Am J Transl Res 2021;13(5):5617-5622
www.ajtr.org /ISSN:1943-8141/AJTR0126357

Original Article

Changes and significance of serum CXCL-16, GDF-15, PLA-2 levels in patients with cerebral infarction

Xiqi Liu

Department of Neurology, Cangzhou Central Hospital, Hebei Province, China

Received November 16, 2020; Accepted December 26, 2020; Epub May 15, 2021; Published May 30, 2021

Abstract: Objective: To explore the changes and significance of serum CXC chemokine ligand 16 (CXCL-16), growth differentiation factor 15 (GDF-15) and lipoprotein-related phospholipase A2 (PLA-2) levels in patients with cerebral infarction. Methods: A total of 87 patients with cerebral infarction between August 2019 and May 2020 in our hospital were selected as the disease group, and 50 healthy patients were selected as the healthy control group. Enzyme-linked immunosorbent assay was used to detect serum CXCL-16, GDF-15, PLA-2 expression levels in all subjects. A comprehensive evaluation was made in terms of changes of various indicator levels in patients while the changes of CXCL-16, GDF-15, PLA-2 in patients with cerebral infarction after effective treatment were monitored. Results: Compared with the healthy group, the expression levels of serum CXCL-16, GDF-15 and PLA-2 in the disease group were up-regulated ($P < 0.001$). Patients with cerebral infarction were divided into subgroups according to the National Institutes of Health Neurological Impairment Score (NIHSS). The expression levels of serum CXCL-16, GDF-15 and PLA-2 in patients with cerebral infarction increased with the increase of the impairment score ($P < 0.001$). The areas of the patient's cerebral infarction were calculated according to the Pullicino formula. The expression levels of serum CXCL-16, GDF-15 and PLA-2 in patients with cerebral infarction increased with the enlargement of the infarct area, and the difference between the groups was statistically significant ($P < 0.001$). The expression levels of serum CXCL-16, GDF-15 and PLA-2 in patients with cerebral infarction when discharged from the hospital after treatment were significantly lower than those before treatment ($P < 0.001$). The AUC areas of serum CXCL-16, GDF-15 and PLA-2 indicators in predicting the therapeutic effect of cerebral infarction were 0.821, 0.883, and 0.711, respectively. Conclusion: Serum CXCL-16, GDF-15, PLA-2 in patients with cerebral infarction were all highly expressed and changed with the disease severity, which can be used as reliable indicators for monitoring the incidence, severity, and prognosis of cerebral infarction.

Keywords: Cerebral infarction, CXCL-16, GDF-15, PLA-2

Introduction

Cerebral infarction is a cerebrovascular disease caused by cerebral ischemia and hypoxia which is triggered by many factors, with high incidence, disability rate and fatality rate. Atherosclerosis is considered a common pathological mechanism for this disease [1]. Inflammatory reaction runs through the whole process of atherosclerosis and plays a vital role [2]. With the constant progress of scientific research, some new markers of inflammation have been detected in the smooth muscle cells, macrophages and vascular endothelial cells at the damaged sites of patients with inflammatory diseases, such as serum CXC chemokine Ligand 16 (CXCL-16), growth differentiation factor 15 (GFF-15), lipoprotein-associated Phospholipase A2 (PLA-2), etc. [3-5]. The levels of CXCL16, GDF15 and PLA2 are closely related to the occurrence of atherosclerosis-related diseases. The most common pathological mechanism of cerebral infarction is atherosclerosis caused by inflammation, and CXCL16, GDF15 and PLA2 all play an important role in the pro-inflammatory response in its occurrence. High levels of CXCL16, GDF15 and Lp-PLA2 can lead to the formation of atherosclerosis, increase in plaque instability, and finally lead to CI, and it may be of certain guiding value to understand the changes in serum CXcl-16, GDF-15 and PLA-2 levels in patients for the diagnosis of cerebral infarction. Therefore, we investigated the expression pat-

terns of CXCl-16, GDF-15 and PLA-2 in peripheral blood serum of patients with cerebral infarction so as to provide a clinical reference for the scientific research on this disease.

Materials and methods

General materials

A total of 87 cerebral infarction patients who were admitted to our hospital from August 2019 to May 2020 were included into the disease group. Inclusion criteria: (1) Patients aged over 18 years and with complete clinical data; (2) Patients who were diagnosed with cerebral infarction by CT and MIR examination after admission; (3) First onset and diagnosis within 1 week of enrollment. No use history of anticoagulants, lipid-lowering or immunosuppressive drugs, etc. prior to the onset of the disease; (4) Those who were informed of the content of this study and signed the informed consent. Exclusion criteria: (1) With aortic dissection, brain tumor or other unknown causes; (2) With autoimmune diseases, trauma or infection; (3) With a history of cerebral hemorrhage and craniocerebral trauma; (4) With acute myocardial infarction. Meanwhile, 50 healthy patients who passed a physical examination in our hospital were selected as the healthy control group. There were 137 patients, including 77 males and 60 females. The disease group was aged 53.1-77.3 years old, with a body weight of 21.7-26.0 kg/m^2. The healthy group was aged 52.7-77.1 years old, with a body weight of 21.8-25.4 kg/m^2. This study has been approved by the Ethics Committee of our hospital.

Methods

Test method: Before admission, 5 mL fasting venous blood was extracted from the disease group, and 5 mL fasting venous blood was extracted from the healthy group on the day of physical examination. The samples were placed in a vacuum anticoagulant tube, gently shaken, centrifuged for 10 min (radius of 13.5 cm) and the serum was separated. Expression levels of CXCL-16, GDF-15 and PLA-2 in the serum were tested by ELISA, and the testing kits were purchased from Thermo Fisher Scientific (China) Inc. The operation was in strict accordance with the kit instructions.

Therapeutic methods: The disease group was given routine antibiotics treatment. Measures were taken to stabilize patients' intracranial pressure and blood pressure. Aspirin (manufacturer: Hebei Ruisen Pharmaceutical Co., LTD., SDFA approval number H20173209) was administered 50 mg once a day for 7 days. At the same time, 10 mg argatroban injection (manufacturer: Tianjin Institute of Pharmaceutical Research Co., Ltd., SDFA approval number H18002905) was diluted with normal saline and given through intravenous infusion with micropump for 3 hours each time for 7 consecutive days.

Evaluation methods: The severity of cerebral infarction in 87 patients was evaluated by the National Institutes of Health Neurological Deficit Score (NIHSS) [6]. Patients whose NIHSS score < 5 were included in the mild group; patients with 5-15 scores were divided into the moderate group; and those with 16-42 scores were the severe group. The Pullicino formula was used to calculate the cerebral infarction area of patients. The small infarction group included patients with an infarct area < 3 cm^3; the middle infarction group consisted of patients with an infarct area 3-5 cm^3; and the large infarction group was composed of patients with an infarct area of > 6 cm^3.

Statistical methods

Discrete variables were expressed as counts and percentages, and continuous variables as means and standard deviation (SD); and differences between groups were assessed using Chi-square test for categorical variables, and Student *t*-test or Variance (F) analysis for continuous variables. The efficacy of CXCL-16, GDF-15 and PLA-2 in predicting the therapeutic effect of cerebral infarction was evaluated by area under the ROC curve (AUC). The significance level of all the analyses was defined as $P < 0.05$. SPSS 19.0 was the software used for the analysis.

Results

Comparison of the general data

The gender, age and body mass between the two groups were similar ($P > 0.05$, **Table 1**).

Comparison of serum CXCL-16, GDF-15 and PLA-2 levels between the groups

Compared with the healthy group, the expression levels of serum CXcl-16, GDF-15 and PLA-2

Table 1. Comparison of general materials between the groups [n (%), $\bar{x}$±sd]

Group	Cases	Gender (Male/Female)	Age (years old)	Body mass(kg/m^2)	systolic pressure(mmHg)	diastolic pressure(mmHg)
Disease group	87	48/39	65.56±11.73	23.84±2.11	135.4±10.1	87.9±5.4
Healthy group	50	29/21	64.93±12.16	23.59±1.79	135.1±10.3	87.8±5.3
χ^2/t		0.103	0.298	0.704	0.315	0.146
P		0.748	0.766	0.482	0.874	0.689

Table 2. Comparison of serum CXCL-16, GDF-15 and PLA-2 levels between the groups ($\bar{x}$±sd)

Group	Cases	CXCL-16(ng/ml)	GDF-15(ng/ml)	PLA-2(mmol/L)
Disease group	87	2.73±0.36	1.41±0.27	31.53±6.74
Healthy group	50	1.68±0.25	0.87±0.19	14.72±3.26
t		18.240	12.470	16.540
P		< 0.001	< 0.001	< 0.001

Table 3. Comparison of serum CXcl-16, GDF-15 and PLA-2 levels among patients with different NIHSS scores ($\bar{x}$±sd)

Group	Cases	CXCL-16(ng/ml)	GDF-15(ng/ml)	PLA-2(mmol/L)
Mild group	29	2.21±0.28	1.13±0.24	24.58±5.49
Moderate group	35	2.78±0.31[a]	1.45±0.27[a]	31.96±6.47[a]
Severe group	23	3.31±0.34[a,b]	1.70±0.29[a,b]	39.64±7.36[a,b]
F		82.190	30.140	35.460
P		< 0.001	< 0.001	< 0.001

Note: Compared to the mild group, [a]*P* < 0.001, compared to the moderate group, [b]*P* < 0.001.

were all up-regulated in the disease group (P < 0.001, **Table 2**).

Comparison of serum CXcl-16, GDF-15 and PLA-2 levels among patients with different NIHSS scores

The expression levels of serum CXCL-16, GDF-15 and PLA-2 in patients with mild, moderate and severe cerebral infarction were significantly different (P < 0.001). The expression levels of serum CXCL-16, GDF-15 and PLA-2 in cerebral infarction patients increased with the increase of the score. Pairwise comparison between the groups showed statistically significant differences (P < 0.001), as shown in **Table 3**.

Comparison of serum CXCL-16, GDF-15 and PLA-2 levels in patients with different infarction areas

The expression levels of serum CXcl-16, GDF-15 and PLA-2 in patients with small, medium and large infarction areas were significantly different (P < 0.001), and the levels increased with the enlargement of infarction areas. Pairwise comparison between the groups showed statistically significant differences (P < 0.001), as shown in **Table 4**.

Monitoring the expression levels of serum CXCL-16, GDF-15 and PLA-2 in cerebral infarction patients when discharged

After treatment, the expression levels of serum CXCL-16, GDF-15 and PLA-2 in cerebral infarction patients were significantly decreased (P < 0.001), as shown in **Table 5**.

ROC curve

The AUC areas of serum CXCL-16, GDF-15, and PLA-2 in predicting the therapeutic effect of cerebral infarction were 0.821, 0.883, and 0.711, respectively. The cutoff value of CXCL-16 was 2.32 ng/ ml, the sensitivity was 81.5%, and the specificity was 84.4%. The cutoff value of GDF-15 was 1.20 ng/ml, the sensitivity was 69.5%, and the specificity was 72.4%. The cutoff value of PLA-2 was 21.75 mmol/L, the sen-

Table 4. Comparison of serum CXCL-16, GDF-15 and PLA-2 levels in patients with different infarction areas ($\bar{x}$±sd)

Group	Cases	CXCL-16 (ng/ml)	GDF-15 (ng/ml)	PLA-2 (mmol/L)
Small infarction group	26	2.18±0.27	1.09±0.23	23.89±5.25
Medium infarction group	36	2.83±0.33[a]	1.42±0.26[a]	32.17±6.69[a]
Large infarction group	25	3.16±0.35[a,b]	1.73±0.31[a,b]	38.55±7.07[a,b]
F		62.930	36.610	33.601
P		< 0.001	< 0.001	< 0.001

Note: Compared to the mild group, [a]*P* < 0.001, compared to the moderate group, [b]*P* < 0.001.

Table 5. Changes of serum CXCL-16, GDF-15 and PLA-2 in patients with cerebral infarction after effective treatment ($\bar{x}$±sd)

Group	Cases	CXCL-16 (ng/ml)	GDF-15 (ng/ml)	PLA-2 (mmol/L)
Before treatment	87	2.73±0.36	1.41±0.27	31.53±6.74
After treatment	87	1.81±0.24	1.12±0.21	18.34±2.72
t		19.830	7.908	16.930
P		< 0.001	< 0.001	< 0.001

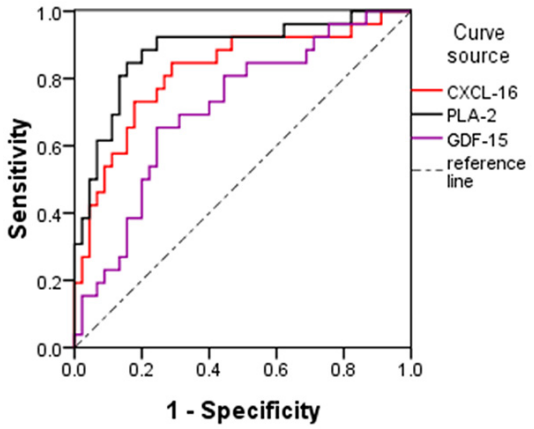

Figure 1. ROC curve to evaluate the efficacy of serum CXCL-16, GDF-15, PLA-2 indicators in predicting the therapeutic effect of cerebral infarction.

sitivity was 87.3%, and the specificity was 90.2%. See **Figure 1**.

Discussion

Cerebral thrombosis caused by atherosclerosis is the main cause of cerebral infarction, in which the inflammatory response plays a vital role [7-9]. Cerebral ischemia will trigger a series of stress responses in the body, which will stimulate inflammatory cells to secrete more inflammatory factors, interfere with the formation of brain stromal proteases, enhance the blood-brain barrier permeability, increase the inflammatory response, accelerate the apoptosis of normal cells, and increase the neuronal damage in the cerebral infarction area and ischemic penumbra [10, 11]. It is critical to find measurable markers to improve the prevention and treatment of cerebrovascular disease.

The study found that the expression levels of CXCL-16, GDF-15 and PLA-2 in patients with cerebral infarction were all higher than those in the healthy group, which is similar with the results of Ma et al. [12] that CXCL16 was significantly increased in patients with positive and negative microembolic signals. Therefore, higher levels of CXCL16, GDF-15 and PLA-2 may be a biomarker predicting the incidence of stroke and may cause plaque instability. Meanwhile, the results suggested that the expression levels of these indicators were correlated with the occurrence and development of cerebral infarction. CxCL-16 is a chemokine, essentially a small molecule secreted protein that links the body's inflammatory response and immune response. It can combine with T lymphocytes to produce chemotaxis on T lymphocytes [13-15], promote the transfer of T lymphocytes to cerebral ischemia and hypoxia, the formation of atherosclerotic plaques and the occurrence and development of cerebral infarction. GDF-15 is a secreted protein in the body [16], which is expressed in small amounts in tissues and organs in the normal physiological state [17, 18]. When the body has a strong inflammatory or immune response, its expression level will see a rapid rise. When there is ischemia, hypox-

ia, arteriosclerosis and other conditions in the cerebral tissue of patients with cerebral infarction, the body will cause a strong inflammatory response, increasing GDF-15 expression level. PLA-2 is a new vascular specific inflammatory marker, which can reflect the inflammation of the body's vascular walls. The mechanism of action between PLA-2 and cerebral infarction is to generate oxidized free fatty acids through the hydrolysis of oxidative lipoprotein in the body, which promotes the release of a large number of inflammatory factors in the body and promotes the gradual aggregation of monocytes and their evolution into macrophages [19]. These cells will bind to the damaged vascular endothelial cells, resulting in smaller vascular lumen, ischemia in brain tissue, and thus cerebral infarction. CXCL-16, GDF-15 and PLA-2 may play different roles in the onset, development, formation and instability of cerebral infarction through different action pathways. Some scholars have reported that the expression levels of CXCL-16 and PLA-2 in stable plaques and vulnerable plaques in patients with acute cerebral infarction were different, and the expression levels also increased with the increase of plaque vulnerability [20]. Animal experiments demonstrated that the expression of GDF-15 mRNA in mice with middle cerebral artery occlusion was significantly up-regulated after 3-24 h, and a large amount of GDF-15 expression was found in the infarct site; the more severe the infarct site, the higher the expression level of GDF-15 [21]. It was found in this study that serum CXCL-16, GDF-15 and PLA-2 levels increased with the aggravation of cerebral infarction and the enlargement of the infarction area. After monitoring the expression levels of serum CXcl-16, GDF-15 and PLA-2 in patients with cerebral infarction on the day of discharge, it was noted that the levels were significantly decreased compared with those before treatment. In consequence, serum CXCL-16, GDF-15 and PLA-2 indexes can be potential indicators for monitoring the condition and prognosis of patients with malignant blood diseases. When mapping CXCL-16, GDF-15 and PLA-2 indexes to predict the therapeutic effect of cerebral infarction, it was found that the three indexes all had high sensitivity and specificity, which is similar to the results of many studies [12, 22, 23] that CXCL16 has high sensitivity and specificity for patients with cerebral infarction, and GDF-15 and PLA-2 are independent predictors of cerebral infarction. However, because this study was a single-center study, the results may be inevitably somehow biased. In the future, multi-center studies need to be conducted to further reinforce the findings.

In summary, serum CXCL-16, GDF-15 and PLA-2 were all highly expressed in patients with cerebral infarction and changed with the disease severity. Therefore, they can be used as reliable indicators for assessing the incidence, severity, and prognosis of cerebral infarction patients.

Disclosure of conflict of interest

None.

Address correspondence to: Xiqi Liu, Department of Neurology, Cangzhou Central Hospital, No. 50, West Xinhua Rd, Yunhe District, 061000, Hebei Province, China. Tel: 86-15903175527; E-mail: xiqi_liu@163.com

References

[1] van Os HJ, Mulder IA, van der Schaaf IC, Kappelle LJ, Velthuis BK, Broersen A, Vos JA, Terwindt GM, Schonewille W, Ferrari MD, Algra A, van Walderveen MA and Wermer MJ. Role of atherosclerosis, clot extent, and penumbra volume in headache during ischemic stroke. Neurology 2016; 87: 1124-1130.

[2] O'Neal WT, Kamel H, Zhang ZM, Chen LY, Alonso A and Soliman EZ. Advanced interatrial block and ischemic stroke: the atherosclerosis risk in communities study. Neurology 2016; 87: 352-356.

[3] Binazzi A, Ferrante P and Marinaccio A. Occupational exposure and sinonasal cancer: a systematic review and meta-analysis. BMC Cancer 2015; 15: 49.

[4] Wei L, Ke Z, Zhao Y and Cai Z. The elevated lipoprotein-associated phospholipase A2 activity is associated with the occurrence and recurrence of acute cerebral infarction. Neuroreport 2017; 28: 325-330.

[5] Yilmaz H, Çelik HT, Gurel OM, Bilgic MA, Namuslu M, Bozkurt H, Ayyildiz A, Inan O, Bavbek N and Akcay A. Increased serum levels of GDF-15 associated with mortality and subclinical atherosclerosis in patients on maintenance hemodialysis. Herz 2015; 40 Suppl 3: 305-312.

[6] Yaghi S, Herber C, Boehme AK, Andrews H, Willey JZ, Rostanski SK, Siket M, Jayaraman MV, McTaggart RA, Furie KL, Marshall RS, Lazar RM and Boden-Albala B. The association

between diffusion MRI-defined infarct volume and NIHSS score in patients with minor acute stroke. J Neuroimaging 2017; 27: 388-391.
[7] Kapoor A, Lanctot KL, Bayley M, Herrmann N, Murray BJ and Swartz RH. Screening for post-stroke depression and cognitive impairment at baseline predicts long-term patient-centered outcomes after stroke. J Geriatr Psychiatry Neurol 2019; 32: 40-48.
[8] Zang RS, Zhang H, Xu Y, Zhang SM, Liu X, Wang J, Gao YZ, Shu M, Mei B and Li HG. Serum C-reactive protein, fibrinogen and D-dimer in patients with progressive cerebral infarction. Transl Neurosci 2016; 7: 84-88.
[9] Shi D, Xia T, Feng H and Cheng Q. Evaluating the diagnostic value of vWF:Ag, D-D and FDP in patients with acute cerebral infarction using ROC curves. Exp Ther Med 2014; 7: 1573-1577.
[10] Tai W, Garcia M, Mlynash M, Kemp S, Albers GW and Olivot JM. Lipoprotein phospholipase A2 mass and activity are not associated with the diagnosis of acute brain ischemia. Cerebrovasc Dis (Basel, Switzerland) 2014; 38: 324-327.
[11] Joshi AU, Orset C, Engelhardt B, Baumgart-Vogt E, Gerriets T, Vivien D and Kanse SM. Deficiency of Factor VII activating protease alters the outcome of ischemic stroke in mice. Eur J Neurosci 2015; 41: 965-975.
[12] Ma AJ, Yang SN, Wang Y, Wang X and Pan XD. Increase of serum CXCL16 level correlates well to microembolic signals in acute stroke patients with carotid artery stenosis. Clin Chim Acta 2016; 460: 6-71.
[13] Kim SH, Lee JY, Park SH, Jang HC, Lim EJ, Chang SJ and Lee SS. Plasma B-type natriuretic peptide level in patients with acute cerebral infarction according to infarction subtype and infarction volume. Int J Med Sci 2013; 10: 103-109.
[14] Ueland T, Smedbakken LM, Hallén J, Atar D, Januzzi JL, Halvorsen B, Jensen JK and Aukrust P. Soluble CXCL16 and long-term outcome in acute ischemic stroke. Atherosclerosis 2012; 220: 244-249.
[15] Ma A, Pan X, Xing Y, Wu M, Wang Y and Ma C. Elevation of serum CXCL16 level correlates well with atherosclerotic ischemic stroke. Arch Med Sci 2014; 10: 47-52.
[16] Artz A, Butz S and Vestweber D. GDF-15 inhibits integrin activation and mouse neutrophil recruitment through the ALK-5/TGF-βRII heterodimer. Blood 2016; 128: 529-541.
[17] Wang FF, Chen BX, Yu HY, Mi L, Li ZJ and Gao W. Correlation between growth differentiation factor-15 and collagen metabolism indicators in patients with myocardial infarction and heart failure. J Geriatr cardiol 2016; 13: 88-93.
[18] Hagström E, Held C, Stewart RA, Aylward PE, Budaj A, Cannon CP, Koenig W, Krug-Gourley S, Mohler ER 3rd, Steg PG, Tarka E, Östlund O, White HD, Siegbahn A and Wallentin L. Growth differentiation factor 15 predicts all-cause morbidity and mortality in stable coronary heart disease. Clin Chem 2017; 63: 325-333.
[19] Heriansyah T, Nafisatuzzamrudah N, Aini FN, Ridwan M, Primardhika RF, Wijayanti M, Bekti RS and Wihastuti TA. Reduction in vasa vasorum angiogenesis by Lp-PLA2 selective inhibitor through the HIF-1α and VEGF expression under dyslipidemic conditions in atherosclerosis pathogenesis. Cardiovas Hematol Agents Med Chem 2018; 16: 114-119.
[20] Hagström E, James SK, Bertilsson M, Becker RC, Himmelmann A, Husted S, Katus HA, Steg PG, Storey RF, Siegbahn A and Wallentin L. Growth differentiation factor-15 level predicts major bleeding and cardiovascular events in patients with acute coronary syndromes: results from the PLATO study. Eur Heart J 2016; 37: 1325-1333.
[21] Xu XY, Nie Y, Wang FF, Bai Y, Lv ZZ, Zhang YY, Li ZJ and Gao W. Growth differentiation factor (GDF)-15 blocks norepinephrine-induced myocardial hypertrophy via a novel pathway involving inhibition of epidermal growth factor receptor transactivation. J Biol Chem 2014; 289: 10084-10094.
[22] Wang XJ, Zhu L, Wu Y, Sun K, Su M, Yu LP, Chen JZ, Li WJ, Yang J, Yuan ZY and Hui RT. Plasma growth differentiation factor 15 predicts first-ever stroke in hypertensive patients. Medicine (Baltimore) 2016; 95: e4342.
[23] Wei LL, Ke ZY, Zhao Y and Cai ZY. The elevated lipoprotein-associated phospholipase A2 activity is associated with the occurrence and recurrence of acute cerebral infarction. Neuroreport 2017; 28: 325-330.