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ORIGINAL ARTICLE

The Value of Serum Complement C1q in the Diagnosis of Acute Ischemic Stroke

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SUMMARY

Background: **To investigate the relationship between the levels of serum complement C1q and the risk and severity of acute ischemic stroke, a total of 154 patients with acute ischemic stroke and 42 healthy volunteers as normal controls were enrolled in the present study.**
Methods: **According to the onset time of stroke, patients were divided into three groups. Using an immune transmission turbidity method, the levels of serum complement C1q were detected to investigate the relationship between the level of serum complement C1q and the incidence and severity of acute ischemic stroke. The risk factors of these groups were calculated using a conditional logistic regression model. The assessment of neurological function impairment was carried out according to the National Institute of Health Stroke Scale. Then correlation analysis was carried out between the level of serum complement C1q among patients with acute ischemic stroke and the degree of neurological function impairment.**
Results: **The results showed that the level of serum complement C1q was higher in the ischemic stroke group than in the control group. Using a conditional logistic regression model it was discovered that serum complement C1q was the independent pathogenic factor of cerebral infarction. There also was a decreasing trend in the level of serum complement C1q with the extension of the onset time and an increasing trend in the level of serum complement C1q with the increase in the maximum diameter of infarction volume.**
Conclusions: **Serum complement C1q is an independent risk factor for acute outbreak of ischemic stroke, whose level is closely related to the outbreak and infarct size and neurological function impairment.**
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KEY WORDS

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INTRODUCTION

Cerebral infarction is one of the most common cerebrovascular diseases worldwide with the highest mortality rate [1]. It is also the second most common factor that causes death and disability in developing countries. The most common cerebral infarction is acute ischemic stroke that accounts for about 80%, and the main cause of this disease is thrombus caused by atherosclerosis. More and more evidence demonstrates that acute systemic inflammation occurs after ischemic stroke [2,3].

Meanwhile, the activation of complement also exists during the formation and development of atherosclerosis, and ischemia and reperfusion injury caused by the formation and rupture of atherosclerotic plaque are partly mediated by the activation of complement. So, the activation of the complement system is indispensable in the occurrence of ischemic stroke whose main cause is atherosclerosis. This discovery is also the basis for the application of complement inhibitors in the clinical treatment of ischemic stroke. It has been confirmed in recent research that the activation of complement C1q plays an important part in promoting the outbreak of ischemic cerebrovascular diseases. In this study, the level of serum complement C1q in 154 patients with acute ischemic stroke was detected and compared with 42 healthy volunteers, in order to investigate the relationship between the level of serum complement C1q and the risk and severity of acute ischemic stroke, and to provide an experimental basis for its clinical diagnosis.

MATERIALS AND METHODS

Ethics

Written informed consent was obtained from every participant in this study for the use of samples. This project was approved by the Clinical Research Ethics Committee of Liaocheng People's Hospital.

Patients

154 patients with acute ischemic stroke who were sent to Liaocheng People's Hospital within 72 hours of the onset of stroke from January to June 2015 were chosen. Among these patients, there were 89 males and 65 females, whose average age was 62.41 ± 11.87. New infarct lesions were found in all these patients after CT or MRI examination, and the maximum diameters of infarctions were measured by imaging professionals. All the above cases are in accordance with Chinese Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke (2010). Exclusion criteria: serious disease of the heart, liver, kidney or blood system, and autoimmune disease. At the same time, 42 healthy volunteers (22 males and 20 females) from the Physical Examination Center of Liaocheng People's Hospital were randomly selected as the normal control group, whose average was 66.05 ± 10.8. According to the onset time of stroke, patients were divided into three groups, i.e., < 24 hours group (83 patients), 24 hours - 48 hours group (59 patients), and > 48 hours group (12 patients). By measuring the maximum diameter of the infarct size, patients were divided into three groups: large size group (maximum diameter > 3 cm, 60 patients), medium size group (maximum diameter 1.5 - 3 cm, 52 patients), and small size group (maximum diameter < 1.5 cm, 42 patients).

Reagents and Instruments

Using an immune transmission turbidity method, the concentration of serum complement C1q was detected. The detection reagents were purchased from Shanghai Beijia Biochemical Co. Ltd., and, after careful parameter setting and quality control, the detection was carried out using an automatic biochemical analyzer by Beckman Instruments, Inc. USA., which was also used along with its original reagents in the detection of blood-lipid level, including total cholesterol (CHOL), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C).

The collection and storage of specimens

On the second morning of admission, all the specimens were collected, and 3 mL was then placed in pro-coagulant tubes and centrifuged at 1200 x g for 6 minutes. The extracted sera were put into consecutively numbered EP tubes and then stored at -80°C.

Statistical Methods

SPSS 17.0 was used in the statistical analysis of this study. The normally distributed variables of each group were all shown in the form of mean value ± standard deviation, including age, CHOL, TG, HDL, LDL, systolic blood pressure (SBP), diastolic blood pressure (DBP), and C1q. Two sample *t*-test was used in the comparison between two groups, conditional logistic regression model was used in the calculation of risk factors of cerebral infarction among the three groups, and Pearson's correlation analysis was used to investigate the correlation between the level of serum complement C1q and the degree of neurological function impairment.

RESULTS

The comparison of baseline indexes between patient group and control group

From Table 1, we can see that there was no statistically significant difference in gender, age or HDL of baseline indexes between patient group and control group, but the differences in CHOL, LDL, SBP, DBP and C1q showed statistical significance ($p < 0.05$) (Table 1).

Analysis on the risk factors of patients with acute ischemic stroke

In the patient group, the level of serum complement C1q was increased. Considered from the view of the relative risk of various monitoring indicators, the factors related to cerebral infarction were C1q, CHOL, TG, HDL, SBP, and DBP. Using a conditional logistic regression model, it was found that serum complement C1q, TG, and SBP are independent pathogenic factors of cerebral infarction, while the level of HDL shows a negative correlation with cerebral infarction (see Table 2).

Table 1. The comparison between the baseline indexes of the patient group and control group.

Baseline indexes	Patient group	Control group	p-value
Gender (male/female)	89/65	22/20	0.343
Age	62.41 ± 11.87	66.05 ± 10.8	0.076
TG (mmol/L)	1.38 ± 0.0.43 *	0.94 ± 0.37	< 0.001
CHOL (mmol/L)	4.79 ± 1.25 *	4.47 ± 0.7	0.039
HDL (mmol/L)	1.41 ± 0.27	1.43 ± 0.27	< 0.001
LDL (mmol/L)	3.03 ± 0.8 *	2.17 ± 0.6	< 0.001
SBP (mmHg)	155.28 ± 23.03 *	122.31 ± 19.12	< 0.001
DBP (mmHg)	91.67 ± 14.72 *	75.9 ± 12.42	< 0.001
C1q (mg/L)	181.38 ± 48.86 *	163.68 ± 34.42	0.009

Compared with the control group, $p < 0.05$.

Table 2. Logistic regression analysis.

Related factors	Risk partial regression coefficients	S.E.	Wald x 2	p-value	OR value	95% CI	
						lower	upper
TG	0.887	0.457	3.963	0.049	2.427	1.002	5.943
HDL-C	-2.488	0.626	15.809	< 0.001	0.083	0.024	0.283
CH	1.139	1.483	0.589	0.443	3.123	0.171	57.159
Diastolic pressure	0.006	0.029	0.046	0.830	1.006	0.950	1.066
Systolic pressure	0.079	0.020	15.349	< 0.001	1.082	1.040	1.126
LDL-C	-0.002	0.008	0.075	0.784	0.998	0.983	1.013
Complement C1q	4.433	0.958	21.425	< 0.001	84.161	12.881	549.885
Constant term	-12.489	3.306	14.274	0.000	0.000	-	-

Compared with the control group, $p < 0.05$.

Table 3. The relationship between the level of serum complement C1q and cerebral infarction.

Group	Number of cases	Complement C1q	F	p-value
< 24 hours	83	178.37 ± 51.40	5.116	0.007
24 - 48 hours	59	158.69 ± 53.93 [a]	-	-
> 48 hours	12	135.37 ± 29.78[a]	-	-

[a] Compared with the group within 24 hours, $p < 0.05$.

The relationship between the level of serum complement C1q and the onset time of acute ischemic stroke

The level of serum complement C1q showed a decreasing trend with extended onset time of acute ischemia stroke. In the < 24 hours group, the level of serum complement is 178.37 ± 51.40 mg/L, significantly higher than that of the 24 - 48 hours group and > 48 hours group, and the differences are statistically significant ($p < 0.05$) (see Table 3).

Table 4. The relationship between the level of serum complement C1q and the size of cerebral infarction.

Group	Number of cases	Complement C1q	F	p-value
Small size group	60	152.65 ± 56.23	5.227	0.007
Medium size group	52	163.30 ± 45.79 [a]	-	-
Large size group	42	189.10 ± 55.45 [a]	-	-

[a] Compared with the group of serious illness, $p < 0.05$.

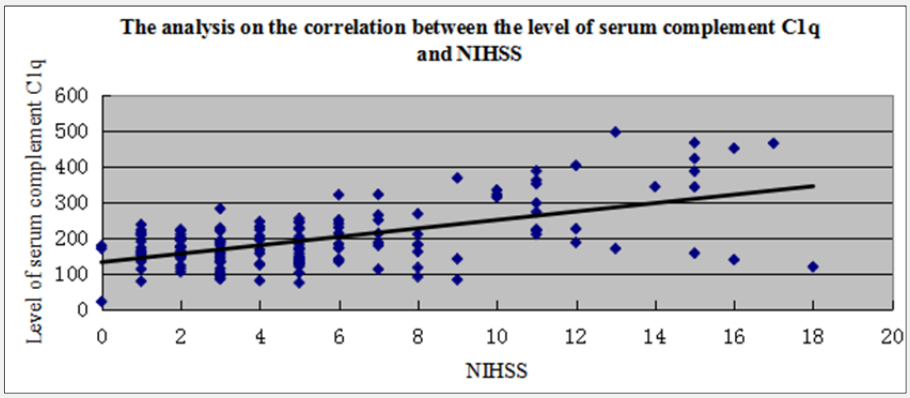

Figure 1. The analysis on the correlation between the level of serum complement C1q and NIHSS.

$r = 0.576$, $p < 0.05$.

The relationship between the level of serum complement C1q and the size of cerebral infarction

The level of serum complement C1q has shown an increasing trend with the increase of the size of cerebral infarction. In the large size group, the level of serum complement C1q is 189.10 ± 55.45 mg/L and significantly higher than that of the medium and small size group, and the differences are statistically significant ($p < 0.05$). The difference between the medium and small size group is not statistically significant. (see Table 4).

The analysis of the correlation between the level of serum complement C1q and the degree of neurological function impairment

Pearson's correlation analysis shows that there is a moderate degree of correlation between serum complement C1q and the score of neurological function impairment (NIHSS) of patients with acute ischemic stroke ($r = 0.576$, $p < 0.05$) (see Figure 1).

DISCUSSION

The basic cause of ischemic stroke is atherosclerosis, which is essentially a chronic inflammation according to recent studies [4]. The most common one is focal cerebral ischemia, whose epidemiological characteristics are high incidence, high disability, and high mortality [5]. At present, there are many theories on the pathogenesis of ischemic stroke, e.g., energy metabolism disorder theory, free radical and lipid peroxidation theory, intracellular Ca^{+} overload theory, inflammatory immune theory, excitatory amino acid toxicity theory, etc. [6]. In recent years, complement mediated inflammatory immune theory has received much attention. The complement system is an important part of congenital immunity and adaptive immunity, which consists of a group of proteins with enzymatic activity on the surface of blood, body fluids, and cell membranes. It has biological function in regulating phagocytosis and clearing aging and apoptotic cells, participating in the immune response as well as mediating inflammatory reactions [7]. More and more evidence shows that immune factors and inflam-

mation plays an important role in the pathophysiology of stroke, and the activation of the complement system is one of the most important factors in the pathological mechanism of brain injury in ischemic cerebral vascular disease. Thus, as a part of the innate immune system, the complement system plays an important part in the process of initiating and regulating the inflammatory response [8,9]. It has been confirmed in previous studies that when wild rat has transient occlusion surgery on the focal cerebral artery, its expression of C1q, C3, C3aR, and C5aR increased in the injured area. But when rats' C3 gene is knocked out and has the same surgery, there is a significant decrease in the size of cerebral infarction and brain water content compared with wild rats [10]. Moreover, C1q and C3 deposition has been detected in animal stroke models [10,11]. Through further animal experiments, Luo Hao et al. found that there is a positive correlation between the expression of CD11b and the level of C1q and C3 in the brain tissue of rat which has brain injury caused by ischemia and reperfusion. When this injury occurs, the innate immune response in the brain is initiated, and complement C1q, C3 and microglia cells are activated, thus playing a protective part in this injury [12]. In acute glomerulonephritis, lupus nephritis, and cardiovascular diseases, the missing of C1q cannot activate the complement cascade or eliminate immune complexes, resulting in its deposit in organs. When the above diseases occur, there will be no assist for macrophages to eliminate apoptotic cells in time, which causes the release of auto-antigens in apoptotic bodies, stimulates B cells to produce auto-antibodies, and exacerbates inflammation. As the initial activator of the classical activation pathway, C1 consists of three kinds of protein, i.e., C1q, C1r, and C1s, of which C1q is the biggest. It has a specific receptor and function and participates in the formation of C3 invertase. The knockout of C1q gene of new-born rats could have a significant long-term protective effect, which indicates that the lack of C1q could play a more complete inhibitory role in the cascade reaction.

In this experiment, through the analysis of 154 patients with acute ischemic stroke, it was found that the level of serum complement C1q is significantly higher than that of the control group ($p < 0.05$). A conditional logistic regression model showed that serum complement C1q was the independent pathogenic factor of acute ischemic stroke. Besides, it was found that with the extension of onset time, there was a downward trend in the level of serum complement C1q, and the comparisons between each group were statistically significant ($p < 0.05$). Thus, serum complement C1q is expected to be the early clinical indicator of monitoring acute ischemic stroke. In addition, with the increase in the size of infarction, the level of serum complement C1q in the large size group is significantly higher than that of medium and small size groups ($p < 0.05$), which indicates its relationship to the size of cerebral infarction. Therefore, it can be used as a potential indicator of the severity of disease and effect of treatment. Pearson's correlation analysis showed that there is a medium correlation ($r = 0.576$, $p < 0.05$) between the level of serum complement C1q among patients with acute ischemic stroke and the degree of neurological function impairment (NIHSS). However, more samples are needed to verify this correlation. Compared with animal experiments, the research on the activation of complement in the process of human acute ischemic stroke is currently limited. In this experiment, a preliminary study was carried out on the level of serum complement C1q in patients with acute ischemic stroke, which could confirm the expression and significance of serum complement C1q in acute ischemic stroke and provide a more reliable laboratory basis for its clinical diagnosis.

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Declaration of Interest:
There is no conflict of interest in the submission of this manuscript, which is approved by all authors for publication. All co-authors declare that the work described was original research that has not been published previously and is not under consideration for publication elsewhere.

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