A RESEARCH ON THE RISK FACTORS FOR THE FORMATION AND STABILITY OF CAROTID ATHEROSCLEROTIC PLAQUE

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ABSTRACT

Objective*: Intend to explore the risk factors of carotid plaque formation and the factors affecting plaque stability by screening carotid plaque by color Doppler.*

Methods: *Color Doppler ultrasound examination of carotid artery was performed in 150 subjects. Logistic regression analysis was used to screen out the variables with statistical significance and the predominance ratio of each variable to carotid atherosclerotic plaque compared with (OR). In addition, we intended to evaluate the risk of carotid atherosclerotic plaque formation. According to the ultrasonic characteristics of plaques, multi-factor Logistic regression analysis was carried out, and the indexes with statistical significance were obtained.*

Results: *The results of univariate regression analysis and multivariate Logistic regression showed that age, history of hypertension, history of diabetes, triglyceride (TG), low density lipoprotein (LDL), C-reactive protein (CRP) were closely related to the formation of carotid plaque. In the meanwhile, it was concluded that age, history of hypertension and history of diabetes were independent risk factors for the formation of carotid plaque. Those with a history of diabetes had the highest risk of carotid plaque. It was concluded that CRP was a risk factor affecting plaque stability, and the higher the CRP, the more unstable the plaque through the multivariate regression analysis of carotid plaque stability.*

Conclusion: *Color Doppler ultrasound of carotid artery can be used as a window to reflect the degree of atherosclerosis because of its non-invasive, economical, convenient and easy to repeat. By screening carotid plaque and analyzing the stability of plaque, we are guided to explore the risk factors of carotid plaque combined with clinical and laboratory indicators. It contributes to indirectly evaluate the potential risk of cardiovascular disease and reduce the incidence of cardiovascular accidents.*

Keywords: *Ultrasonography, carotid atherosclerotic plaque, risk factors, plaque stability.*

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Introduction

In recent years, cardiovascular and cerebrovascular diseases have become the leading cause of human death with the continuous development and progress of society, the gradual improvement of living standards, the acceleration of the pace of life, the gradual change of dietary structure, the change of living habits, and the gradual aging of the population, the incidence of hypertension, diabetes and obesity has increased year by year. As a consequence, people pay more and more attention to atherosclerosis as the pathological basis of cardio-cerebrovascular diseases. Carotid artery is a common site of atherosclerosis. Various examination methods have been applied to the detection of carotid plaque with the rapid development of medical examination technology, including non-invasive (such as color Doppler ultrasound, ultrasound elastography, contrast-enhanced ultrasound, neck CT and MRI) and invasive (such as DSA and intravascular ultrasound, intravascular MRI). Carotid ultrasonography has the advantages of non-radiation, low price, high resolution, fast and simple imaging. As a consequence, it is recognized as a routine method for the diagnosis of carotid atherosclerotic plaque[(1)]. It can not only

detect the changes of intima in the early stage of carotid atherosclerosis, but also has a high detection rate for early small protuberant plaque. In addition, it can also analyze the stability of plaques through the echo characteristics and blood flow of plaques. For patients with carotid artery stenosis caused by larger plaques, the degree of carotid artery stenosis can be analyzed by measuring the lumen diameter and combined with hemodynamic changes. In the meanwhile, the detection of carotid plaque can reflect the degree of systemic atherosclerosis early, accurately and indirectly. As a consequence, carotid artery screening plays an important role in the prevention and treatment of systemic atherosclerosis.

The formation of carotid atherosclerotic plaque is the result of external environmental factors and abnormal regulation of internal polygenes. The development of carotid atherosclerotic plaque is a dynamic equilibrium process[2]. The internal structural characteristics of carotid atherosclerotic plaques are the key factors affecting the stability of carotid atherosclerotic plaques. It is found that the main cause of these emergencies is not vascular stenosis and blockage caused by atherosclerotic plaques, but the rupture and shedding of unstable plaques with the continuous understanding of the pathogenesis of acute cerebral infarction and ischemic stroke. Unstable plaque is prone to plaque rupture because of its pathological characteristics, and after plaque rupture, the rough surface and exposure of some procoagulant substances can easily lead to thrombosis, resulting in ischemic cerebrovascular disease and acute cerebral infarction. As a consequence, unstable plaque is an important risk factor for a series of emergencies. Various studies have shown that many factors are closely related to the formation and progression of carotid plaque, such as age, sex, smoking history, blood pressure, blood glucose, serum C-reactive protein and blood lipids, etc. As a consequence, by screening the formation of carotid atherosclerotic plaques, early identification of unstable plaques that may lead to cerebral ischemic emergencies can be used for early detection and early treatment of high-risk groups. It plays an important role in preventing acute cerebral infarction.

Referring to a large number of literatures, the author found that there is no comprehensive statistical analysis on the indicators that determine the formation of plaque and affect its stability. As a consequence, in this study, the carotid artery was detected by ultrasonography in 150 patients, and the stability of plaque was judged according to the detection of carotid plaque and CTA detection results. In the meanwhile, the relevant clinical and laboratory indexes were collected for statistical analysis.

The independent risk factors that determine the formation of carotid plaque were obtained to guide the early prevention and control of high-risk population, and the risk factors affecting plaque stability were obtained by statistical analysis. The research intended to provide a more comprehensive theoretical basis for clinical treatment and early prevention of acute cerebral infarction.

Materials and methods

Research objects

A total of 150 patients who underwent carotid ultrasonography in our hospital from April 2018 to April 2019 were selected, including 82 males and 68 females, aged from 40 to 80 years, with an average of (63.12±3.7) years.

Inclusion criteria:

All subjects' medical history collection should meet the following conditions:

• Smoking history: continuous or cumulative smoking for 6 months or more;

• Hypertension history: in line with the diagnostic criteria of the revised Chinese guidelines on Prevention and treatment of Hypertension (2010);

• Diabetes history: in accordance with the 1999 World Health Organization (WHO) diagnostic criteria;

• All subjects did not take hypolipidemic drugs within 1 month.

92 cases of carotid atherosclerotic plaque were examined by CTA. Combined with the results of ultrasound and CTA, 92 cases of carotid atherosclerotic plaque were further divided into stable plaque (n=67) and unstable plaque (n=25).

Research methods

All subjects took fasting venous blood 3ml. Triglyceride (TG), total cholesterol (TC) and low density lipoprotein (LDL), were detected by Hitachi 7180 automatic biochemical analyzer. C-reactive protein (CRP) was detected by American BeckmanArray360 automatic specific protein analyzer. In addition, general data such as age, sex, smoking history, hypertension and diabetes should be recorded in detail.

Ultrasonography was performed with Hitachi HITACHI HIVISION AVIUS color Doppler ultrasound diagnostic instrument, L74m high frequency probe and probe frequency 5-13MHz.

The detection methods were as follows

The subjects took the supine position, exposed the neck and raise the chin, first tilted the head to the left to check the blood vessels of the right neck, and the probe was from the bottom to the top of the neck. Scan one by one according to the shape of the blood vessels: common carotid artery, carotid bifurcation, namely bursa, internal carotid artery, external carotid artery, and then scan the opposite side in this order. The normal three-layer structure of blood vessel wall was carefully observed, and the thickness of intima-media (IMT) was measured in the process of detecting blood vessels. For those with uneven intima thickening, different parts should be measured many times to get the average value. For the detected plaques, multi-section scanning should be carried out to observe the internal echo characteristics, whether the surface is smooth and the blood flow inside the plaques. For the carotid plaque that causes carotid artery stenosis, not only the plaque size but also the lumen diameter should be measured, and the stenosis rate of the lumen should be calculated combined with color Doppler imaging. Finally, the stability of the plaque and the degree of lumen stenosis were analyzed by the location, size and echo characteristics of the plaque. For those with multiple plaques, the larger 1-2 plaques were selected for statistics. It can be divided into homogeneous echo plaque and heterogeneous echo plaque according to the characteristics of plaque echo. The former can be shown as low echo, equal echo and strong echo, the latter is heterogeneous mixed echo, strong echo is usually calcification, medium echo and low echo are usually lipid-rich plaque core. The risk of plaque is mainly reflected in the stability of plaque, so plaque is usually divided into stable plaque and unstable plaque.

Its ultrasonographic features are as follows

• Unstable plaque: internal echo is changeable, often showing mixed echo with low echo, the core of lipid in plaque is hypoechoic, and intra-plaque hemorrhage is anechoic. The surface of the incomplete fiber cap shows that the surface of the patch is uneven, and some cracks can be seen. A little punctate and short rod blood flow can be detected in some plaques, suggesting the formation of neovascularization in the plaques.

• Stable plaque: the internal echo of the plaque is uniform, there is no low echo of the lipid core, most of them are uniform strong echo, the surface can be irregular, but smooth and complete. Some plaques can cause significant changes in the diameter of the carotid artery. The blood flow velocity, vascular diameter and area can be measured by two-dimensional and color Doppler flow imaging so as to comprehensively evaluate the degree of carotid artery stenosis.

Subjects were divided into two groups: 150 subjects were detected by carotid artery color Doppler, carotid plaque was detected in 92 cases and no plaque was detected in 58 cases. According to the characteristics of color Doppler and CTA, 92 patients with plaque were further divided into stable plaque (n=67) and unstable plaque (n=25).

Statistical methods

All data were processed by SPSS13.0 statistical software, independent sample t-test was used for comparison between groups, and x2 test was used for counting data. Taking carotid plaque as dependent variable, sex, age, smoking history, hypertension history, diabetes history, TC, TG, LDL and CRP as independent variables, the independent variables with statistical significance in univariate analysis were included in multivariate analysis. Binary logistic regression was used in multivariate analysis to analyze the influencing factors and compare the odds ratio (OR) of each variable.

The results of carotid color Doppler and CTA were statistically analyzed to explore the significance of carotid color Doppler in identifying plaque stability. In the meanwhile, plaque stability was taken as dependent variables, age, hypertension, diabetes, TG, LDL and CRP were selected as independent variables for multivariate logistic regression, and the risk factors affecting plaque stability were analyzed.

The risk factors of atherosclerotic plaque were analyzed and compared among non-plaque group, stable plaque group and unstable plaque group. Spearsman correlation was used to analyze the relationship between the stability of atherosclerotic plaque and risk factors.

Results

Univariate regression analysis of carotid plaque formation

Taking carotid plaque as dependent variable, sex, age, history of smoking, history of hypertension, history of diabetes, TC, TG, LDL and CRP as independent variables were selected for univariate analysis. The results showed that age, history of hypertension, history of diabetes, TG, LDL and CRP entered the equation (see Table 1 for details).

Variable	Plaque group	Non-plaque group	OR	95%CI of OR	P value
Gender Female Male	 41 51	 27 31	0.183	0.083-16.918	0.902
Age ≤60 years old >60 years old	 20 72	 33 25	0.945	0.909-0.981	0.003
Smoking history No Yes	 64 38	 48 10	1.278	0.413-3.956	0.671
Hypertension history No Yes	 37 45	 37 21	0.527	0.352-0.791	0.002
Diabetes history No Yes	 53 39	 53 5	5.628	1.736-18.251	0.004
TC ≤5.8mmol/L >5.8mmol/L	 84 8	 55 3	0.296	0.028-3.157	0.314
TG ≤1.86mmol/L >1.86mmol/L	 70 32	 38 20	1.659	1.071-2.570	0.023
LDL ≤3.36mmol/L >3.36mmol/L	 88 4	 55 3	8.496	1.352-4.666	0.004
CRP ≤4.93mg/L >4.93 mg/L	 44 38	 48 10	7.571	0.470-0.983	0.006

Table 1: Univariate regression analysis of carotid plaque formation.

Multivariate Logistic regression analysis of carotid plaque formation

The results of univariate analysis showed that there was no significant difference in gender, smoking history and TC. After exclusion, multivariate Logistic regression analysis was used to screen variables. The results showed that age, history of hypertension and history of diabetes were statistically significant, which were independent risk factors for the formation of carotid plaque (see Table 2 for details).

The odds ratio (OR) from large to small was the history of diabetes, age and hypertension. The Logistic regression equation was: Logit (P)=-0.056X2-0.613X4+1.577X5+0.239.

Variable	B	S.E	x^2 value	OR	95%CI of OR	P value
Age	-0.056	0.020	7.594	0.946	0.909-0.984	0.006
Hypertension history	-0.613	0.223	7.540	0.542	0.350-0.839	0.006
Diabetes history	1.577	0.611	6.662	4.842	1.462-16.038	0.010
Constant term	0.239	2.649	0.008	1.270		0.928

Table 2: Univariate regression analysis of carotid plaque formation.

Analysis of risk factors affecting plaque stability

The stability of plaque was taken as dependent variable (0 for stable plaque and 1 for unstable plaque). Age, history of hypertension, diabetes, TG, LDL and CRP were taken as independent variables. Multivariate Logistic regression analysis was carried out. The results showed that CRP was a risk factor affecting plaque stability (see Table 3).

Variable	B	S.E	x^2 value	OR	P value
Age	-0.702	0.623	1.268	0.496	0.260
Hypertension history	6.564	3.497	3.524	708.913	0.060
Diabetes history	3.867	2.875	1.809	47.797	0.179
TG	-2.615	2.104	1.544	0.073	0.214
LDL	-6.294	5.387	1.365	0.002	0.243
CRP	9.895	5.050	3.839	19834.170	0.050
Constant term	-65.803	37.334	3.107	0.000	0.078

Table 3: Table 3 Multi-factor regression analysis of plaque stability.

Spearsman correlation analysis of carotid plaque stability and CRP

It was concluded that the stability of atherosclerotic plaque was positively correlated with the level of CRP (the correlation coefficient was 0.734 and p=0.000) by analyzing and comparing the CRP of stable plaque and unstable plaque between the two groups.

Statistical analysis results

Through the univariate regression analysis of the formation of carotid atherosclerotic plaque, it was concluded that age, history of hypertension, history of diabetes, TG, LDL and CRP were closely related to the formation of carotid plaque. Further multivariate regression showed that age, history of hypertension and history of diabetes were independent risk factors for the formation of carotid atherosclerotic plaque. Compared the odds ratio of each factor, and got rid of the influence of other factors, it was found that elderly people with a history of diabetes or hypertension can lead to the formation of carotid atherosclerotic plaque, in which the OR value of diabetes history was the highest, indicating that people with a history of diabetes have the highest risk of carotid plaque. Through the multivariate regression analysis of carotid plaque stability, it was concluded that CRP was a risk factor affecting plaque stability.

In the meanwhile, through Spearsman correlation analysis, it was further concluded that there was a positive correlation between CRP and carotid plaque stability. In other words, the higher the CRP, the more unstable the plaque.

Discussion

As the most common and important vascular disease in arteriosclerosis, atherosclerosis is a vascular disease that can affect multiple systems of the whole body. its formation and development is the result of changes in the environment and multiple factors in the body.

The results showed that age, history of hypertension, history of diabetes, TG, LDL and CRP were statistically significant characteristic variables, among which there were significant differences in age, history of hypertension and history of diabetes, suggesting that patients with carotid plaque were more likely to develop carotid plaque than those with a history of hypertension and diabetes. When there are more of these risk factors, the more likely it is to form carotid plaque. This study also determines the degree of risk of carotid plaque by comparing the odds ratio (OR) of risk factors, that is, the greater the OR value, the greater the possibility of carotid plaque. In this study, diabetes had the highest OR and the highest risk of carotid plaque. It shows that diabetes, as a high risk factor for the formation of carotid atherosclerotic plaque, can still show a strong pathogenic effect even after excluding other risk factors. The specific mechanism may be through the activation of protein kinase C to activate nuclear transcription factors, stimulate the mitochondrial electron transport chain to produce too much superoxide, induce endothelial damage and dysfunction, leading to the formation of atherosclerotic plaques[3-4].

The carotid artery in patients with hypertension is one of the most easily involved vessels after the occurrence of atherosclerosis. The occurrence of carotid plaque is closely related to hypertension, and hypertension is an important factor in accelerating the development of atherosclerosis. The injury of vascular wall endothelium caused by hypertension leads to the function of flow-mediated endothelial dilation is significantly lower than that of normal blood pressure, which leads to the increase of collagen synthesis of intimal smooth muscle cells in arterial vessels. As a result, the ability of arterial wall to remove cholesterol and low density lipoprotein decreased, vascular elasticity decreased, and further caused lipid deposition to form atherosclerotic plaques.

Studies have shown that the formation of atherosclerotic plaques is closely related to age. With the increase of age, arteriosclerosis gradually develops and aggravates, and finally forms vascular stenosis or occlusion[5]. In the meanwhile, with the increase of age, blood pressure changes gradually, systolic blood pressure increases, diastolic blood pressure decreases, pulse pressure increases, indicating that vascular hardness increases significantly, vascular compliance decreases, and intimal thickening further reduces vascular elasticity, resulting in atherosclerosis and the formation of atherosclerotic plaques. This shows that carotid atherosclerosis is positively correlated with the increase of age, and the elderly should be the key population for the prevention of carotid atherosclerosis. In the meanwhile, it also suggests that carotid atherosclerosis is the normal physiological change of human aging, which is consistent with the fact that age is an independent risk factor for the formation of carotid plaque in this study.

In this study, univariate analysis showed that triglyceride (TG) and low density lipoprotein (LDL) were closely related to carotid atherosclerotic plaque, which was determined by the mechanism of atherosclerotic plaque formation. As a consequence, routine carotid ultrasonography is performed in such high-risk groups, and timely measures are taken to actively reduce lipids, which plays an important role in the early prevention and treatment of carotid atherosclerosis. In the multivariate regression analysis of this study, triglyceride and low density lipoprotein finally failed to become independent risk factors for the formation of carotid plaque, which seemed to be inconsistent with the clinic. The reason may be that both diabetes and hypertension can lead to lipid metabolism disorders, resulting in lipid deposition, while hyperlipidemia is mainly ingested from food, but does not cause impaired blood glucose regulation and elevated blood pressure. In addition, clinical studies have shown that the normal value of LDL in patients with carotid plaque should be reduced to 3.1 mmol / L, which needs to be further studied and demonstrated.

Carotid atherosclerosis is one of the important risk factors for acute cerebral infarction. Atherosclerotic lesions are often accompanied by chronic inflammatory process. Some scholars believe that C-reactive protein is involved in the occurrence and development of atherosclerosis, and its increased content is an independent risk factor for acute cerebral infarction[6]. In this study, univariate analysis showed that there was a correlation between C-reactive protein and the formation of carotid atherosclerotic plaque. However, it could not be reflected in multivariate regression analysis, which may be

related to the limited sample size. It needs to be further explored and studied in the clinical work in the future. Many studies have shown that smoking can increase blood viscosity and increase the content of plasma fibrinogen; nicotine can also stimulate sympathetic nerve excitation to cause arterial contraction, increase blood pressure and increase vascular endothelial permeability. The above factors can lead to the formation of atherosclerotic plaque. Literature[7] points out that smoking is an independent risk factor for atherosclerotic plaque, but it has not been reflected in this study, which may be due to the large number of female subjects in this study and none of them have a history of smoking. Coupled with the strengthening of national awareness of health care, there are more and more people who quit smoking.

In this study, the gender factor did not enter the regression equation and failed to get the statistical significance of gender. It may be related to the sample size, or because the female subjects were older, most of them were menopausal, and the protective effect of estrogen was less. Further research is needed in combination with clinical practice.

Plaque instability is the result of multiple factors. In this study, through multivariate regression and Spearsman correlation analysis of carotid plaque stability, it is concluded that CRP is an independent risk factor affecting plaque stability, and the higher the CRP, the more unstable the plaque. It shows that C-reactive protein, as the most iconic factor in the process of inflammation, is considered to be a very sensitive index in the occurrence and development of atherosclerosis[8].

CRP directly affects the stability of carotid plaque through the following aspects:

• The immune stimulation of CRP further promotes the lipid deposition in the plaque, which gradually increases and shifts;

• C-reactive protein causes damage to smooth muscle cells on the plaque surface, thus thinning the fibrous cap and increasing the instability of the plaque;

• The effect on inflammation. CRP can degrade the main components of extracellular matrix, thus increasing the brittleness of fibrous cap and promoting the development of unstable plaques.

Two-dimensional ultrasound can clearly show the inner diameter of arterial lumen, intima thickness in vascular wall, location, size, shape, internal echo characteristics and plaque surface smoothness of atherosclerotic plaques with the continuous improvement of ultrasound image quality. Color Doppler can show the blood flow filling of vascular lumen, measure the lumen area, and analyze the stability of atherosclerotic plaques through the blood flow characteristics of plaques, especially for unstable plaques with irregular surface and rich blood vessels. Early detection and diagnosis can be made. Color Doppler is also a good method to detect anechoic plaques which are easy to be missed by two-dimensional ultrasound. Spectral Doppler can be used to evaluate the degree of carotid artery stenosis by measuring the hemorheological parameters of carotid artery. Although color Doppler ultrasound has significant advantages in determining the nature of plaques, it still has disadvantages compared with CTA. The main reason is that ultrasound is subjectively influenced by the operator, and the examination is limited for obese people and patients with short and thick neck. In the meanwhile, internal carotid artery blood reflux and ultrasonic eddy current caused by carotid bifurcation will also affect the accuracy of examination. In addition, the neovascularization examination of unstable plaques is limited and prone to omission.

Generally speaking, the formation of carotid atherosclerotic plaque is an important factor in the pathogenesis of cerebrovascular diseases. Carotid artery screening plays an important role in the prevention and treatment of systemic arteriosclerosis. In this paper, we screen carotid plaque by color Doppler ultrasound, and analyze the stability of carotid plaque.It guides us to explore the risk factors of carotid plaque combined with clinical and laboratory indicators so as to reduce the occurrence of cardiovascular accidents.

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