VALUE OF SERUM LP-PLA2, ADAMTS4 AND FGF23 IN EVALUATING THE STABILITY OF CAROTID PLAQUE

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ABSTRACT

Objective: *The objective is to investigate the value of Lp-PLA2, ADAMTS4, and FGF23 serum in evaluating the stability of carotid plaque.*

Methods: *From August 2018 to August 2019, 64 patients with carotid stenosis were admitted to our hospital and enrolled. According to the pathological tissue types, the patients were divided into a stable plaque group (n = 34) and an unstable plaque group (n = 30). The patients who were selected for physical examination in our hospital during the same time were the control group (n = 30). The levels of Lp-PLA2, ADAMTS4, and FGF23 serum in each group were detected using an enzyme-linked immunosorbent assay. The Lp-PLA2, ADAMTS4, and FGF23 serum levels were analysed using the receiver operating characteristic (ROC) curve analysis to evaluate the value of the carotid plaque stability.*

Results: *The Lp-PLA2, ADAMTS4, FGF23, IL-6, and hs-CRP serum levels were significantly higher in the unstable plaque group than in the stable plaque group and control group. The Lp-PLA2, ADAMTS4, FGF23, IL-6, and hs-CRP levels in the stable plaque group were significantly higher than those in the control group, and the difference was statistically significant (P<.01). In the stable plaque group, the neovascular grading was primarily Grades I and II, accounting for 67.65%. The unstable plaque group was primarily Grades III and IV, accounting for 70.00%. Significant differences were found in the grades of neovascularisation between the two groups (P<.05). A multivariate logistic regression analysis showed that Lp-PLA2, ADAMTS4, FGF23, IL-6, and hs-CRP levels are independent risk factors for carotid plaque stability (P<.05). Using the ROC curve analysis, the area under the curve (AUC) for the Lp-PLA2 evaluation of carotid plaque stability was 0.952. The optimal cutoff value was 134.26 μg/L. The sensitivity was 82.45%, and the specificity was 76.49%. The stability of the carotid plaque was evaluated using ADAMTS4 with an AUC of 0.931, with an optimal cutoff value of 98.26 ng/mL. The sensitivity was 79.58%, and specificity was 85.61%. The stability of carotid plaque evaluated using FUC23 showed an AUC of 0.915. The cutoff value was 55.79 pg/mL. The sensitivity was 81.64%, and the specificity was 76.48%.*

Conclusion: *The Lp-PLA2, ADAMTS4, and FGF23 levels have a certain value in evaluating the stability of carotid plaque and can be widely used in the clinic.*

Keywords: *Lp-PLA2, ADAMTS4, FGF23, carotid plaque stability.*

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Introduction

Carotid plaque is the most common manifestation of carotid atherosclerosis, primarily in the bifurcation of the common carotid artery. Age, smoking, hypertension, diabetes, and hyperlipidaemia can cause carotid artery necrosis. Intracranial hypoperfusion and plaque detachment form an embolus, which eventually leads to the occurrence of an intracranial arterial embolism[1]. Clinical manifestations are transient unilateral limb dyskinesia, aphasia, monocular blindness, headache, dizziness, syncope, and other symptoms, which, if severe, can seriously endanger the patient's life[2].

Clinically, the carotid plaque morphology and carotid stenosis were examined to determine the risk of carotid plaque, and targeted treatment was given in time[3]. Lipoprotein-related phospholipase A2 (lipoprotein-associated phosPhohPaseA2, Lp-PLA2), also known as platelet-activating factor acetamidine hydrolase, is a phospholipase secreted by inflammatory cells that promotes the hydrolysis of oxidised

phospholipids and is a member of the phospholipase A2 superfamily. Studies have shown that Lp-PLA2 is highly expressed in patients with carotid stenosis[4]. Platelet reactive protein A disintegrin and metallopeptidase 4 (ADAMTS4) is a $Zn2^{+}$-dependent endocrine metalloproteinase that has been found in recent years, which is closely related to the formation of carotid plaque[5]. Fibroblast growth factor 23 (FGF23) is a newly discovered secreted protein involved in blood phosphorus metabolism, which is of great significance in the metabolism of substances affecting plaque stability[6]. This study was conducted in our hospital to evaluate the value of the Lp-PLA2, ADAMTS4, and FGF23 serum in evaluating the stability of carotid plaque.

Data and methods

General data

From August 2018 to August 2019, 64 patients with carotid stenosis were admitted to our hospital and enrolled. The inclusion criteria were based on the criteria of the American Institute of Ultrasound Conference. For all patients, carotid artery stenosis was found using carotid artery ultrasonography, with a degree not less than 50%.

Patients with the following criteria were excluded:

• Stenosis combined with a malignant tumour, infectious disease, autoimmune disease, or rheumatism;

• Severe liver and kidney dysfunction;

• Severe oedema;

• Hypothyroidism or those who were taking thyroid hormone;

• Osteoarthritis, synovitis, or osteoporosis;

• Coronary stenosis or peripheral arterial stenosis;

• Fibromuscular dysplasia, aortitis and post-radiation fibrosis, and other non-arteriosclerotic carotid stenoses;

• Recently applied immunosuppressive agents, oestrogen, glucocorticoids, and other drugs

• Blood diseases and serious heart disease.

The wall echo, plaque size, and location and type of carotid artery stenosis were observed. Plaque echo enhancement, sound and shadow, and smooth surface showed hard spots. Low echo, rough surfaces, and mixed plaques were soft spots. Soft spots and mixed plaques were collectively referred to as unstable plaques, and hard spots and flat plaques were collectively referred to as stable plaques. According to the patient's plaque type, patients were divided into a stable or unstable plaque group. There were 34 patients in the stable plaque group, consisting of 18 males and 16 females with an average age of 62.35±7.46 years and an average BMI of 20.13±1.03 Kg/m^2. Of these, there were 5 cases of diabetes and 4 cases of smoking history.

Thirty patients comprised the unstable plaque group, consisting of 16 males and 14 females with an average age of 61.98±7.33 years and a mean BMI of 20.12±1.11 Kg/m2. Moreover, of these, there were 6 cases of diabetes and 5 cases of smoking history. The patients who were selected for physical examination were the control group. In total, 30 patients were in the control group, including 15 males and 15 females. The average age was 62.16±7.25 years old, and the average BMI was 20.09±1.05 Kg/m^2.

In this group, there were 4 cases of diabetes and 5 cases of smoking history. No significant differences were found in age, gender, and BMI between the groups (P>.05; Table 1).

Groups	Age (years)	Gender (cases)		BMI value (Kg/m^2)	Diabetes (cases)	Smoking history (cases)
		Male	Female			
Control (n = 30)	62.16±7.25	15	15	20.09±1.05	5	4
Stable plaque (n = 34)	62.35±7.46	18	16	20.13±1.03	6	5
Unstable plaque (n = 30)	61.98±7.33	16	14	20.12±1.11	4	5
F/χ^2	0.200	0.081		0.010	0.238	0.133
P	.980	.960		.988	.888	.936

Table 1: Comparison of general data of subjects in each group ($\bar{x}\pm s$).

Observation indices

From all subjects, 5 ml of fasting venous blood was taken in the morning and centrifuged at 2500 r/min for 15 min. The serum was carefully separated and refrigerated in an environment of 80°C to avoid repeated freezing and thawing. The Lp-PLA2, ADAMTS4, FGF23, interleukin-6 (IL-6), and hypersensitive C-reactive protein (hs-CRP) serum levels were measured using an enzyme-linked immunosorbent assay. Ultrasound examination and contrast-enhanced ultrasonography were performed on all subjects, and the angiographic grading in carotid plaques was evaluated according to the degree of signal enhancement in the plaques:

• Grade 1: no plaque enhancement;

• Grade II: plaque internal or peripheral enhancement, mainly in multiple-point distribution;

• Grade III: plaque internal or peripheral enhancement, mainly in the form of point or line distribution;

• Grade IV: plaque internal or peripheral enhancement, mainly in diffused grade linear distribution.

The neovascularisation of the carotid plaque of subjects in each group was graded.

Statistical methods

The data in this study were analysed using the SPSS20.0 software package. All the measurement data were compared using $\bar{x}\pm s$. The comparison between groups was performed via the F test. The enumeration data were expressed as percentages. The comparison between groups was performed using the chi-square (χ^2) test. The ranking data were compared using the ridit test. Multivariate logistic regression was used to analyse the independent risk factors of carotid plaque stability. The ROC curve was used to analyse the value of Lp-PLA2, ADAMTS4, and FGF23 in evaluating the stability of carotid plaque. The statistical results were statistically significant at P<.05.

Results

Comparison of serum Lp-PLA2, ADAMTS4, FGF23, IL-6, and hs-CRP levels in each group

The levels of the Lp-PLA2, ADAMTS4, FGF23, IL-6, and hs-CRP serum in the unstable plaque group were significantly higher than those in the stable plaque group and control group. The Lp-PLA2, ADAMTS4, FGF23, IL-6, and hs-CRP serum levels in the stable plaque group were significantly higher than those of the control group, and the differences were statistically significant (P<.01; Table 2).

Groups	Lp-PLA2 (μg/L)	ADAMTS4 (ng/mL)	FGF23 (pg/mL)	IL-6 (pg/mL)	hs-CRP (mg/L)
Control (n = 30)	51.03±15.36	46.13 ±18.36	32.16±6.15	23.15±4.61	2.85±1.26
Stable plaque (n = 34)	95.11±32.51[a]	72.31±21.43[a]	46.15±7.45[a]	45.76±5.41[a]	5.22±1.34[a]
Unstable plaque (n = 30)	198.45±45.42[ab]	111.21±18.42[ab]	73.85±8.45[ab]	71.45±6.48[ab]	7.59±2.01[ab]
F	155.810	84.280	246.570	569.500	68.930
P	<.001	<.001	<.001	<.001	<.001

Table 2: Comparison of the Lp-PLA2, ADAMTS4, FGF23, IL-6, and hs-CRP serum levels in each group ($\bar{x}\pm s$).
Notes: a Compared with the control group, P<.05; b Compared with the stable plaque group, P<.05.

Comparison of neovascularisation of carotid plaques in each group

In the stable plaque group, the neovascular grading was primarily Grades I and II, accounting for 67.65%. The unstable plaque group was primarily Grades III and IV, accounting for 70.00%. Significant differences were found in the grades of neovascularisation between the two groups (P<.05; Table 3).

Groups	Grade I plaque	Grade II plaque	Grade III plaque	Grade IV plaque
Stable plaque (n = 34)	11(32.35%)	12(35.29%)	7(20.59%)	4(11.76%)
Unstable plaque (n = 30)	3(10.00%)	6(20.00%)	9(30.00%)	12(40.00%)
χ^2	10.613			
P	.014			

Table 3: Comparison of neovascularisation of carotid plaques in each group (cases, %).

Multivariate logistic regression analysis of independent risk factors for carotid plaque stability

A multivariate logistic regression analysis showed that the Lp-PLA2, ADAMTS4, FGF23, IL-6, and hs-CRP levels were independent risk factors for carotid plaque stability (P<.05; Table 4).

Indices	B	SE	95%CI	*P*
Lp-PLA2	2.48	0.95	4.658-85.461	0.015
ADAMTS4	2.46	0.86	3.569-86.521	0.026
FGF23	1.86	0.82	4.568-84.159	0.038
IL-6	1.26	0.79	2.018-16.524	0.041
hs-CRP	1.24	0.56	8.471-98.421	0.025

Table 4: Multivariate logistic regression analysis of independent risk factors for carotid plaque stability.

Receiver operating characteristic curve analysis of the value of Lp-PLA2, ADAMTS4, and FGF23 in evaluating the stability of carotid plaque

Using the ROC curve analysis, the AUC of the Lp-PLA2 evaluation of carotid plaque stability was 0.952, and the optimal cutoff value was 134.26 μg/L. The sensitivity was 82.45%, and the specificity was 76.49%. The AUC of the carotid plaque stability evaluated via ADAMTS4 was 0.931, and the optimal cutoff value was 98.26 ng/mL.

The sensitivity was 79.58%, and the specificity was 85.61%. The stability of carotid plaque evaluated by FUC23 with an AUC was 0.915, and the best cutoff value was 55.79 pg/mL. The sensitivity was 81.64%, and the specificity was 76.48%. See the results in Table 5.

Indices	AUC	95% CI	Best cutoff value	Sensitivity	Specificity
Lp-PLA2	0.952	0.856-0.984	134.26	82.45%	76.49%
ADAMTS4	0.931	0.843-0.974	98.26	79.58%	85.61%
FGF23	0.915	0.862-0.971	55.79	81.64%	76.48%
IL-6	0.743	0.668-0.782	56.89	70.18%	71.29%
hs-CRP	0.716	0.695-0.773	6.41	69.48%	72.43%

Table 5: Receiver operating characteristic curve analysis of the value of Lp-PLA2, ADAMTS4, and FGF23 in evaluating the stability of carotid plaque.

Conclusion

Cerebrovascular disease is one of the diseases with higher global morbidity and mortality. The change in carotid plaque formation and stability is the pathological basis of cerebrovascular diseases, such as ischaemic stroke[7]. Many studies have found that cerebral ischaemia and hypoxia caused by carotid stenosis can induce cerebrovascular disease, such as ischaemic stroke, to a certain extent, but it is not proportional to the incidence of cerebrovascular diseases[8]. Plaque stability is of great significance in influencing the occurrence of cerebrovascular disease and has received extensive attention from scholars in the medical field. In this study, the angiographic grading of patients with stable plaque was predominantly Grades I and II, accounting for 67.65%. The unstable plaque group was primarily Grades III and IV, accounting for 70.00%. Significant differences were found in the grades of neovascularisation between the two groups (P<.05), suggesting that the plaque fragility of patients with Grades III and IV is larger than that of patients with Grades I and II. The degree of plaque instability is serious, and the incidence of cerebrovascular diseases, such as coronary heart disease and ischaemic stroke, is high.

The main methods for clinically examining the stability of carotid plaque are the Doppler ultrasound, transcranial Doppler ultrasound, computed tomography (CT) angiography, nuclear magnetic angiography, and digital subtraction angiography, but they are expensive and have certain requirements for patients' conditions[9]. Therefore, it is urgent to find a test index that can evaluate the stability of carotid plaque.

Moreover, Lp-PLA2 is a newly discovered vascular-specific inflammatory marker, which is mainly synthesised and secreted by lymphocytes and macrophages in plaques. It can regulate apoptotic macrophages by inflammatory mediators, increase the thickness of the arterial wall, promote the formation of arterial plaque, and accelerate the patient's course[10, 11]. The US Food and Drug Administration (FDA) has listed Lp-PLA2 as a test index for predicting cerebrovascular disease in patients with coronary heart disease and ischaemic stroke[12].

The structure of ADAMTS4 and the hydrolysis of extracellular matrix proteins are similar to those of MMPs, but ADAMTS4 can directly react with the extracellular matrix, and the effect is more obvious[13]. More studies have shown that ADAMTS4 expression levels are elevated in atherosclerotic carotid and carotid plaques[14]. In addition, FGF23 plays an important role in the vascular calcification mechanism, leading to the occurrence of vascular dysfunction and atherosclerosis to a certain extent[15].

In this study, the Lp-PLA2, ADAMTS4, FGF23, IL-6, and hs-CRP serum levels in the unstable plaque group were significantly higher than those in the stable plaque group and control group. The Lp-PLA2, ADAMTS4, FGF23, IL-6, and hs-CRP levels in the stable plaque group were significantly higher than those of the control group, and the difference was statistically significant (P<.01). In the multivariate logistic regression analysis, Lp-PLA2, ADAMTS4, FGF23, IL-6, and hs-CRP levels were independent risk factors for carotid plaque stability (P<.05), suggesting that the Lp-PLA2, ADAMTS4, FGF23, IL-6, and hs-CRP serum levels are related to the stability of carotid plaque, to a certain extent. The Lp-PLA2, ADAMTS4, and FGF23 levels also induce plaque instability to a certain extent. Using the ROC curve analysis, the AUC of the Lp-PLA2 evaluation of carotid plaque stability was 0.952, and the optimal cutoff value was 134.26 μg/L. The sensitivity was 82.45%, and the specificity was 76.49%. The carotid plaque stability of the AUC evaluated by ADAMTS4 was 0.931, and the optimal cutoff value was 98.26 ng/mL. The sensitivity was 79.58%, and the specificity was 85.61%. The stability of carotid plaque evaluated by FUC23 with an AUC was 0.915, and the best cutoff value was 55.79 pg/mL. The sensitivity was 81.64%, and the specificity is 76.48%, indicating that the levels of Lp-PLA2, ADAMTS4, and FGF23 can evaluate the stability of carotid plaque and can be used as a serological index to evaluate the stability of carotid plaque. In conclusion, the levels of serum of Lp-PLA2, ADAMTS4, and FGF23 vary with the severity of carotid plaque stability and induce the instability of plaque to a certain extent. These can be widely used as serological indices to evaluate the stability of carotid plaque.

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