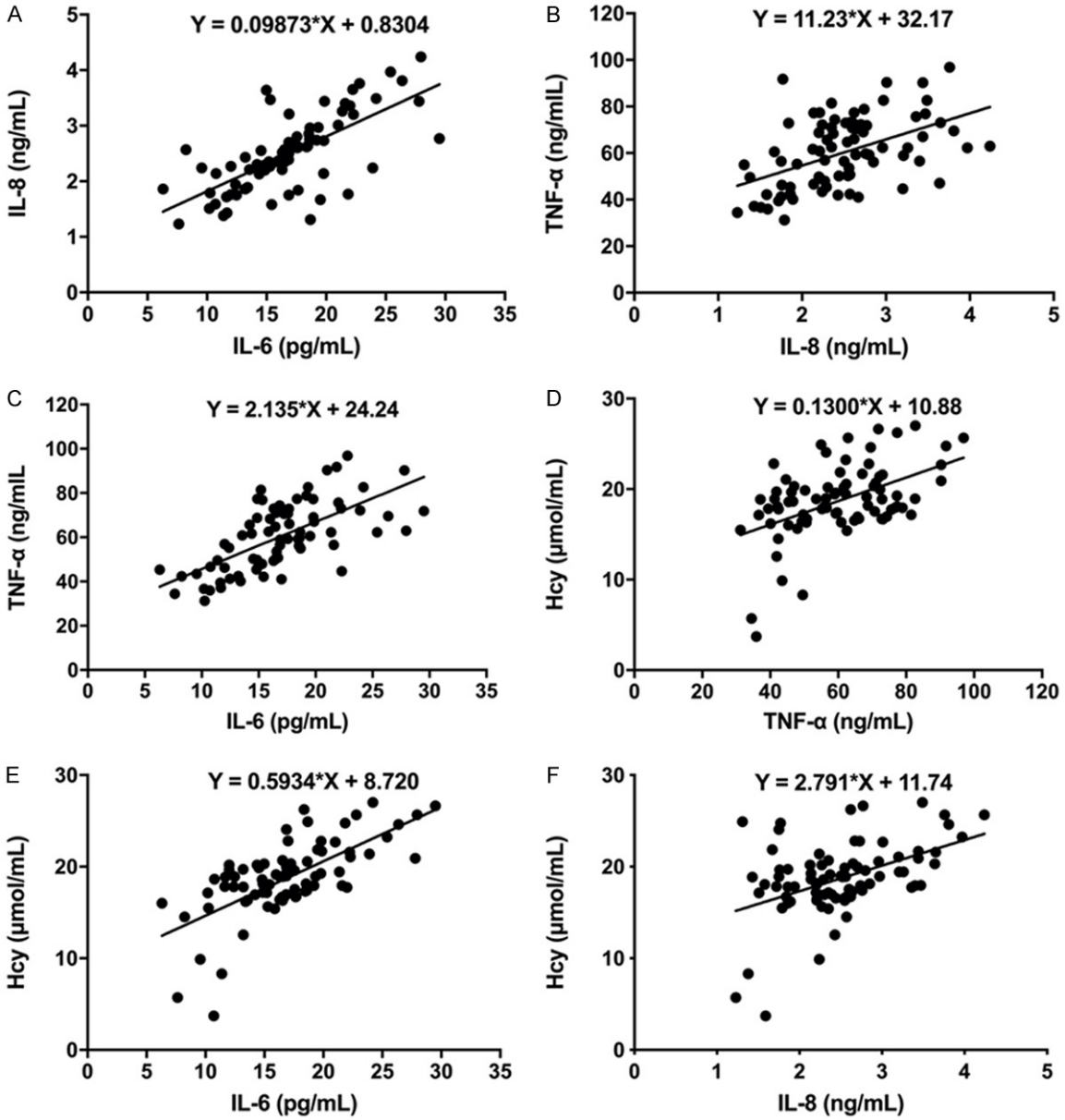

Figure 1. Correlation among inflammatory factors and Hcy in patients with acute cerebral infarction and hypertension. A. IL-6 vs. IL-8. B. IL-8 vs. TNF-α. C. IL-6 vs. TNF-α. D. TNF-α vs. Hcy. E. IL-6 vs. Hcy. F. IL-8 vs. Hcy.

Table 4. Expression of inflammatory factors in serum of patients with different infarct volume

Index	Normal group	Small volume	Medium volume	Mass volume
IL-6 (pg/mL)	7.19±1.43	10.11±4.19*	13.69±7.85*	19.57±3.19*,#,&
IL-8 (ng/mL)	0.52±0.09	1.35±0.61*	2.02±1.01*,#	3.03±0.79*,#,&
TNF-α (ng/mL)	18.35±6.71	45.81±11.90*	55.71±13.98*,#	64.39±14.47*,#,&

Note: *compared to Normal group, P<0.05. #compared to Small volume, P<0.05. &compared to Medium volume, P<0.05.

The data showed that the average value of IL-6, IL-8, TNF-α and Hcy in the study group was significantly higher than that in the cerebral infarction Group and normal group, while these levels in the cerebral infarction group were further significantly higher than those in the normal group. Hcy is a sulfur-containing amino acid produced by methionine and is an intermediate

metabolite of methionine. Its action on blood vessels is extremely complicated. It is believed that Hcy reduces the elasticity of blood vessels by affecting the structure and function of blood vessel walls. High plasma Hcy levels can accelerate the dissolution of elastic fibers in the vessel wall and promote the synthesis of collagen fibers. This change leads to an imbalance of fiber components in the vessel wall and a decrease in vascular elasticity, suggesting that Hcy has a great influence on vasoconstriction and diastolic function [14]. Hcy inhibits and degrades the effect of vascular endothelia cells, and also increases the synthesis of endothelial endothelin, which causes an imbalance of endothelin. The impact of Hcy on diastolic function of blood vessels promotes an increase in pulse pressure and arterial systolic pressure. In addition, Hcy has strong oxidation functions, which damages vascular endothelial cells, and induces the formation of atherosclerotic plaque. High level of Hcy stimulates the proliferation of vascular smooth muscle cells, which will reduce vascular compliance. It can also cause vasoconstriction by increasing the concentration of calcium ions in smooth muscle cells [15]. In this study, we found that 92.7% of hypertensive patients with acute cerebral infarction, had Hcy levels that were significantly higher than normal (the Hcy level was generally considered to be normal below 6 μmol/L). There was also evidence of abnormally high Hcy levels in the study group and cerebral infarct group, demonstrating the role of Hcy in cerebral infarction and its synergistic effects with hypertension.

IL-6 is characterized as a cytokine with multiple immunoregulatory functions. In addition to neurotrophic and neuroprotective effects, it can also mediate inflammation, demyelination and gliosis, and contributes to maintaining a physiological balance in the body. Previous findings showed that the level of IL-6 in serum and cerebrospinal fluid was increased significantly, along with the elevation of matrix 1 expression, where brain damage was aggravated owing to the reperfusion of Metalloproteinases [16]. IL-8 is chemotactic signal for all known types of migratory immune cells. IL-8 also produces active oxidized metabolites that can directly damage tissues and cells [17]. Tumor necrosis factor alpha is a polymorphic cytokine, which is mainly secreted by activated mononuclear C macrophages, and the appropriate secretions can play immunomodulatory roles. However, excessive secretion or imbalance leads to damage of the blood-brain barrier and vascular endothelial cells. It also has an impact on increasing the adhesion of leukocytes to the endothelium, enhanced clotting activity of endothelial cells, which further leads to neurotoxicity and aggravation of cerebral infarction damage [18]. In this study, we found a significant correlation between inflammatory factors and Hcy, while the increased levels of IL-6, IL-8 and TNF-α in the study group were implicated with an increase of infarct volume. It is suggested that IL-6, IL-8 and TNF-α not only participate in the occurrence and development of cerebral infarction, but also participate in the inflammatory reactions of the inflammatory response in the early stages of hypertension, and are involved in plaque formation [19, 20]. It is speculated that, due to long-term damage caused by high pressure and abnormal blood flow in the vascular endothelial cells, and shedding, the macrophages and monocytes in the lesioned area are deposited and break into the vascular intima of the blood vessels to form lipid plaques. While monocytes, mononuclear C macrophages and vascular endothelial cells are activated to produce large amounts of oxygen free radicals, elevated levels of IL-6, IL-8, and TNF-α, leading to high oxidative stress and accelerated atherosclerosis [21, 22].

Some limitation in this study still exists in that the clinical effect of these factors as biomarkers should be further validated among a larger number of patients, while potential targeting therapy against acute cerebral infarction and hypertension requires further investigation, based on our initial findings regarding the changes and correlation of Hcy, inflammation level and cerebral infarction in hypertensive patients.

Conclusion

In summary, the expression of IL-6, IL-8, TNF-α and HCY are abnormally increased in patients with hypertension and acute cerebral infarction. Significant correlations are found between Hcy and inflammatory factors IL-6, IL-8, and TNF-α, which are implicated to correlate to the size of infarct volume.

Disclosure of conflict of interest

None.

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