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High Serum Concentration of Sulfatide is a Risk Factor for Restenosis in Patients with Coronary Heart Disease after Percutaneous Coronary Intervention

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Abstract

Objective: Restenosis after Percutaneous Coronary Intervention (PCI) is regarded as the result of a combination of various pathological events. The mechanisms are complex and not completely understood. This study aims to determine the correlation between the concentration of serum sulfatide and restenosis after PCI.

Method: We studied 68 consecutive patients with CHD of single-vessel disease who successfully underwent PCI. All patients were evaluated by a follow-up angiography approximately 6.5 months after the PCI and were divided into two groups, the restenosis (20 patients) and the nonrestenosis (48 patients). We measured and compared serum sulfatide levels and conventional cardiovascular risk factors in those two groups.

Result: The serum sulfatide concentration (18.73 ± 3.81 μmol/L) in the restenosis group was significantly higher than that (11.52 ± 3.37 μmol/L) in the nonrestenosis group ($p<0.01$). Multiple logistic regression analysis for risk factors revealed a significant correlation between serum sulfatide and restenosis after PCI ($p<0.05$). The concentration of serum sulfatide was positively correlated with the coronary percent stenosis at the time of follow-up angiography ($r=0.32$, $p<0.05$).

Conclusion: High concentration of serum sulfatide is therefore a risk factor for restenosis after PCI in patients with CHD.

Keywords: Serum sulfatide; Percutaneous coronary intervention; Restenosis

Introduction

Percutaneous Coronary Intervention (PCI) is an established myocardial revascularization procedure. However, restenosis after successful PCI remains a significant problem, which occurs in approximately one-third of patients within 6 months [1]. Restenosis is regarded as the result of a combination of various pathophysiologic events including artery atherosclerosis, inflammatory reaction, thrombogenesis, and the proliferation of vascular components such as the extracellular matrix in an injured vessel [2]. The mechanisms are complex and not completely understood. Therefore, identification of the novel risk factors would enable us to implement a more effective therapeutic strategy to ameliorate the outcome of PCI.

Sulfatide is ester of sulfuric acid with galactosylceramides at C3 of the galactosyl residue [3]. Lots of experimental and clinical studies have shown that serum sulfatide is related to Atherosclerosis (AS) [2,4] and Coronary Heart Disease (CHD), may be a novel biomarker for cardiovascular disease in patients with end-stage renal failure [5]. And serum sulfatide is also closely related to inflammatory reaction and thrombogenesis, even the proliferation of the extracellular matrix in an injured vessel [2,4,6].

In this study, we examined the relationship between serum sulfatide concentration and restenosis after PCI to investigate whether serum sulfatide is a novel predictor of restenosis after PCI in patients with CHD.

Methods

Patients: It has been found that one lesion of coronary artery shows restenosis whereas the other lesions do not in multiple vessel coronary angioplasty. In such instance evaluation of the influence of patient-specific factors on restenosis after PCI becomes difficult [7]. Therefore, in order to reduce the heterogeneity of the study population, we limited our study to patients with single-vessel disease. The subjects included 68 patients with stable atherosclerotic coronary artery disease who underwent a planned single coronary stent implantation in Hebei General Hospital. The patients' characteristics are shown in Table 1. All of the patients received standard daily oral medications for angina, including 100 mg of aspirin, and none of these medications were discontinued or exchanged during the stent procedure or the post-

	Restenosis group	Nonrestenosis group	
No.of patients	20	48	
Age (years)	65.6 ± 6.6	65.0 ±7.1	NS
Gender (male) (%)	75.0	70.8	NS
BMI (kg/m^2)	24.8 ± 2.0	24.7 ± 1.7	NS
SBP (mmHg)	147±13	140 ±15	NS
DBP (mmHg)	80 ±11	78 ± 8	NS
Coronary risk factors			
Hypertension (%)	55.0	39.6	NS
Diabetes mellitus (%)	35.0	25.0	NS
Smoking (%)	60.0	52.1	NS
Family history (%)	30.0	20.8	NS
Hyperlipidemia (%)	65.0	50.0	NS

NS: not significant

Table 1: Baseline and Clinical data of restenosis and nonrestenosis groups.

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stent follow-up period. The patients also received 75 mg of daily oral clopidogrel starting 3 days before stent implantation, and this therapy was continued at least for one year after stent implantation as a specific post-stent anti-platelet regimen. The hospital ethics committee for human subjects approved the study. Written informed consent was obtained from all patients before experimentation began.

Blood sampling: Blood samples from fasting patients on admission were obtained from all subjects early in the morning prior to the PCI. Clinical data such as total cholesterol, triglyceride, High-Density Lipoprotein (HDL) cholesterol and Low-Density Lipoprotein (LDL) cholesterol in blood samples, were measured by an autobiochemical analysis system (AU2700, Olympus, Japan). For the measurement of the sulfatide, the serum was stored at -80°C until analyzed.

Measurement of serum sulfatide: Sulfatide was extracted from specimens using a hexane- isopropanol mixture and analyzed as lyso-forms (sulfatides without fatty acids) using our developed method [8]. Briefly, the total lipids were extracted from 50 μl of serum with n-hexane: isopropanol (3:2 v/v). After dried and hydrolyzed with 0.1 N NaOH in 90% methanol at 150°C for 30 min, sulfatide was converted to lyso-sulfatide. Subsequently, samples were desalted by Mono-tip C18 tips (GL Sciences, Tokyo, Japan) and analyzed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry with delayed ion extraction using a Voyager Elite XL (6.5 m flight length in the reflector mode) Biospectrometry Workstation (PerSeptive Biosystems, Framingham, MA, USA). A nitrogen laser (337 nm) was used for ionization and negative ion mode detection was employed.

Quantitative coronary angiographic measurements: Coronary angiography was performed with Judkins' catheters via the right femoral artery using a digital angiographic system. Angiography, coronary angioplasty, stent implantation and follow-up angiography were performed by the experienced investigators who were unaware of the patients' clinical and analytic data. All patients received heparin at the dose required to maintain the activated clotting time above 300 seconds throughout the procedure. Related parameters were determined and are shown in Table 2. Quantitative Coronary Angiographic (QCA) measurements were performed before and after stent implantation and had a follow-up angiography approximately 6.5 months after the PCI. The angiographic criteria for restenosis after PCI was defined by the follow-up angiography associated with ≥50 percent stenosis at the site of angioplasty. Patients with restenosis were categorized as the restenosis group and those without restenosis as the nonrestenosis group.

Statistical analysis: The data is presented as a means standard. Univariate analysis with the unpaired student's t test was performed for continuous clinical and serum variables in the restenosis and nonrestenosis groups; chi-square tests were performed for categorical variables. The differences of serum sulfatide in the two groups were analyzed by Wilcoxon ranksum test. Multiple logistic regression analysis was used to simultaneously evaluate the relations of several factors with restenosis versus nonrestenosis. A correlation between the serum concentration of sulfatide and the coronary artery percent stenosis at the time of follow-up angiography was assessed by linear regression analysis. A *P* value <0.05 was considered statistically significant.

Results

Baseline and Clinical Data: As shown in Table 1, the 68 patients were classified into the restenosis group (20 patients) and the nonrestenosis group (48 patients). The incidence of restenosis was 20 of 68 patients (29%). There were no significant differences in age, gender, body mass index or blood pressure between the restenosis group and nonrestenosis group at baseline. The proportion of hypertensive, hyperlipidemic and diabetes patients tended to be higher in the restenosis group than in the nonrestenosis group, but there were no significant differences (P>0.05).

In the acute phase there were no significant differences between the 2 groups in target lesion, reference diameter, reperfusion time, stenosis before and after PCI, left ventricular ejection fraction (Table 2).

Serum lipid, serum sulfatide concentration and distribution: Table 3 shows that there were no significant differences in serum concentrations of total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol between the restenosis and nonrestenosis groups. Although the total cholesterol concentration and LDL-cholesterol concentration tended to be high in the restenosis group. Only the serum sulfatide concentration (18.73 ± 3.81 μmol/L) in the restenosis group was significantly higher than that (11.52 ± 3.37 μmol/L) in the nonrestenosis group (p<0.01).

In the total patient population, the mean serum sulfatide concentration was (13.64 ± 4.80 μmol/L). The minimum and maximum of serum sulfatide concentration were 3.60 μmol/L and 27.71 μmol/L, respectively (Figure 1).

Multiple logistic regression analysis: The results of multiple logistic regression analysis for risk factors in restenosis were shown in Table 4. There were significant correlations between restenosis and serum sulfatide concentration (p<0.01); while there were no correlations between restenosis and other factors, including age, male, hypertension, diabetes mellitus, smoking, family history, and hyperuricemia and concentrations of total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol.

Linear regression analysis: Figure 1 shows that there was positive correlation between serum sulfatide concentration and the percent

	Restenosis group(n=20)	Nonrestenosis group(n=48)	
Target vessel			
LAD	9	24	NS
RCA	6	14	NS
LCX	5	10	NS
Referencediameter(mm)	3.10 ± 0.45	3.12 ± 0.31	NS
Reperfusion time(min)	235 ± 182	282 ± 245	NS
Stenosis before PCI(%)	89 ± 15	90 ± 13	NS
Stenosis after PCI(%)	32 ± 11	30 ± 10	NS
LVEF(%)	56.9 ± 17.5	55.7 ± 16.9	NS

LAD: Left Anterior Descending Artery; RCA: Right Coronary Artery; LCX: Left Circumflex Artery; LVEF: Left Ventricular Ejection Fraction; PCI: Percutaneous Coronary Intervention

Table 2: Clinical characteristics in the Acute Phase.

	Restenosis group(n=20)	Nonrestenosis group(n=48)	
Total cholesterol(mmol/l)	5.16 ± 0.65	4.86 ± 0.65	NS
Triglyceride(mmol/l)	1.53 ± 0.55	1.41 ± 0.42	NS
HDL-C(mmol/l)	0.96 ± 0.18	1.05 ± 0.17	NS
LDL-C(mmol/l)	3.25 ± 0.49	2.98 ± 0.52	NS
Sulfatide(μmol/l)	18.73 ± 3.81	11.52 ± 3.37	<0.001

HDL-C: High Density Lipoprotein- Cholesterol; LDL-C: Low-Density Lipoprotein-Cholesterol

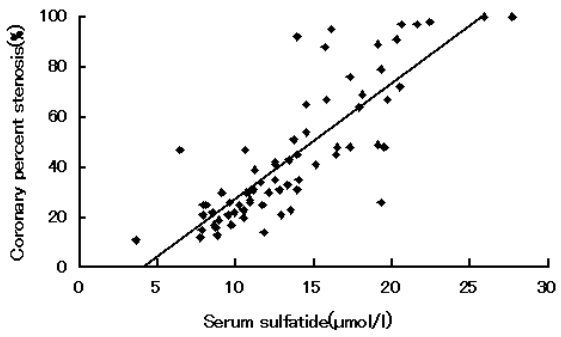
Table 3: Serum lipid and sulfatide concentrations between restenosis and nonrestenosis groups.

		Standard error	p Value
Age	-0.041	0.039	0.391
Male	-0.985	0.799	0.235
Total cholesterol	-0.003	0.010	0.851
Triglyceride	-0.007	0.006	0.532
HDL-C	-0.011	0.018	0.521
LDL-C	1.161	0.663	0.072
Sulfatide	0.058	0.020	0.003*
Hypertension	0.172	0.581	0.755
Diabetes mellitus	1.101	0.742	0.145
Smoking	0.451	0.650	0.495
Family history	-0.763	0.645	0.565
Hyperuricemia	0.710	0.851	0.406

*Significant.

HDL-C: High Density Lipoprotein- Cholesterol; LDL-C: Low-Density Lipoprotein-Cholesterol

Table 4: Multiple logistic regression analysis for risk factors in restenosis.



Graph shows significant correlation between serum sulfatide concentration and coronary percent stenosis at time of follow-up angiography (n =68; y =4.58x-17.98; r = 0.68, p <0.01).

Figure 1: The distribution of values for serum sulfatide level.

stenosis of coronary artery at the time of the follow-up angiography (y=4.58x-17.98; r=0.68, p<0.01). From Figure 1, we hypothesized that restonesis would be likely to happen (coronary percent stenosis >50%) when the serum sulfatide concentration was more than 14.84μmol/L in the patients with CHD after PCI.

Discussion

We assessed whether there are positive correlations between serum sulfatide and restenosis in patients with CHD after PCI. The serum sulfatide concentration in the restenosis group was significantly higher than that in the nonrestenosis group. The incidence of other risk factors did not significantly differ between the two groups. Multiple logistic regression analysis for risk factors in restenosis revealed a significant correlation between restenosis and serum sulfatide concentration. Furthermore, the serum sulfatide concentration was positively correlated with the coronary percent stenosis at the follow-up angiography. These data findings therefore suggest that a high serum concentration of sulfatide may be a risk factor for restenosis in patients with CHD after PCI.

Although the mechanisms of restenosis after PCI are complex and not completely understood, which are related with the later pathophysiologic process: On the basis of severe atherosclerosis in coronary artery [9], balloon inflation and stent implantation induce vascular damage [10]. A substantial inflammatory reaction and thrombogenesis happen in the local injured vessel wall [2,6]. These conditions are followed by the proliferation of vascular components such as the extracellular matrix [11,12]. Studies have revealed that serum sulfatide is closely related to artery atherosclerosis [4], inflammatory reaction and thrombogenesis [2], even the proliferation of the extracellular matrix [6]:

In atherosclerosis: Sulfatide exists in serum lipoprotein of various mammals including humans [8]. Studies have revealed that the increased sulfatide in both lipoprotein and atherosclerosis plaques is intimately correlated with the development of atherosclerosis in WHHL rabbit-an animal model for human familial hypercholesterolemia. The serum sulfatide content in WHHL rabbit is markedly increased by 40-fold over the normal rabbit level. Furthermore, the lipid analysis of atherosclerosis aorta of WHHL rabbits revealed that a large amount of sulfatide was accumulated there, while normal aorta contained no sulfatide at all [4,13].

In inflammatory reaction: Sulfatide was demonstrated to play an important physiological role in the inflammation observed in vascular injury associated with the development of atherothrombosis or atherosclerosis [2]. In the inflammatory process after vascular injury result from stent implantation, activated leukocytes, neutrophils as well as monocytes, and platelets play an important role [14]. A layer of platelets and fibrin are first deposited at the injured and de-endothelialised vessel surface. A sequential adhesion model of leukocyte attachment to and transmigration across surface-adherent platelets has been proposed. Activated platelets on the injured vessel surface express P-selectin. The initial tethering and rolling of leukocytes on platelets are mediated by P-selectin binding to leukocyte receptors [15,16]. Leukocytes then adhere firmly to the vessel surface through leukocyte Mac-1 via direct attachment to platelet receptors [17]. It has been known that sulfatides are expressed on the membrane surface of, and are also excreted by, neutrophils [18,19]. Since neutrophil activation occurred after stent deployment [20], lots of sulfatides were released from activated neutrophils. Sulfatides are also native ligands of P-selectin [21]. Studies have found that sulfatides could activate platelets through an agonistic effect for P-selectin and enhance platelet-leukocyte aggregation at the injured vessel sites [3]. Therefore, activated neutrophils and platelets may result in increased serum sulfatides in the inflammatory process after vascular injury resulting from stent implantation.

In thrombogenesis: Sulfatides have been reported to be able to activate blood coagulation factor XII and is believed to be one of the important factors in the initiation of the intrinsic coagulation pathway [22]. Studies have found that sulfatide could markedly enhance thrombogenesis in the rat deep vein thrombosis model (vein ligation in rat), while it could not induce thrombosis in normal rats [6].Which shows sulfatides have thrombogeneic activity in condition of vessel damage. The underlying mechanism might be: Sulfatides as a native ligand of P-selectin could activate platelets through P-selectin, thus enhancing platelet and platelet-leukocyte aggregation [2].

In proliferation of extracellular matrix: Studies have demonstrated that sulfatide could specifically bind to extracellular matrix components such as laminin, thrombospondin, and von Willebrand factor (vWF) [6]. The procedure of balloon inflation and stent implantation induced vessel endothelium damage, exposing and stimulating the extracellular matrix. Along with the proliferation of extracellular matrix, more and more sulfatides, as an adhesive molecule, would increase and gather around the local injured vessel.

Study Limitations: Only 68 patients participated in this study; thus in the restenosis group analysis, the number of patients was small. Therefore, there were marginally significant differences in some factors such as total cholesterol, HDL cholesterol, LDL cholesterol and plasma glucose between restenosis and nonrestenosis groups. The relatively small amount of clinical data may have limited the statistical power in the analysis of the efficacy of stenting.

In conclusion, we reveal: There are positive correlations between serum sulfatide and restenosis after PCI. A high serum concentration of sulfatide may be a risk factor for restenosis in patients with CHD after PCI. Although it is still premature to conclude, we believe that the determination of the level of serum sulfatides could provide a new means for the diagnosis and prevention of restenosis after PCI, which would contribute to an improvement in patients' quality of life and to an increase in their life span. Large-scale clinical studies to confirm and expand on our findings are currently ongoing.

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