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PTX3 expression in the plasma of elderly ACI patients and its relationship with severity and prognosis of the disease

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Abstract. – OBJECTIVE: This study aims to investigate the expression of pentraxin3 (PTX3) in elderly patients with acute cerebral infarction (ACI) and to analyze the relationship of PTX3 with the severity and prognosis of ACI.

PATIENTS AND METHODS: Between June 2014 and August 2015, 96 elderly patients with first-onset of ACI admitted to our institution were enrolled in the present study. Also, 70 healthy elderly subjects were included as controls in this study. Levels of PTX3, C-reactive protein (CRP), tumor necrosis factor (TNF)-α, homocysteine (Hcy), fibrinogen (FIB), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were determined in all patients both pre-therapeutically and 30 days post-therapeutically. Moreover, the severity of ACI was evaluated using the National Institute of Health stroke scale (NIHSS), and the prognosis was evaluated using the Modified Rankin Scale (mRS). The differences in the levels of above parameters were compared between groups, and the relationship between PTX3 and above biochemical parameters as well as the scores were investigated.

RESULTS: PTX3 levels in the plasma of ACI patients were significantly higher than those of healthy controls ($p < 0.05$). Compared to low score group, mRS and levels of all parameters except Hcy and HDL-C were significantly increased in the patients of the high score group. In addition, plasma levels of HDL-C in the patients of the high score group were significantly lower than those in the low score group ($p < 0.05$). Biochemical parameters, NIHSS scores and mRS scores were significantly higher in the patients of the high concentration group than the low concentration group ($p < 0.05$), while no significant differences were observed in the plasma HDL-C levels between these two groups ($p > 0.05$). NIHSS scores and levels of all the biochemical parameters except HDL-C were significantly lower in the patients of the good prognosis group than in the patients of the poor prognosis group. HDL-C levels were significantly higher in the good prognosis group than in the poor prognosis group ($p < 0.05$). PTX3 levels were positively correlated with levels of CRP, TNF-α, Hcy, TC, TG and LDL-C as well as NIHSS score and mRS score, respectively, (r = 0.814, 0.682, 0.704, 0.726, 0.699, 0.734, 0.746, 0.753, p = 0.008, 0.043, 0.034, 0.027, 0.036, 0.024, 0.021, 0.019). However, no significant correlations were observed between FIB levels and HDL-C levels (r = 0.326, 0.626, p = 0.392, 0.071).

CONCLUSIONS: Plasma levels of PTX3 are significantly elevated in elderly ACI patients, and the levels increase along with the exacerbation of the disease and deterioration in the prognosis. Also, PTX3 levels are positively correlated with levels of inflammatory markers as well as lipids. Therefore, PTX3 can be used as a biomarker for predicting and evaluating clinical conditions of ACI in elderly patients.

Key Words:
Elderly, Acute cerebral infarction, Pentraxin-3, Severity, Prognosis.

Introduction

Acute cerebral infarction (ACI), a cerebrovascular event commonly occurred in the elderly, refers to necrosis of brain tissue resulting from cerebral ischemia and hypoxia caused by insufficient blood supply to the brain[1]. ACI is frequently complicated by atherosclerosis, diabetes mellitus and coronary heart disease with high morbidity and mortality as well as a high propensity for relapse, having a serious impact on patients themselves and their families as well. Therefore, early and timely diagnosis, assessment and treatment of ACI have important implications for improving therapeutic efficacy and ameliorate prognosis[2]. Multiple factors are involved in the initiation and development of ACI, including apopto-

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sis, oxidative stress and inflammation, etc. Inflammation response is an important event leading to the injury of affected area[3]. Initiation of ACI induces the release of a large number of cytokines, which aggravate the severity of brain damage through upregulating the expression of adhesion molecules or chemokines[4]. Acute phase reaction protein, which can accelerate the release of inflammatory mediators and activate the immune system, is closely associated with the degree of damage and, thereby, can serve as an important biomarker for the evaluation of the initiation and development of ACI[5]. C-reactive protein (CRP) is a common acute phase reactive protein to be used to assess inflammation in clinical settings; however, CRP, as a type of marker for systemic inflammation, results in poor specificity[6]. Pentraxin 3 (PTX3), a member of the pentraxin superfamily as well as an acute phase reactive protein, is produced by vascular endothelial cells as well as macrophages and secreted from blood vessels into lesion site, directly reflecting conditions of vascular inflammation[7]. However, few studies on PTX3 are available in China. In this regard, the present study is designed to explore the implication of PTX-3 in the diagnosis and treatment of ACI by assessing PTX3 expression in elderly ACI patients and investigating its relationship with the severity and prognosis of ACI.

Patients and Methods

Patients

Between June 2014 and August 2015, 96 elderly patients with first-onset of ACI admitted to our institution were enrolled in the present study. All patients met the criteria of the Chinese Guidelines of Diagnosis and Treatment for Acute Ischemic Stroke 2010. All ACI cases were confirmed by head CT and MRI. The inclusion criteria are listed as follows. (1) First-onset of ACI ≤ 72h. (2) Age ≥ 60 years. (3) Not having received any anti-inflammatory or lipid-lowering medications within half a month. (4) With complete medical record. Exclusion criteria include the following conditions. (1) Comorbidities affecting the liver, the kidney, and other vital organs. (2) Experiencing infections, autoimmune diseases, and malignancies. (3) Having undergone surgery or experienced trauma within three months. (4) Presenting severe cognitive impairment and being incapable of performing activities of daily living. This study included 65 males and 31 female patients with an age range of 60-74 years and a mean age of 65.18 ± 3.22 years. Also, 70 healthy elderly subjects (48 males, 32 females, age range 61-76 years, mean age 65.77 ± 4.24 years) seeking health check-up at our institution during the same period were also included as controls in this study. No significant differences were found in the gender and age of patients between two groups ($p > 0.05$). The study protocol was approved by the Medical Ethics Committee of our institution and written consent was obtained from all patients.

Outcome Measures

Four milliliters of fasting venous blood were collected from all patients the next morning of admission. Blood samples were placed in EDTA-treated tube centrifuged for 10 min at a speed of 3500 rpm. The supernatant containing the plasma was collected and stored at -80°C. PTX3, CRP, tumor necrosis factor-alpha (TNF)-α and homocysteine (Hcy) in the plasma were measured by using ELISA (BD-Biosciences, San Jose, CA, USA). Levels of fibrinogen (FIB), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were determined by using Olympus AU400 automated chemistry analyzer (Olympus, Tokyo, Japan). All these parameters were examined in patients 30 days after symptomatic treatment.

Groups of Patients

The severity of ACI was evaluated using National Institute of Health stroke scale (NIHSS)[8] and, thereby, patients were divided into high score group (NIHSS ≥ 5) and low score group (NIHSS < 5). Moreover, according to the median concentration of PTX3 (6.02 μg/L) patients were further divided into PTX3 high concentration group (PTX3 ≥ 6.02 μg/L) and low concentration group (PTX3 < 6.02 μg/L). Prognosis at 30d after ACI onset was evaluated using Modified Rankin Scale (mRS)[9], based on which patients were divided into good prognosis group (mRS ≤ 2) and poor prognosis group (mRS > 2). Levels of all biochemical parameters were compared across group pairs. No significant differences were observed in gender or age between different group pairs (Table I).

Classification Criteria

NIHSS assesses neurological outcome and degree of recovery for stroke patients based on the

Table I. Clinical characteristics of patients in various groups.

Group	n	Gender (M/F)	χ^2	p	Age (years)	t	p
High score group	47	32/15	1.063	0.273	65.23 ± 2.64	1.085	0.281
Low score group	49	33/16			65.17 ± 1.89		
High concentration group	46	30/16	1.142	0.084	63.22 ± 3.20	1.136	0.259
Low concentration group	50	35/15			66.35 ± 2.87		
Poor prognosis group	42	28/14	1.217	0.069	64.27 ± 4.13	1.451	0.150
Good prognosis group	54	37/17			66.07 ± 5.24		

levels of consciousness, extra ocular movement, visual-field loss, facial paresis, motor strength, ataxia, sensory loss, language, dysarthria and neglect. Ratings for each item are scored with 0 to 2 grads and 0-3 grades with 0 as normal and higher scores define stroke with more severity.

mRS is classified into six categories: (0) no symptoms at all; (1) no significant disability, despite symptoms able to carry out all usual duties and activities; (2) slight disability, unable to carry out all previous activities but able to look after their own affairs without assistance; (3) moderate disability, requiring some help but able to walk without assistance; (4) moderate to severe disability, unable to walk without assistance, unable to attend to their own body without assistance; (5) severe disability, requiring constant nursing care and attention.

Statistical Analysis

All statistical analyses were performed using SPSS software version 19.0 (SPSS Inc., Chicago, IL, USA). Quantitative data were expressed as mean ± SD. Differences between groups were analyzed using *t*-test. Qualitative data were expressed as percentages or ratios and data were compared between groups using χ^2-test. The relationship between PTX3 and other parameters, NIHSS scores and mRS scores were analyzed using Spearman rank correlation. $p < 0.05$ was considered statistically different.

Results

PTX3 Levels in ACI Patients and Healthy Controls

PTX3 levels in ACI patients were significantly higher than those in healthy controls [(6.02 ± 1.87) μg/L *vs.* (1.51±0.21) μg/L, $t = 2.956$, $p = 0.004 < 0.05$].

Biochemical Parameters and mRS Scores in Patients with Different NIHSS Scores

Compared to the low score group, mRS and levels of all parameters except Hcy and HDL-C were significantly increased in patients of the high score group. In addition, plasma levels of HDL-C in patients of the high score group were significantly lower than those in the low score group ($p < 0.05$) (Table I).

Table II. Biochemical parameters and mRS scores in patients with different NIHSS scores ($\bar{x} \pm s$).

Group	n	PTX-3 (μg/L)	CRP (mg/L)	TNF-α (ng/mL)	Hcy (μmol/L)	FIB (g/L)
High score group	47	6.87 ± 0.93	5.02 ± 1.18	1.62 ± 0.41	15.26 ± 2.74	5.17 ± 1.02
Low score group	49	4.26 ± 0.88	3.22 ± 0.71	1.29 ± 0.33	14.37 ± 1.88	4.02 ± 0.83
t		1.998	2.004	2.571	1.323	2.225
p		0.049	0.048	0.012	0.189	0.029
Group	**n**	**TC (mmol/L)**	**TG (mmol/L)**	**LDL-C (mmol/L)**	**HDL-C (mmol/L)**	**mRS (grade)**
High score group	47	1.69 ± 0.31	4.27 ± 1.02	2.55 ± 0.63	1.19 ± 0.27	3.39 ± 0.54
Low score group	49	1.14 ± 0.25	2.25 ± 0.58	1.49 ± 0.26	1.39 ± 0.24	1.97 ± 0.42
t		2.384	2.612	2.155	2.583	2.407
p		0.019	0.011	0.034	0.011	0.018

Biochemical Parameters, NIHSS Scores and mRS Scores in Patients with Different PTX3 Levels

Biochemical parameters, NIHSS scores and mRS scores were significantly higher in patients of high concentration group than low concentration group ($p < 0.05$), while no significant differences were observed in plasma HDL-C levels between these two groups ($p > 0.05$) (Table III).

Biochemical Parameters and NIHSS Scores in Patients with Different mRS Scores

Levels of all biochemical parameters except HDL-C and NIHSS scores were significantly lower in patients of good prognosis group than in patients of poor prognosis group. HDL-C levels were significantly higher in good prognosis group than in poor prognosis group ($p < 0.05$) (Table IV).

Relationship Between PTX3 and Other Outcome Measures

PTX3 levels were positively correlated with levels of CRP, TNF-α, Hcy, TC, TG and LDL-C as well as NIHSS score and mRS score, respectively, (r = 0.814, 0.682, 0.704, 0.726, 0.699, 0.734, 0.746, 0.753, p = 0.008, 0.043, 0.034, 0.027, 0.036, 0.024, 0.021, 0.019). However, no significant correlations were observed between FIB levels and HDL-C levels (r = 0.326, 0.626 p = 0.392, 0.071) (Table V).

Discussion

ACI has become one of the major causes of increased mortality worldwide, seriously threatening people's life. Therefore, early prevention, diagnosis and treatment, as well as prognosis evaluation, can effectively decrease the incidence and mortality of ACI[10]. ACI can be diagnosed by using multiple methods, such as MRI and CT scan. However, considering the low availability of advanced equipment in several countries, developing simple and efficacious assessment indicators becomes the key to the diagnosis and treatment of ACI. In recent years, biomarkers have provided important evidence for diagnosing diseases of all systems except the brain. Due to the complexity of the brain, rapid and efficacious diagnostic standards are still lacking for evaluating the severity of brain damage and cell pathology in ACI patients. Hence, research on biomarkers for the diagnosis of brain damage of ACI patients is desirable[11]. Atherosclerosis, the pathological basis of cerebral infarction, is closely associated with inflammatory response. Inflammatory cytokines, produced by both immune cells and non-immune cells, can mediate inflammation and pathological processes. As a result, studies were performed on many inflammatory factors, such as CRP and TNF-α[12]. However, these factors resulted in low specificity. The recently discovered PTX3 is in low level in normal human body. However, when inflammation is initiated, PTX3 is produced and released by vascular endothelial cells and macrophages, thereby mediating inflammatory signaling pathways[13]. Furthermore, abnormal lipid metabolism induces atherosclerosis and, thereby, triggers ACI. Therefore, dyslipidemia plays an important role in the initiation and development of ACI[14]. In the present work, levels of plasma PTX3, inflammatory factors

Table III. Biochemical parameters, NIHSS scores and mRS scores in patients with different PTX3 levels ($\bar{x} \pm s$).

Group	n	CRP (mg/L)	TNF-α (pg/mL)	Hcy (μmol/L)	FIB (g/L)	TC (mmol/L)
High concentration group	46	4.97 ± 1.13	1.63 ± 0.33	14.96 ± 2.04	5.22 ± 1.14	1.57 ± 0.28
Low concentration group	50	3.24 ± 0.74	1.26 ± 0.24	11.15 ± 1.87	4.15 ± 0.96	1.22 ± 0.37
t		2.038	2.105	2.237	2.517	2.413
p		0.023	0.038	0.028	0.014	0.018
Group	**n**	**TG (mmol/L)**	**LDL-C (mmol/L)**	**HDL-C (mmol/L)**	**NIHSS (grade)**	**mRS (grade)**
High concentration group	46	4.31 ± 1.07	2.49 ± 0.47	1.21 ± 0.32	7.83 ± 1.12	3.25 ± 1.02
Low concentration group	50	2.28 ± 0.62	1.52 ± 0.32	1.23 ± 0.19	4.62 ± 1.03	1.88 ± 0.39
t		2.038	2.019	2.001	2.732	2.183
p		0.044	0.046	0.048	0.008	0.032

Table IV. Levels of biochemical parameters and NIHSS scores in patients with different mRS scores ($\bar{x} \pm s$).

Group	n	PTX-3 (μg/L)	CRP (mg/L)	TNF-α (pg/mL)	Hcy (μmol/L)	FIB (g/L)
Poor prognosis group	42	6.19 ± 1.15	4.22 ± 1.06	1.48 ± 0.33	13.87 ± 2.63	5.15 ± 1.06
Good prognosis group	54	4.11 ± 1.23	3.04 ± 1.19	1.11 ± 0.29	9.53 ± 1.24	4.02 ± 0.89
t		2.154	2.009	2.371	2.654	2.348
p		0.034	0.047	0.020	0.009	0.021
Group	**n**	**TC (mmol/L)**	**TG (mmol/L)**	**LDL-C (mmol/L)**	**HDL-C (mmol/L)**	**NIHSS (grade)**
Poor prognosis group	42	1.49 ± 0.26	4.19 ± 1.21	2.41 ± 0.47	1.22 ± 0.28	7.52 ± 1.14
Good prognosis group	54	1.16 ± 0.33	2.11 ± 0.63	1.42 ± 0.38	1.47 ± 0.36	4.25 ± 0.97
t		2.203	2.314	2.446	2.402	2.573
p		0.030	0.023	0.016	0.018	0.012

and lipids were measured to investigate their impact on elderly ACI patients, and the relationships between PTX3 and other factors were analyzed.

The study showed that plasma levels of PTX3 were significantly increased in ACI patients than in healthy controls ($p < 0.05$), indicating that PTX3 may be involved in the pathological process of ACI and may be used as a biomarker for predicting PCI. In addition, other inflammatory factors, including CRP, TNF-α and Hcy also plays important roles in the pathogenesis of ACI. CRP and PTX3 are members of the pentraxin family. The long chain pentraxin (PTX3) and the short chain pentraxin (CRP) are both acute phase reactive proteins. CRP is massively produced and secreted from stem cells stimulated by pro-inflammatory cytokines secreted by macrophages during acute inflammation[15]. TNF-α, as one of the earliest inflammatory factors produced during the occurrence of inflammation, can induce the release of other inflammatory factors. Mainly produced by macrophages, TNF-α exhibits anti-inflammatory function, increases endothelial permeability and stimulates the release of many neurotoxic factors, aggravating the severity of brain injury[16]. Meanwhile, some paper showed that a high expression of Hcy is an independent risk factor for ischemic encephalopathy, which can accelerate the proliferation of the vascular smooth muscle cells, it cause the thickening of the vessel wall and, thereby, it decrease vascular elasticity. Besides, it can cause injury to vascular endothelium through oxidative stress, thereby leading to the development of cerebral thrombosis and cerebral infarction. FIB, a coagulation factor as well as an acute phase reactive protein produced by the liver, is involved in blood coagulation[17]. The higher concentration of FIB results in a higher viscosity of whole blood, accelerated platelet aggregation and thrombosis, thereby deteriorating cerebral infarction[18]. The results of the present research demonstrated that mRS scores and levels of all inflammatory factors except Hcy were significantly higher in patients of high score group than in low score group. Also, levels of these factors and NIHSS scores were significantly lower in patients of good prognosis group than in poor prognosis group ($p < 0.05$), indicating that inflammatory factors can serve as one of the biomarkers for the diagnosis and the prognosis evaluation of ACI.

Table V. Correlation between PTX-3 and other outcome measures.

Outcome measures	Correlation coefficient®	*p*
CRP	0.814	0.008
TNF-α	0.682	0.043
Hcy	0.704	0.034
FIB	0.326	0.392
TC	0.726	0.027
TG	0.699	0.036
LDL-C	0.734	0.024
HDL-C	0.626	0.071
NIHSS score	0.746	0.021
mRS score	0.753	0.019

The initiation and development of ACI are closely associated with hyperlipidemia. Evidence has been shown that a large amount of cholesterol accelerates the progression of atherosclerosis and plaque formation, thereby resulting in cerebral infarction; meanwhile, inflam-

mation is accompanied by lipid accumulation[19]. TC, TG, LDL-C and HDL-C are common indicators of lipids. The elevation in the first three indicators results in the exacerbation of cerebral infarction as well as unfavorable prognosis[20,21]. However, HDL-C can remove excessive cholesterols and transported them to the liver for degradation[22]. Besides, it also exhibits anti-atherosclerosis function through exerting anti-oxidation and protecting vascular endothelium[23]. This report showed that mRS scores, NIHSS scores and levels of all lipids except HDL-C were significantly higher in patients of both high score group and poor prognosis group than in low score group, but plasma HDL-C levels were significantly lower than those of low score group ($p < 0.05$), suggesting that lipids can also be used as auxiliary biomarkers for assessing the condition and prognosis of ACI.

Comparison of other parameters, mRS scores and NIHSS scores between ACI patients with different PTX3 concentrations showed that levels of these parameters, NIHSS scores and mRS scores were significantly higher in patients with high PTX3 concentrations than in patients with low PTX3 concentrations ($p < 0.05$). Further linear regression analysis revealed that along with the increase in PTX3 levels, levels of CRP, TNF-α, Hcy, TC, TG and LDL-C as well as NIHSS scores and mRS scores were significantly increased ($p < 0.05$), but no significant correlation was observed between FIB level and HDL-C level ($p > 0.05$). These findings suggested that PTX3 is involved in the initiation and development of ACI likely through synergistic collaboration with other inflammatory factors and lipids, thereby aggravating the disease and affecting prognosis[24]. The most significant correlation between PTX3 and CRP may be attributed to the presence of homologous carboxyl-terminals in the molecular structures of these two factors[25].

Conclusions

In summary, plasma PTX3 levels are significantly elevated in elderly ACI patients. Inflammatory factors and lipids play an important role in predicting the development and prognosis of ACI. Also, PTX3 is positively correlated with multiple inflammatory factors and hence, can effectively and rapidly reflect the severity and prognosis of ACI.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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