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Serum ANGPTL4 and SIRT1 factor levels and the Carotid Atherosclerotic plaque stability relationship analysis

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ABSTRACT

This study was to investigate the relationship between the levels of Angiopoietin-Like Protein 4 (ANGPTL4) and Silent Mating-type Information Regulation 2 Homolog 1 (SIRT1) and the stability of carotid atherosclerotic plaque. For this purpose, 108 patients with coronary heart disease in our hospital from Jan 2021 to May 2022 were selected as the coronary heart disease (CHD) group and 80 patients with the healthy examination as the control group. Patients' serum levels of ANGPTL4 and SIRT1 were collected, and their stability of carotid atherosclerotic plaque was determined by carotid ultrasound. According to their stability results, patients were divided into three subgroups: No plaque, Stable plaque, and Unstable plaque. The serum ANGPTL4 and SIRT1 levels were analyzed in different groups, and the correlation between their serum levels and the stability of carotid atherosclerotic plaque was analyzed by rank correlation. Results showed that the CHD group's serum ANGPTL4 and SIRT1 levels were lower, with statistical significance (P<0.05); A statistically significant difference in serum ANGPTL4 and SIRT1 levels were observed among patients with No plaques, Stable plaques, and Unstable plaques (P<0.05); A negative correlation was observed between serum levels of ANGPTL4 and SIRT1 and the stability of carotid atherosclerotic plaque (r=-0.438, -0.717, P<0.001); Serum ANGPTL4 and SIRT1 can be used as the evaluation method of carotid atherosclerotic plaque stability. When ANGPTL4 ≤ 30.17mg/L and SIRT1 ≤ 6.91μg/L, patients were more likely to develop unstable plaques; When ANGPTL4 ≤ 30.40mg/L and SIRT1 ≤ 6.87μg/L, patients were more likely to develop plaques (instability and/or stability). In conclusion, the serum levels of ANGPTL4 and SIRT1 in patients with CHD decreased. ANGPTL4 and SIRT1 will participate in the formation and development of carotid plaque, which can be used as a serological evaluation index to evaluate the occurrence and carotid atherosclerotic plaque's stability.

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Introduction

A survey report from 204 countries and regions showed that there have been 197.2 million cases of ischemic heart disease and 9.1 million deaths worldwide, which curbs human health development. Coronary atherosclerosis, a key type of ischemic heart disease, represents a significant cause of mortality (1). Despite the extensive advantages of modern revascularization methods, statin therapy, and successful efforts to address modifiable risk factors like smoking and exercise over the last four decades, a residual risk remains evident, as revealed by the high number of new cases that emerge annually (2). One of the main characteristics of atherosclerosis is its systematic and progressive nature. The pathogenesis, morphology, evolution, and segmental (bifurcation) stability of plaque determine the occurrence and development of complications of carotid and coronary atherosclerosis in patients (3). Research showed that an association was observed between the neovascularization and stability of carotid artery plaques and the progression of coronary artery disease (4). Katano et al. (5) believed that serum ANGPTL4 was involved in the upregulation of anti-angiogenic regulatory function in hypercalcified plaques. SIRT1 was found to regulate the cross-linking between endothelial and vascular smooth muscle cells for preventing adverse arterial remodeling and maintaining vascular homeostasis. This was achieved by downregulating acetylated LKB1 protein through HERC2 (6). The objective of this study was to investigate the association between serum levels of ANGPTL4 and SIRT1 factors and the stability of carotid atherosclerotic plaque (CAP). The findings are as follows.

Materials and Methods

Materials

A total of 108 CHD patients who were admitted to our hospital between January 2021 and May 2022 were included in the CHD group, while 80 healthy individuals who visited during the same period were selected as the control group. Inclusion criteria: ① Patients with coronary atherosclerotic heart disease diagnosed by echocar-

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Table 1. Inter-group materials comparison.

G (*n*)	Sex		Age	BMI (kg/m^2)	Complication		
	M	F			Hypertension	Hyperlipidemia	Diabetes
Control (80)	47	33	65.75±6.98	21.57±0.59	26	28	28
CHD (108)	58	50	65.57±6.88	21.53±0.60	42	42	40
χ^2/t	0.475		0.172	0.368	0.813	0.297	0.083
P	0.491		0.863	0.714	0.367	0.586	0.774

diography; ② Age: 18-75 years old; ③ Patients with a good mental state and able to cooperate with this study. Exclusion criteria: ① Patients with a history of carotid artery stent implantation; ② Patients with a history of vascular lesions such as peripheral multiple arteritides and congenital arterial stenosis; ③ Patients with concomitant cerebrovascular, liver, and kidney diseases. The patients in the control group had no evidence of CHD and carotid atherosclerotic plaque except by echocardiography; Other inclusion criteria refer to the CHD group. There was no statistically significant difference in sex, age, BMI, and underlying disease between the CHD group and the control group (*P*>0.05) (Table 1).

Methods

Materials collection

A self-made general information questionnaire filled out by the patients was used for their gender, age, BMI, and comorbidities. The investigator distributed 188 questionnaires and was on-site for the questions about the form content, with a recovery rate of 100%.

Carotid atherosclerotic plaque stability evaluation and subgroup classification

GE730 color Doppler ultrasound blood flow imaging instrument and 7.5~10.0MHz high-frequency linear array probe were used for diagnosis by two ultrasonic physicians. A supine position was used with a pillow on his shoulder and his head slightly tilted back. The front of the neck was fully exposed. The internal carotid artery diameter and the artery intima echo intensity were measured. The presence of plaque in the lumen, the characteristics of plaque, and the degree of stenosis of the lumen were evaluated. According to the stability evaluation, the results were divided into three subgroups: No plaque, Stable plaque, and Unstable plaque. Referring to relevant literature (7), the arterial intima-media thickness greater than 1.5 mm indicated plaque formation. According to the nature of plaques, they are divided into stable plaques (hard plaques) and unstable plaques (soft plaques, flat plaques, mixed plaques). The control group was classified according to the stability of CAPs during physical examination, and all patients in the control group in this study were classified as having No plaque.

Serological ANGPTL4 and SIRT1 indicators examination

5ml of the morning fasting elbow venous blood of the two groups was collected and centrifugated at 3500r/min with a high-speed centrifuge for 10min for the serum. Enzyme-linked immunosorbent assay detected ANGPTL4 and SIRT1 by the detection reagents from Wuhan Feien Technology Company. The inspectors of our hospital operated according to the kit instructions and recorded their expression levels.

Statistical methods

SPSS 21.0 software was used for statistical analysis, with measurement data represented by $\bar{x}\pm s$ and counting data represented by case numbers. Independent sample t-tests or variance analysis were used, respectively χ^2 Test, and Spearman correlation analyzed the correlation. Medcalc software was used to draw the ROC curve and analyze the correlation standard, sensitivity, specificity, Youden index and correction level of serum ANGPTL4 and SIRT1 levels in predicting the different stability of CAP α=0.05 and *P*<0.05 were statistically significant differences.

Results

Patients baseline data comparison with different carotid atherosclerotic plaque stability

There was no statistically significant difference in sex, age, BMI, and comorbidities among the three subgroups of patients (*P*>0.05) (Table 2).

Serum ANGPTL4 and SIRT1 levels

The serum ANGPTL4 and SIRT1 levels in the CHD group were lower, with statistical significance (*P*<0.05); There was a statistically significant difference in serum ANGPTL4 and SIRT1 levels among the three subgroups of patients (*P*<0.05) (Table 3).

Spearman correlation analysis results

There was a negative correlation between the serum ANGPTL4 and SIRT1 levels and the CAP stability (r=-

Table 2. Patients baseline data comparison.

G (*n*)	Sex		Age	BMI (kg/m^2)	Complication		
	M	F			Hypertension	Hyperlipidemia	Diabetes
No plaques (110)	62	48	66.08±6.57	21.52±0.60	39	41	41
Stable plaques (36)	17	19	65.53±6.84	21.53±0.49	15	12	10
Unstable plaques (42)	26	16	64.62±7.82	21.63±0.66	14	17	17
χ^2/F	1.723		0.687	0.573	0.642	0.423	1.493
P	0.423		0.504	0.565	0.725	0.809	0.474

Table 3. Serum ANGPTL4 and SIRT1 levels Comparison.

G (*n*)	ANGPTL4 (mg/L)	SIRT1 (μg/L)
Control (80)	33.71±6.33	10.58±2.64
CHD (108)	28.89±6.04	6.92±2.75
t	5.299	9.187
P	<0.001	<0.001
No plaques (110)	33.35±6.14	10.47±2.49
Stable plaques (36)	28.61±6.17	6.47±1.87
Unstable plaques (42)	26.63±5.15	5.02±1.48
F	22.880	113.357
P	<0.001	<0.001

Table 4. Spearman correlation.

G (*n*)	*r*	*P*
ANGPTL4	-0.438	<0.001
SIRT1	-0.717	<0.001

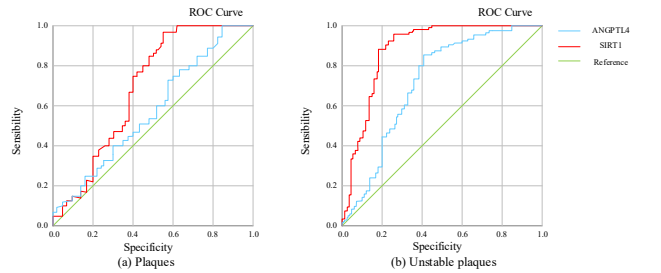

Figure 1. ROC curve of patients with plaques and unstable plaques.

0.438, -0.717, *P*<0.001) (Table 4).

ROC curve analysis results

Serum ANGPTL4 and SIRT1 can be used as the evaluation method of carotid atherosclerotic plaque stability. When ANGPTL4 ≤ 30.17mg/L and SIRT1 ≤ 6.91 μg/L, patients were more likely to develop unstable plaques, with ANGPTL4 ≤ 30.40mg/L and SIRT1 ≤ 6.87μg/L, patients were more likely to develop plaques (instability and/or stability) (Table 5 and Figure 1).

Discussion

Most cardiovascular events are caused by vulnerable plaques, which are mostly caused by the new blood vessels. The formation and development of vulnerable plaques are a series of pathological processes that coexist in multiple parts of the vascular bed (8). It is unclear whether the characteristics of carotid artery plaques examined by ultrasound are associated with risk factors for cardiovascular disease (CVD) or predict future CVD events (9). The coronary artery calcification score of patients with CHD can predict their cardiovascular events, and its predictive ability is better than that of carotid artery wall plaques (10). The carotid artery in tima-media thickness is a commonly used non-invasive examination for evaluating atherosclerotic plaques and has become a substitute indicator for subclinical cardiovascular diseases and a predictive indicator of cardiovascular events (11). Epicardial fat thickness may be a risk factor and biomarker for early atherosclerosis (12). Ultrasonic changes of carotid atherosclerotic plaque are weakly influenced by the degree of coronary artery disease in patients with CHD, and plaque stability is a risk factor for CHD (13,14). Therefore, the CAP stability in patients with CHD should be used as a clinical monitoring indicator. The CAP stability is usually determined by contrast-enhanced ultrasound imaging (15), but contrast-enhanced ultrasound requires the injection of a contrast agent, which will have some adverse effects on the kidney. Therefore, it is necessary to find a safer method for monitoring plaque stability.

Plasma ANGPTL4 levels and ANGPTL4 variants are significantly independent of predicting cardiovascular events from traditional cardiovascular risk factors (16). ANGPTL4 is a bifunctional protein that affects plasma triglyceride levels and has angiogenic properties (17). High levels of triglycerides can cause subclinical arteriosclerosis and vascular inflammation. The change in NGPTL4 is not only related to coronary atherosclerosis but also related to the change in triglycerides, which can be used as a predictor of coronary atherosclerosis (18). At the same time, ANGPTL4 can avoid potential atherogenic effects and other complications (19). Current research indicated that the reduction of ANGPTL4 function had beneficial effects on blood lipid parameters and overall cardiovascular disease risk, and ANGPTL4 may even become a new therapeutic target for treating cardiovascular diseases (20). The role of ANGPTL-4 in assessing the severity of coronary heart disease is limited (21). However, this study found that serum ANGPTL4 was lower in patients with CHD, which is similar to Aryal et al. (22) study. At the same time, there was a statistically significant difference in serum ANGPTL4 levels among the subgroups of patients with No plaques, Stable plaques, and Unstable plaques

Table 5. Efficacy in Evaluating the CAP Stability.

Type	Index	Sensibility	Specificity	Youden index	AUC	Potency
Unstable plaques	ANGPTL4	85.71	66.44	0.522	0.759	≤30.17mg/L
	SIRT1	90.48	79.45	0.699	0.906	≤6.91μg/L
Plaques	ANGPTL4	73.08	72.73	0.458	0.757	≤30.4mg/L
	SIRT1	80.77	94.55	0.753	0.944	≤6.87μg/L

(P<0.05). There was a negative correlation between the serum level of ANGPTL4 and the CAP stability (r=-0.438, *P*<0.001); Serum ANGPTL4 can be used as a way to assess the CAP stability. When ANGPTL4 ≤ 30.17mg/L, patients were more likely to have unstable plaque. When ANGPTL4 ≤ 30.40mg/L, patients were more likely to have plaque (instability and/or stability). ANGPTL4 was a participating factor in the formation of coronary atherosclerotic plaque in patients with CHD, and the level of ANGPTL4 can be used to assess CAP stability in patients with CHD. The decline of ANGPTL4 level would weaken the inflammatory factor reaction of macrophage activator in the carotid artery, cause the progression of carotid atherosclerosis, and then aggravate the degree of coronary artery stenosis.

The phenotype changes of vascular smooth muscle cells are a key link in the occurrence and development of vascular remodeling diseases. SIRT1 is an important expression factor in the AMPK/SIRT1/FOXO3a signaling pathway, which can promote the differentiation of vascular smooth muscle cell phenotype (23). In smooth muscle cells, inhibition of SIRT1 activity will reduce the expression of TIMP3, while overexpression of SIRT1 will increase the promoter activity of TIMP3. TIMP3 is an important factor involved in human carotid atherosclerosis and its expression has a slight negative trend among advanced atherosclerotic plaques and has a certain impact on plaque stability (24). When SIRT1 activity decreases, TIMP3 decreases and plaque stability decreases accordingly. From the research results, the serum SIRT1 level of patients with CHD was lower, and there was a significant difference between different plaque stability subgroups. There was a negative correlation between the serum SIRT1 level and the stability of carotid atherosclerosis plaque (r=-0.717, *P*<0.001). Serum SIRT1 can be used as a way to evaluate CAP stability, when SIRT1 ≤ 6.91 μg/L, had a higher probability of developing unstable plaques, when SIRT1 ≤ 6.87 μg/L, patients had a higher probability of developing plaques (instability and/or stability). SIRT1 can reduce the level of oxidative stress in endothelial cells and inhibit the expression of endothelial type NO synthase, it is related to protecting endothelial cell function. However, due to the lack of follow-up of patients in this study and the fact that the control group included elderly individuals, it was not possible to completely exclude the study grouping error caused by carotid ultrasound examination, which caused data bias issues. Further multicenter studies can be conducted in the future to explore the impact of decreased ANGPTL4 and SIRT1 levels on cardiovascular adverse events.

Conclusion

The serum ANGPTL4 and SIRT1 levels in patients with CHD decreased. ANGPTL4 and SIRT1 will participate in the formation and development of carotid plaque, which can be used as a serological evaluation index to evaluate the occurrence and CAP stability in patients with CHD.

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