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Correlations of carotid intima-media thickness, plasma protein z, and fibrinogen in patients with cerebral infarction.

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Abstract

The study aim was to investigate the situations of the carotid Intima-Media Thickness (cIMT) and atherosclerotic plaques in the patients with Cerebral Infarction (CI) as well as their correlations with Plasma protein Z (PZ) and Fibrinogen (FIB). 75 CI patients and 35 non-CI patients were performed carotid ultrasonography to measure their cIMT and to observe the situations of atherosclerotic plaques for the comparative analysis; the venous blood was sampled within 24 h after the admission for the detection of PZ and FIB. The bilateral cIMT values in the study group were significantly higher than the control group (P<0.01); the values of PZ and FIB in the study group were significantly higher (P<0.05). The increasing of cIMT in the study group was positively correlated with PZ and FIB (r=2.365, P<0.01). The incidence rate of atherosclerotic plaques in the study group was significantly higher than the control group (P<0.01). The increasing of cIMT was positively correlated with PZ and FIB; the cIMT increasing together with the levels of PZ and FIB was the important risk factor of CI.

Keywords: Cerebral infarction, Carotid intima-media thickness, Plasma protein Z, Fibrinogen.

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Introduction

Cerebral Infarction (CI) is a common and frequently-occurring disease in the Department of Neurology; one statistical study [1] showed that stroke had become the first cause of death in Chinese populations as well as the most common cause of disability in human diseases currently [2]. Studies had shown [3-6] that atherosclerosis was a common cause of ischemic cerebrovessels. Cerebral atherosclerosis mainly occurs in the vessels wider than 500 μm in diameter, and carotid artery is the site frequently involved. Carotid Intima-Media Thickness (cIMT) has already been widely used as the marker of systemic atherosclerosis and an auxiliary marker of coronary artery diseases [7], and the roles of the cIMT changes in coronary heart disease and vascular cognitive impairments had been reported many times [8-11], however, the roles of cIMT in the occurrence of stroke and its correlations with Plasma protein Z (PZ) and Fibrinogen (FIB) have not been finalized yet; therefore, we detected cIMT, PZ, and FIB in the patients with CI, aiming to investigate the correlations among these three factors as well as their correlations with the onset of CI. Previous study suggested that cIMT, PZ, and FIB might exist certain links with CI and its prognosis, and certain study had shown that cIMT changed before the atherosclerotic plaques formed, and the atherosclerosis plaques-derived thromboembolism was the main pathogenesis of ischemic stroke in the patients with large artery atherosclerosis [12]. However, whether detecting cIMT could play the roles towards the prevention and treatment of CI still needed to be further explored. PZ is a vitamin K dependent protein acting as the cofactor of the protein Z dependent inhibitor, in the inhibition of activated factor X bound on the phospholipids [13]. Early studies showed that PZ could mediate the thrombin to bind the phospholipid on the endothelial cell surface, thus promoting the blood clotting. FIB participates in the function of blood coagulation and is a hemostatic marker. FIB production in the liver is regulated by cytokines and is greatly enhanced in response to different inflammatory processes. FIB may promote carotid atherosclerosis through increasing blood viscosity, promoting fibrin formation, enhancing platelet-platelet interactions and other mechanisms [14]. Some studies [15] showed that smoking may modify the association between fibrinogen and carotid atherosclerosis. This study detected cIMT, PZ, and FIB in the patients with CI, aiming to investigate their roles towards the onset of CI as well as the correlations among these three factors.

Materials and Methods

Subjects

A total of 75 patients with TOAST-classification-confirmed acute CI hospitalized in our department from June 2011 to June 2013 were selected, including 43 males and 32 females, aged 36 to 77 years, mean age 63.38 ± 12.26 years old. All the patients met the diagnostic criteria revised in the Fourth

National Conference of cerebrovascular diseases, were diagnosed as the anterior circulation infarction by brain Magnetic Resonance Imaging (MRI), and met the following criteria: 1) without serious hepatonephric dysfunction, hypothyroidism, serious cardiac dysfunction, autoimmune disease, gout, cancer, serious systemic infection, or trauma/ surgery within 4 weeks before the onset of CI; 2) admitted within 48 h after the onset; 3) not administrated immunosuppressants such as methotrexate, oral contraceptives, estrogen, inflammation inhibitors, anticoagulants, or anti-epileptic drugs such as carbamazepine and phenytoin sodium recently. The 35 patients divided into the control group (the patients hospitalized for the non-cerebrovascular diseases during the same period) included 19 males and 16 females, aged 36 to 75 years, mean age 60.08 ± 10.32 years old. The inclusion criteria were the same as the above. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Wannan Medical College. Written informed consent was obtained from all participants.

Detection of PZ

2 ml of fasting venous blood was sampled from the patient within 24 h after the admission and then placed into 0.129 mol/l sodium citrate-containing anticoagulation tube for 20 min centrifugation at 3000 r/min (within two hours after the sampling at room temperature). The plasma was then collected and stored at -80°C. The blood samples of the control group were also obtained and stored using the same method within 24 h after the admission. The plasma PZ level in both groups was then simultaneously detected using the ELSIA method (the detection kit was provided by Shanghai China Wines da industrial Co., Ltd).

Detection of FIB

The fasting venous blood was sampled within 24 h after the admission and then sent to the laboratory for the FIB detection.

Detection of cIMT

All the patients were performed the color Droppler ultrasonography towards the carotid vessels within 3 days after the admission to measure cIMT and to detect the situations and types of plaques, which could be divided into wall thickening, hard plaque, mixed plaque, and soft plaque according to the carotid plaques' shapes, among which the mixed plaque and soft plaque were vulnerable. cIMT was measured at the end-diastolic phase along the vertical distance between the leading edge of the first and second echogenic lines of the far wall of the common carotid artery. IMT was measured at three points (IMT-tpm) along the roughly 20 mm region: both ends and the midpoint of the region. The maximal IMT value was defined as the greatest IMT value along the region. Atherosclerotic plaques are divided into stable plaques and instable plaques. The instable plaques refer to those with larger lipid center inside the plaques or the lipid center was close to the surface of the vessel lumen, the fibrous cap was thin. Those exhibited low or iso-echo in the ultrasonography are the soft plaques and mixed plaques, while strong echo referred to hard plaque. Carotid artery plaque was defined as an IMT-max>1.0 mm or an area of focal wall thickening that was 100% or greater than the IMT of neighboring sites.

Statistical analysis

All data were analysed using the SPSS13.0 statistical software; the counting data were compared using the χ^2 test; the measurement data were expressed as $\bar{x} \pm s$; the intergroup comparison was firstly performed the test of homogeneity of variance, followed by the group t test; the intragroup comparison was performed the paired t test. All the correlated factors were analysed using the Pearson correlation analysis, and the level of the test significance was $\alpha=0.05$.

Results

The main characteristics of the study population and comparison of main risk factors between patients and controls

The main characteristics as well as main risk factors of the study population were listed in Tables 1 and 2. The main characteristics of the study population included sex, age, blood pressure, blood glucose and blood lipid. And the main risk factors included smoking history, hypertension, diabetes, hyperlipemia. Smoking history between patients and controls had no statistical significance, while other risk factors including hypertension, diabetes, hyperlipemia between patients and controls had remarkable statistical significance ($P<0.05$, Table 6).

Table 1. *The main characteristics of the study population.*

Item	Study group (n=75)	Control group (n=35)
Sex(M/F)	43/32	19/16
Age	63.38 ± 12.26	60.08 ± 10.32
Blood pressure (mmHg)		
SBP	145.60 ± 18.41	130.7 ± 13.13
DBP	86.79 ± 11.59	82.66 ± 9.61
Blood glucose (mmol/L)	6.04 ± 2.42	4.80 ± 0.81
Hyperlipemia (mmol/L)		
TG	1.43 ± 0.88	1.41 ± 1.22
TC	4.09 ± 1.09	4.49 ± 0.87
HDL	1.20 ± 0.23	1.35 ± 0.32
LDL	2.25 ± 0.75	2.37 ± 0.68

Table 2. *Comparison of main risk factors between patients and controls.*

Item	Study group (n=75)	Control group (n=35)	χ^2	p

Smoking history (n)	11	2	1.835	0.149
Hypertension (n)	59	8	31.217	0
Diabetes (n)	28	1	14.611	0
Hyperlipemia (n)	45	8	13.186	0

Comparison of PZ and FIB

The PZ and FIB levels in all the patients of the study group within 24 h after the admission were statistically significantly higher than control group (P<0.05, Table 3).

Table 3. *Comparison of PZ and FIB between the study group and the control group (within 24 h after the admission).*

Group	n	PZ (ng/ml)	FIB (g/l)
Study group	75	13352.96 ± 586.82**	3.82 ± 1.05*
Control group	35	358.32 ± 242.46	3.17 ± 0.96
Compared with the control group, *P <0.05, **P <0.01			

Comparison of cIMT

The study group: left: 1.36 ± 0.16 mm, right: 1.29 ± 0.15 mm; the control group: left: 0.76 ± 0.17 mm, right: 0.71 ± 0.11 mm; the bilateral cIMT values in the study group were significantly higher than the control group (P<0.01, Table 4).

Table 4. *Comparison of cIMT between the study group and the control group.*

Group	n	Left cIMT (mm)	Right cIMT (mm)
Study group	75	1.36 ± 0.16*	1.29 ± 0.15*
Control group	35	0.76 ± 0.17	0.72 ± 0.11

Correlations among cIMT, PZ, and FIB

Because meeting the bivariate normal distribution, the Pearson correlation analysis was used and the results showed that cIMT was positively correlated with PZ and FIB (Table 5).

Table 5. *Correlations among cIMT, PZ, and FIB in the study group.*

Index	IMT	
	Correlation coefficient	P
PZ	0.793	0
FIB	0.822	0

Situations of carotid atherosclerotic plaques

The incidence rate of carotid atherosclerotic plaques in the study group was 70.7% (53/75), among which the incidence rates of the mixed plaques (including soft spots) and hard plaques were 56.0% (42/75) and 14.67% (11/75), respectively; the incidence rate of carotid atherosclerotic plaques in the control group was 34.3% (12/35), among which the incidence rates of the mixed plaques (including soft spots) and hard plaques were 8.57% (3/35) and 25.71% (9/35), respectively. The comparison showed that the incidence rates of the atherosclerotic plaques and mixed plaques (including soft spot) in the study group were significantly higher (Table 6).

Table 6. *Situations of carotid atherosclerotic plaques.*

Group	n	With plagues	Mixed plagues	Ratio	Hard plagues	Ratio
Study group	75	53	42	56	11	14.67
Control group	35	12	3	8.57	9	25.71

Discussion

Atherosclerosis is a common cause of CI [3-5]. It was conventionally considered that the vascular lesions that caused atherosclerotic CI mainly occurred in the intracranial arteries; however, recent studies found that the formation of carotid atherosclerotic plaques was one of the important reasons of CI [6,11]. cIMT had already changed before the carotid atherosclerotic plaques formed. PZ was found by Prowse in 1977, and its roles in the coagulation-fibrinolysis system still remained controversial. Early studies showed that PZ could mediate the thrombin to bind the phospholipid on the endothelial cell surface, thus promoting the blood clotting. Our results showed that the PZ and FIB levels in the study group were significantly higher than the control group, suggesting that the increasing of these two factors could promote the blood clotting, thus leading to the atherosclerotic CI. The increasing of these two indexes was also positively correlated with cIMT, indicating that the increasing might also promote the thickening of the arterial walls and the occurrence of atherosclerosis, followed by the occurrence of atherosclerotic CI. This study investigated the roles of cIMT, PZ, and FIB in the onset of CI as well as their mutual correlations and thus further screened the general and high-risk populations, aiming to provide the basis for the primary prevention of stroke. This study also found that cIMT in the study group was significantly higher than the control group, indicating that the increased cIMT was an important risk factor of CI, basically consistent with previous studies [12,16,17]. Studies had shown that the thickened cIMT could be divided into wall thickening, hard plaque, mixed plaque, and soft plaque according to the carotid plaques' shapes, among which the mixed plaque and soft plaque were vulnerable, and the onset rate of this type of CI was significantly higher than that with normal cIMT [18,19].

Our study found that the carotid atherosclerotic plaques more occurred at the carotid branches, followed by the initial site of the common carotid artery and internal carotid artery; it could be presumed that because the blood flow velocity at the carotid branches was low, so it was easy to form a low-shear vortex area, thus causing the formation of mural thrombus on the vascular intima as well as promoting the formation of the plaques. Previous study [20] considered that the more severe the extent of atherosclerosis, the more obvious the thickening of cIMT, the more obvious the stenosis, and the easier the plaque formation, which then might shed, cause thrombosis, and result in CI. Therefore, the structural properties and biological properties of the atherosclerotic plaques had obtained more and more attentions in recent years [21,22]. In recent years, some scholars divided the atherosclerotic plaques into two categories, namely the stable plaques and the instable plaques; patients with ischemic stroke mainly had the instable plagues [23]. The instable plaques refer to those with larger lipid center inside the plaques or the lipid center was close to the surface of the vessel lumen, the fibrous cap was thin; those exhibited low or iso-echo in the ultrasonography are the soft plaques and mixed plaques. The study found that the detection rate of the carotid intimal plaques in the study group was significantly higher than the control group ($P<0.01$), and these plagues were mainly soft and mixed plaques; it also explained the very close relationships between the occurrence of CI and the features of the carotid atherosclerotic plaques. Papadimitraki and Boumpas [24] reported that the inflammatory responses were involved in the whole pathological process of atherosclerosis; the monocytes and smooth muscle cells in the instable atherosclerotic plaques would express a large number of tissue factors due to local inflammations, and the ruptured atherosclerotic plaques could directly release these activated tissue factors into the blood; the tissue factors would then combine with the blood VII factor to start the extrinsic coagulation system. Meanwhile, the inflammatory cells inside the instable plaques would also secrete certain inflammatory mediators, such as interleukin I and tumor necrosis factor, thus strengthening the expressions of endothelial tissue factors, activating the endothelial cells to produce the platelet-activating factors, which had strong effects to promote the aggregation of the platelets; after injured, the generation of the ADP enzyme on the endothelial cell surface was then reduced. Therefore, the inactivation of ADP was reduced; on one hand, ADP could induce the platelet aggregation; on the other hand, the VII factor contacted with the endothelial cells, thus inducing the pro-enzyme activities in the presence of ADP, activating the XII factor, starting the endogenous coagulation system, undermining the in vivo coagulation-fibrinolysis balance, and leading to the high coagulation status of the blood; meanwhile, the elevated PZ level also promoted the blood clotting, and these two factors worked together to trigger the thrombosis, thus leading to the occurrence of atherosclerotic CI. The results of this study basically achieved the initial targets, but because the case number was relatively small, the results that the cIMT increasing together with the levels of PZ and FIB could be used as the important risk factor of CI and the possible mechanisms still needed further studies for the confirmation. What's more, hypertension, diabetes and hyperlipemia between CI patients and non-CI patients were also found statistically significance in this study, which perhaps indicated that hypertension, diabetes and hyperlipemia were also related with cIMT, that need to be further confirmed by multivariate analysis. Other limitations included that this study was short of comparing PZ, FIB levels among different types of CI according to TOAST-classification and the measurement standard of cIMT were not adjusted to age and sex, which should be taken into account in the future researches.

Acknowledgments

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Conflicts of Interest

All of the authors declare that they have no conflicts of interest regarding this paper.

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