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Relationship between carotid artery atherosclerosis and sulfatide in hypertensive patients

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ABSTRACT. Hypertension is a major traditional risk factor for atherosclerosis, and carotid artery intima-media thickness (IMT) is considered to be an important marker of atherosclerosis. Sulfatides have been shown to play a role in atherogenesis and vascular inflammation, resulting in atherosclerosis. This study aimed to assess the association between serum sulfatide and carotid artery IMT among hypertensive patients. We chose 60 hypertensive patients and 30 matched healthy controls. All subjects had medical examinations at Hebei General Hospital between March 2011 and March 2012. Measurements and other factors compared included serum sulfatide level, carotid artery IMT, and conventional cardiovascular risk factors. Hypertensive patients had higher BMIs (24.4 ± 7.6 to 23.1 ± 3.1 kg/m^2), total cholesterol levels (5.5 ± 0.6 to 5.0 ± 1.1 mM), serum sulfatide levels (3.5 ± 3.9 to 8.3 ± 2.7 μM), and carotid artery IMTs (1.06 ± 0.15 to 0.79 ± 0.07 mm) (all $P < 0.05$)

than control patients. Furthermore, the serum sulfatide level positively correlated with carotid IMT in the hypertensive patients (r = 0.39, P = 0.002). Multiple linear regression analysis showed serum sulfatide was an independent risk factor affecting IMT (P = 0.04). These results suggest that serum sulfatide is more strongly associated with carotid artery IMT than other traditional risk factors in hypertensive patients.

Key words: Carotid artery intima-media thickness; Serum sulfatide; Hypertension.

INTRODUCTION

Hypertension is one of the traditional major risk factors for atherosclerosis. Carotid intima-media thickness (IMT), measured with high-resolution ultrasonography, is thought to be a marker of atherosclerosis that is associated with future cardiovascular events (Shetty et al., 2011). Low-grade inflammation has been implicated in the progression of atherosclerosis (Ridker et al., 2000). Studies have demonstrated a role for sulfatide in atherogenesis and vascular inflammation (Li et al., 2007). Studies have also found a close correlation between low levels of serum sulfatides and a high risk of atherosclerotic cardiovascular disease. Sulfatide may be a novel biomarker for predicting the incidence of atherosclerotic cardiovascular disease in patients (Hu et al., 2007).

In this study, we investigated the association between sulfatide levels and carotid IMT in hypertensive patients who had no major cardiovascular risk factors except hypertension to confirm the possible association between sulfatides and atherosclerosis in hypertensive patients.

MATERIAL AND METHODS

Subjects

For this case control study, we chose 90 participants who had medical examinations at Hebei General Hospital between March 2011 and March 2012 and divided them into two groups. The control group consisted of 30 healthy volunteers who, on the basis of medical history, examination, and selected investigations, had no evidence of disease. The hypertensive group consisted of 60 participants (30 men and 30 women) who were known to have hypertension or had blood pressure measurements >140/90 mmHg on three occasions at rest. Mean blood pressure (MBP) was calculated as follows: MBP (systolic blood pressure + diastolic blood pressure x2) / 3. With each participant clothed only in a lightweight gown, weight and height were measured. We calculated body mass index (BMI) as body weight (kg) divided by the height squared (m^2). Patients having other systemic diseases or taking medications known to affect lipid levels were excluded.

This study was conducted in accordance with the Declaration of Helsinki. Signed informed consent was obtained from all subjects, and the Medical Ethics Committee of Hebei General Hospital approved the study protocols.

Laboratory Examinations

After an overnight fast of 12-14 hours, blood samples were obtained from all subjects early in the morning. Total cholesterol, high-density lipoprotein (HDL), triglycerides, and low-density lipoprotein (LDL) levels in blood samples were measured with an autobiochemical analysis system (AU2700, Olympus, Japan). For measurement of the sulfatides, the serum was stored at -80°C until analysis.

Measurement of serum sulfatides

Sulfatides were extracted from specimens using a hexane-isopropanol mixture and analyzed as lysoforms (sulfatides without fatty acids) using a high-throughput method developed in our laboratory (Li et al., 2007). Briefly, the total lipids were extracted from 50 μL of serum with n-hexane:isopropanol (3:2, v/v). After the solution was dried and hydrolyzed with 0.1M NaOH in 90% methanol at 150°C for 30 min, sulfatides were converted to lysosulfatides. Subsequently, samples were desalted with MonoTip C18 tips (GL Sciences, Tokyo, Japan) and analyzed with matrix-assisted laser desorption ionization time-of-flight mass spectrometry with delayed ion extraction using a Voyager Elite XL Biospectrometry Workstation (PerSeptive Biosystems, Framingham, MA, USA). A nitrogen laser (337 nm) was used for ionization, and negative ion mode detection was employed.

Carotid artery IMT measurements

The carotid arteries were evaluated by a single operator, blind to subject details, with a high-resolution B-mode ultrasonography machine using a 7.5 MHz probe (SSA-270A, Toshiba, Tokyo, Japan). Participants were scanned in the supine position with the neck hyperextended. Both common carotid arteries were thoroughly scanned in a proximal-to-distal direction up to the bifurcation. A frozen photocopy of end-diastolic images in the longitudinal view showing the bifurcation was captured. All images were taken when the inner echoes of both near and far walls were clearly visible. The IMT was measured at the far wall of both common carotid arteries approximately 1 cm proximal to the carotid bulb, and was defined as the mean of the maximal IMT of each common carotid artery.

Statistical analysis

SPSS 13.0 (SPSS Inc., Chicago, IL, USA) was used to perform all statistical analyses. Results are reported as means ± SDs for continuous variables and proportions for categorical variables. Comparison of qualitative variables was done using the chi-square test. Comparison between quantitative variables was performed using a *t*-test to compare two groups and analysis of variance to compare more than two groups. A correlation coefficient was also calculated to find the linear relation between different variables using the *r*-test or the Spearman correlation coefficient. Linear regression estimates the coefficients of the linear equation, involving one or more independent variables that best predict the value of the dependent variable. A P value of < 0.05 was considered to be statistically significant.

RESULTS

Table 1 shows the clinical characteristics of the hypertensive and control groups. No difference was found between the two groups regarding age, smoking status, triglycerides, LDL, or fasting blood sugar (FBS). However, hypertensive patients had higher BMIs, higher total cholesterol levels, and lower HDL levels than the control group patients ($P < 0.05$). Serum sulfatide levels and carotid artery IMTs were both significantly higher in the hypertensive group, than in the control group (both $P < 0.001$).

Table 1. Clinical characteristics of study participants.

	Hypertension	Control	P value
Age (years)	66.4 ± 7.9	65.6 ± 4.6	NS
BMI (kg/m^2)	24.4 ± 7.6	23.1 ± 3.1	<0.05
Smokers (%)	21 (35%)	8 (27%)	NS
Hypertension duration (year)	6.5 ± 6.1	-	-
Total cholesterol (mM)	5.5 ± 0.6	5.0 ± 1.1	<0.05
Triglycerides (mM)	1.4 ± 0.3	1.3 ± 0.2	NS
LDL (mM)	4.1 ± 0.6	3.9 ± 0.7	NS
HDL (mM)	0.9 ± 0.2	1.2 ± 0.3	<0.05
FBS (mM)	4.8 ± 0.4	4.8 ± 0.4	NS
Serum sulfatide (μM)	13.5 ± 3.9	8.3 ± 2.7	<0.001
Carotid artery IMT (mm)	1.06 ± 0.15	0.79 ± 0.07	<0.001

BMI = body mass index; LDL = low-density lipoprotein; HDL = high-density lipoprotein; FBS = fasting blood sugar; IMT = intima-media thickness. NS = not significant.

We performed a Pearson correlation analysis between carotid artery IMT and age, gender, smoking status, BMI, hypertension duration, systolic blood pressure, diastolic blood pressure, MBP, total cholesterol, triglycerides, LDL, HDL, fasting blood sugar, and serum sulfatide level among hypertensive patients. The result showed that carotid artery IMT had a significant positive correlation with serum sulfatide level ($r = 0.382$, $P = 0.002$) and hypertension duration ($r = 0.388$, $P = 0.002$). However, no significant association was seen between carotid artery IMT and the other variables. We then divided the hypertensive patients into two groups, those with short duration of hypertension (<6 months) and those with long duration (>6 months).

Table 2 shows the Pearson correlation analysis between carotid artery IMT and age, BMI, hypertension duration, systolic blood pressure, diastolic blood pressure, MBP, total cholesterol, triglycerides, LDL, HDL, fasting blood sugar, and serum sulfatide level in both hypertensive groups. We found a significant relationship between serum sulfatide level and carotid artery IMT in both hypertensive groups. However, a significant relationship between the duration of hypertension and carotid artery IMT was only seen in the group with hypertension for greater than 6 months.

Table 3 shows multilinear regression analysis using carotid artery IMT as the dependent variable and age, smoking status, hypertension duration, MBP, and levels of LDL, triglycerides, and serum sulfatide as independent variables. Among the independent variables, hypertension duration and serum sulfatide level were more strongly associated with carotid artery IMT than the other variables after adjustment of other factors.

Table 2. Pearson correlations between carotid IMT and study variables among hypertensive patients with duration less than 6 months and greater than 6 months.

	Hypertension <6 months (N = 35)		Hypertension >6 months (M = 25)	
	r	P value	r	P value
Age	0.024	0.932	0.014	0.935
BMI	-0.435	0.070	-0.032	0.843
Hypertension duration	0.091	0.722	0.323	0.035
SBP	0.192	0.446	-0.221	0.162
DBP	-0.118	0.645	-0.142	0.377
MBP	0.012	0.971	-0.206	0.192
Total cholesterol level	0.207	0.413	0.025	0.881
Triglyceride level	0.366	0.141	0.026	0.873
LDL level	0.196	0.444	0.298	0.055
HDL level	-0.025	0.923	0.076	0.635
FBS	-0.329	0.182	-0.072	0.658
Serum sulfatide level	0.390	0.042	0.312	0.041

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; MBP = mean blood pressure; LDL = low-density lipoprotein; HDL = high-density lipoprotein; FBS = fasting blood sugar; IMT = intima-media thickness.

Table 3. Multiple linear regression analysis of the relationship between cardiovascular risk factors and carotid IMT in hypertensive patients.

	All hypertensive subjects		Hypertension <6 months		Hypertension >6 months	
	β	P value	β	P value	β	P value
Age	0.064	0.639	0.059	0.755	0.548	0.123
Smoking	-0.151	0.327	-0.167	0.708	-0.074	0.762
Hypertension duration	0.296	0.038	0.142	0.467	0.234	0.033
MBP	0.15	0.481	0.033	0.975	0.078	0.587
LDL level	0.189	0.148	0.506	0.264	0.506	0.264
Triglyceride level	0.032	0.823	-0.354	0.474	-0.355	0.475
Serum sulfatide level	0.226	0.042	0.160	0.035	-0.051	0.041

MBP = mean blood pressure.

DISCUSSION

Our study assessed the relationship between serum sulfatide and carotid atherosclerosis among hypertensive patients. We demonstrated that hypertensive patients had higher total cholesterol and lower HDL levels than patients in the control group. The finding that hypertension usually occurs in conjunction with dyslipidemia has been supported by another study (Rehman, 2012). In our study, BMI was higher in the hypertensive group than in the control group; however, values in both groups were within the normal range.

Our study also demonstrated that hypertensive patients had a significantly higher carotid artery IMT than members of the control group, a finding that has been supported by another study (Choi et al., 2004). The mechanism by which hypertension leads to atherosclerosis is not clear. One theory is that an elevated pulse pressure causes greater stretching of the arteries, which induces fatigue and fracture of the elastic elements and thus is likely to hasten the development of intimal damage that leads to atherosclerosis. Another theory is that elevated blood pressure promotes inflammatory activation of the arterial wall, which increases the risk of atherosclerosis (Chae et al., 2001; Amer et al., 2011; Anwar et al., 2012).

In our study, we demonstrated that the hypertensive group had higher serum sulfatide

levels than the control group, and elevated serum sulfatide level was more strongly associated with the extent of atherosclerosis than other traditional risk factors. Sulfatides are esters of sulfuric acid with galactosylceramides at C3 of the galactosyl residue. They are widely distributed in the organs and sera of various animals, including humans.

Studies have demonstrated a role for sulfatides in atherogenesis and vascular inflammation resulting in atherosclerosis (Hara et al., 1996; Anwar et al., 2012). An increase in sulfatide was found in both lipoprotein and atherosclerotic plaques in the Watanabe heritable hyperlipidemic rabbit – an animal model for human familial hypercholesterolemia – and was intimately correlated with the development of atherosclerosis (Hara et al., 1996). Conversely, a close correlation was found between low levels of serum sulfatides and a high risk of atherosclerotic cardiovascular disease in patients with end-stage renal failure (Li et al., 2009). Another study found that sulfatides were associated with the progression of atherosclerosis after vascular injury (Inoue et al., 2010).

Sulfatides have been shown to play an important physiological role in the vascular inflammation associated with the development of atherothrombosis and atherosclerosis. In the vascular inflammatory process, activation of leukocytes, neutrophils, and monocytes and their interactions with platelets are mediated by cell adhesion molecules. The crosstalk of leukocyte integrin Mac-1 (CD11b/CD18) and platelet membrane surface P-selectin is known to play an especially important role. The activation and upregulation of Mac-1 on the surface of neutrophils and an increase in the expression of P-selectin on the surface of platelets is related to increased vascular wall thickness (Inoue et al., 2000). Sulfatides are known to be a native ligand of P-selectin and could interact with multiple cell adhesion molecules. Sulfatides are also expressed on the membrane surface of, and excreted by, neutrophils. Because neutrophil activation occurs after vascular inflammation, sulfatides released from activated neutrophils may also act agonistically as a P-selectin ligand and promote P-selectin-Mac-1 crosstalk, resulting in the activation of Mac-1 and a subsequent increase in vascular wall thickness and atherosclerosis (Merten et al., 2005).

A limitation of our study was that the relatively small sample size could not completely reflect the association between serum sulfatide and carotid IMT in hypertensive patients.

In summary, we showed that serum sulfatide level is significantly associated with increased carotid IMT and the progression of atherosclerosis in hypertensive patients. Therefore, the sulfatide-dependent pathway could be a novel target for prevention and/or treatment of atherosclerosis.

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