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Correlations of GGT, Hcy and ABI with carotid atherosclerosis in essential hypertension patients

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ABSTRACT

This study was to explore the correlations of glutamyltransferase (GGT), homocysteine (Hcy) and ankle-brachial index (ABI) with the onset of cervical atherosclerosis (CAS) in essential hypertension (EH) patients. For this purpose, a total of 280 EH patients who were admitted to this hospital or visited the clinic of this hospital were enrolled into the EH group and received the color Doppler ultrasound for carotid artery and biochemical test for blood, and according to the plaques, they were divided into three groups: non-plaque group (n = 113), stable plaque group (n = 102) and non-stable plaque group (n = 65). Simultaneously, 80 healthy subjects who underwent the physical examination were enrolled in the control group. Correlations of GGT, Hcy and ABI with the onset of CAS were analyzed. The results indicated that in the EH group, the prevalence of CAS and Hcy levels were all higher than those in the control group (all $P<0.05$). As compared to the non-plaque group, patients with stable or non-stable plaques had higher levels of GGT and Hcy in serum but lower levels of ABI (all $P<0.05$). Logistic regression analysis revealed that CAS plaques were in positive correlation with the levels of GGT and Hcy in serum, but in negative correlation with ABI ($P<0.05$). In conclusion, ABI is the protective factor of CAS in EH patients, while Hcy and GGT are the negative factors.

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Introduction

Atherosclerosis, as a complex chronic disease, is correlated with various risk factors that play pivotal roles in the development of cardiovascular diseases (1). A variety of studies have identified the factors of atherosclerosis, including smoking, obesity, blood pressure (BP), blood cholesterol and mellitus diabetes (2). Interventions made upon these risk factors could reduce the occurrence of cardiovascular events in the subclinical stage.

γ-glutamyltransferase (GGT), as a kind of membrane-binding enzyme, is critical to the regeneration of intracellular glutathione, a major cellular antioxidant (3,4). Serum GGT is a well-known marker of liver diseases and alcohol abuse (5). Recent studies have shown that serum GGT is correlated with cardiovascular diseases independently (6). Nevertheless, there remains no evidence suggesting a correlation between GGT and carotid atherosclerosis. Homocysteine (HCY), as a kind of amino acid, can reflect vascular injury, and the high level of HCY may be the independent risk factor of cardiac, cerebral and peripheral vascular lesions (7). However, a great body of studies investigating the relationship between HCY and subclinical atherosclerosis in normal populations have yet not reached a consensus. For instance, some studies have shown that homocysteinemia is not related to subclinical atherosclerosis (8-10). On the contrary, a cohort study has demonstrated that mild homocysteinemia is an independent risk factor for the thickening of the carotid artery wall (11). Ankle branchial index (ABI), as reported, is taken as an alternative marker for coronary atherosclerosis and the predictive indicator for the upcoming ischemic event (12,13). Previous findings have shown that ABI at a low level may be associated with intracranial artery stenosis (14,15). However, no evidence has suggested a correlation with carotid atherosclerosis.

In this study, we evaluated carotid atherosclerosis by ultrasound to evaluate the correlations of the characteristics of the vascular wall with the levels of GGT, Hcy and ABI in the serum of EH patients.

Materials and Methods

Subjects

A total of 280 elder EH patients who visited the clinic or were admitted to this hospital between January 2021 and January 2022 were included in the EH group which was further divided into three groups according to the carotid artery ultrasonic performance – non-plaque group (n = 113), stable plaque group (n = 102) and non-stable group (n = 65). Simultaneously, 80 healthy subjects who undertook the physical examination were recruited into the control group. Criteria for inclusion were set according to the *Guidelines for diagnosis and classification of hypertension in China* (16). Criteria for exclusion: Patients with secondary hypertension, acute cardio- or cerebrovascular diseases, liver/kidney dysfunction, diseases in the blood system, liver or gall bladder diseases, or acute infection.

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All subjects of this study were informed of the content of this study, and this study was conducted in accordance with the Standards for Ethics of Affiliated Hospital of Yangzhou University.

Ultrasound for carotid artery

Ultrasonic examination of the carotid artery: A color Doppler ultrasonic diagnostic machine was used, and the probe frequency for the peripheral vessel was set at 7.5 Hz. During the examination, patients were required to stay in the supine position, with the head being lifted backward slightly to determine the inner diameters of an artery in the bifurcation of the common carotid artery and internal carotid artery and the intima-median thickness (IMT) and to detect the plaques and their features. The vascular wall of the distal end of the bilateral common carotid artery and the proximal end of the internal carotid artery that was at 1.0 to 1.5 adjacent to the dilated part of bifurcation was selected to determine the vertical distance between the intimae of the arterial anterior and posterior wall and the carotid arterial IMT. Atherosclerosis was considered for cases with the irregular thickened vascular wall, IMT ≥ 1.2 cm and local structural changes towards the lumen, otherwise cases were considered as no plaque. Plaques were further classified as unstable plaques, including low-echo plaques, plaques with rough surface, heterogenic-echo plaques and ulcer plaques, and stable plaques, including strong-echo plaques, plaques with smooth surface, and mixed echo plaques, mainly the strong echo (17).

Collection and determination of blood samples

At 24 h after admission, 5 mL peripheral elbow venous fasting blood was collected from the patients in the morning into a dry tube, followed by the centrifuge at 3000 r/min for 10 min to collect the supernatant that was later stored at -20°C for following determination of serum indicators. Levels of plasma triglycerides, total cholesterol, low-density lipoprotein, high-density lipoprotein and fasting blood glucose were measured by using an automatic chemical analyzer (Aeroset, Abbott, Holliston, MN, USA), serum Hcy by using the fluorescence polarization immunoassay and serum GGT activity by using a microplate reader (Roche/Hitachi analyzer, Mannheim, Germany). All protocols of measurement were conducted in accordance with the instructions.

Collection of ABI data

Ankle-brachial index (ABI) is the ratio of the systolic blood pressure (SBP) measured at the ankle to that measured at the brachial artery. Prior to the collection, all subjects should rest for at least 10 min in a warm and suitable environment and expose their ankles and humeri. Thereafter, qualified technicians collected the ABIs from patients by using the Omron Colin BP-203RPE III (Omron Health Care, Kyoto, Japan). Meanwhile, the blood pressure of the bilateral ankle and upper arms was also determined. Bilateral ABI was calculated by using the built-in program, and the lower ABI was recorded for the final analysis.

Data analysis

SPSS 19.0 software was used to perform the statistical analysis. Measurement data in normal distribution were expressed in the form of mean ± standard deviation, and the comparison was performed by using the *t*-test. A comparison of the qualitative data was performed by using the chi-square test. Logistic regression analysis was also carried out to validate the correlation. $P<0.05$ suggested that the difference had statistical significance

Results

Clinical features of 280 EH patients and 80 healthy subjects are presented in Table 1. Compared to the control group, patients in the EH group had higher levels of triglyceride and LDL-C but a lower level of HDL-C (all $P<0.05$). Differences in the levels of total cholesterol, glucose and creatinine between the EH group and the control group had no statistical significance (Table 1 and Figure 1).

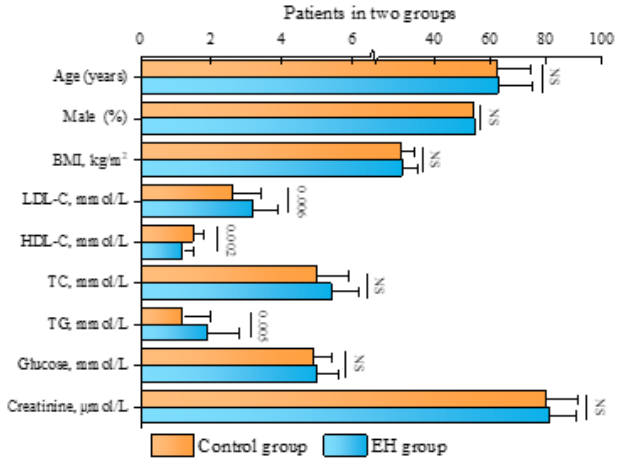

Figure 1. Clinical parameters of patients in two groups. BMI: Body mass index; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; NS: not significant; TC: Total cholesterol; TG: Triglycerides.

Table 1. Clinical parameters of patients in two groups

Items	EH group (n=280)	Control group (n=80)	P
Age (years)	62.8±12.6	62.5±11.8	NS
Male [n (%)]	152 (54.3)	43 (53.8)	NS
BMI, kg/m^2	28.6±5.6	28.3±4.5	NS
LDL-C, mmol/L	3.2±0.7	2.6±0.8	0.006
HDL-C, mmol/L	1.2±0.3	1.5±0.3	0.002
TC, mmol/L	5.4±0.8	5.0±0.9	NS
TG, mmol/L	1.9±0.9	1.2±0.8	0.005
Glucose, mmol/L	5.0±0.6	4.9±0.5	NS
Creatinine, μmol/L	81.2±9.5	79.7±11.6	NS

The prevalence of CAS in EH patients was 59.64%. Differences in the age, EH grade and disease course between the CAS group and non-CAS group had statistical significance. Besides, in the CAS group, patients aged not younger than 70 years, with EH grade ≥ 2 and EH disease course longer than 5 years had a higher proportion, and the difference had statistical significance (P<0.05; Table 2).

In comparison with the non-plaque group, patients in the stable plaque group and non-stable plaque group had higher levels of GGT and Hcy in serum but a lower ABI, and the difference had statistical significance (Table 3 and Figure 2).

With the stability of CAS plaque as the dependent variable and GGT, Hcy and ABI as the independent variable, we performed the logistic regression analysis, and the results showed that the stability of CAS plaque was in positive correlation with the levels of GGT and Hcy in serum but in negative correlation with ABI (Table 4).

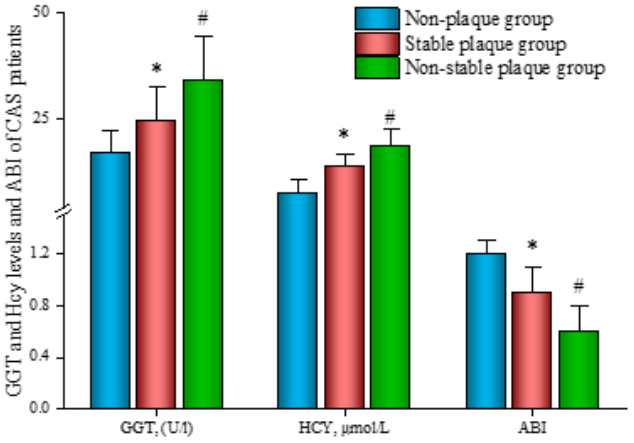

Figure 2. Comparison of GGT and Hcy levels and ABI of CAS patients. *P<0.05, #P<0.01 *vs.* the non-plaque group.

Discussion

In this study, we explored the correlations of carotid AS in EH patients with the levels of GGT and Hcy and ABI, and the results demonstrated that CAS plaque was in positive correlation with the levels of GGT and Hcy in serum but in negative correlation with the ABI.

GGT is a well-known enzymatic marker for alcohol abuse and liver disease. In addition, GGT activity, distributed on the surface of various cells, is involved in the catabolism of glutathione, one of the major antioxidants (18). Existing data have shown that GGT is involved in the pathophysiological process of atherosclerosis (19). Besides, some clinical studies have demonstrated that the activity of serum GGT could predict the risk of atherosclerosis-associated cardiovascular events (20). Our work also indicated that in comparison with the non-plaque group, patients in the stable plaque group and non-stable plaque group had increased GGT in serum, which coincided with the findings above. This is because GGT can hydrolyze the glutathione to release the CYSGLY, a dipeptide, that

Table 2. Clinical features of EH patients in CAS group and non-CAS group.

Items	CAS group (n=167)	Non-CAS group (n=113)	P
Age			
60-69	32 (19.2)	59 (52.2)#	0.015
70-79	46 (27.5)*	34 (30.1)*	0.043
≥ 80	89 (53.3)#	20 (17.7)	0.012
EH grade			
Grade 1	35 (21.0)	63 (55.8)#	0.026
Grade 2	42 (25.1)*	35 (31.0)*	0.045
Grade 3	90 (53.9)#	15 (13.2)	0.008
EH disease course			
<5 years	25 (15.0)	43 (38.2)#	0.029
5-9 years	32 (19.2)*	35 (30.9)#	0.034
10-15 years	49 (29.3)#	25 (22.1)*	0.047
>15 years	61 (36.5)#	10 (8.8)	0.018

*P<0.05, #P<0.01 *vs.* the patients in the same group.

Table 3. Comparison of GGT and Hcy levels and ABI of CAS patients.

Indexes	Non-plaque group (n=113)	Stable plaque group (n=102)	Non-stable plaque group (n=65)
GGT, (U/l)	16.9±5.1	24.5±8.2*	34.0±10.5#
HCY, μmol/L	7.5±3.2	13.8±2.9*	18.6±3.8#
ABI	1.2±0.1	0.9±0.2*	0.6±0.2#

*P<0.05, #P<0.01 *vs.* the non-plaque group.

Table 4. Logistic regression analysis.

Independent variables	β	SE	OR	95%CI	P
GGT	0.076	0.098	1.18	0.92~1.45	0.034
HCY	0.035	0.058	1.32	0.72~0.86	0.026
ABI	-0.187	0.083	1.64	0.98~1.39	0.043

can reduce the ferric iron to ferrous iron, with the production of reactive oxygen, superoxide anion and hydrogen peroxide (21).

Hcy is an acceptable risk factor for cardiovascular diseases. A body of studies have shown that increased Hcy in plasma is related to atherosclerosis (22-24). Hu *et al*. reported that in comparison with the non-hypertension patients, patients with hypertension or hyperhomocysteinemia presented more severe damage to the structure and function of the carotid artery, with increases in the levels of inflammatory factors (25). Likewise, we noted that in comparison with the non-plaque group, patients in the stable plaque group and non-stable plaque group had an increase in the level of Hcy, suggesting that Hcy has a pivotal role in the pathogenesis of atherosclerosis, which may relate to the oxidative stress, endothelial dysfunction and inflammatory reaction.

ABI, as an indicator applied in the non-invasive screening of atherosclerosis that was initially used to evaluate the lower extremity arterial disease, plays a key role in reflecting the development and severity of acute inflammation in the peripheral artery (26). The latest evidence has shown that ABI is closely associated with the risk factors of cardiovascular diseases, so it can be used as the independent factor for predicting these diseases (27,28). In a study on the risk factors of cardiovascular diseases, Li et al. (13) found that ABI might be a potential marker. Furthermore, according to a study on patients who underwent coronary artery angiogram, ABI has a negative correlation with the degree of coronary arterial disease (29). In this study, patients in the non-plaque group had a lower ABI as compared to their counterparts in the stable plaque group and non-stable plaque group, consistent with the findings above, suggesting the close relation between ABI and atherosclerosis.

Overall, ABI is a protective factor for CAS in EH patients, while Hcy and GGT are the adverse factors. Early findings and management of these factors are conducive to delaying the progression of subclinical atherosclerosis to clinical status and cardiovascular events.

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