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Lipoprotein (a) as a risk factor for silent cerebral infarction in hemodialysis patients

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Abstract

In patients with chronic renal failure undergoing hemodialysis (HD), silent cerebral infarctions (SCIs) are associated with high mortality. Levels of lipoprotein (a) (Lp[a]) increase with renal dysfunction and may be a novel predictor for cerebrovascular events. We tested the hypothesis that increased Lp(a) levels correlate with the occurrence of SCI in HD patients. Using cranial magnetic resonance imaging findings, we divided 62 Japanese patients undergoing HD into with-SCI group (61 ± 7 years, mean ± SD, n = 34) and without-SCI group (60 ± 6 years, n = 28). We compared the sex, body mass index, metabolic profiles, Lp(a) levels, and smoking habits between the 2 groups. The following observations were noted: (1) The number of patients with diabetes or hypertension did not differ between the 2 groups. (2) The levels of Lp(a) were higher in the with-SCI group in comparison with the without-SCI group ($P < .0001$). (3) The proportion of smokers was higher in the with-SCI group than in the without-SCI group ($P < .05$). (4) Plasma levels of high-density lipoprotein cholesterol were lower, whereas uric acid was higher, in the with-SCI group than in the without-SCI group ($P < .001$ and $P < .05$, respectively). (5) Multiple logistic regression analysis identified Lp(a) levels as being significantly associated with the presence of SCI (odds ratio, 1.23; 95% confidence interval, 1.09-1.38; $P < .0001$). This study indicates that patients with chronic renal failure, who are maintained on HD, exhibit an increased prevalence of SCI and that Lp(a) is significantly associated with the presence of SCI in HD patients.

1. Introduction

The mortality related to cerebrovascular events in chronic hemodialysis (HD) patients is 4 to 10 times higher compared with the general population [1]. Stroke in HD patients is characterized by a high rate of intracerebral hemorrhage, and hypertension is a significant risk factor for stroke in this group [2-5].

Lipoprotein (a) (Lp[a]) consists of a low-density lipoprotein particle covalently linked by a disulfide bond with a large, unique glycoprotein called *apo(a)* [6]. This Lp(a) has attracted much attention as a potential risk factor for atherosclerotic coronary heart disease and stroke in the general population [7-9]. In addition, Lp(a) levels are independent indicators of the future risk of fatal coronary heart disease in HD patients [10,11].

Silent cerebral infarction (SCI) can underlie or occur concomitantly with clinical subcortical brain infarction or brain hemorrhage [12]. In most cases, SCI is discovered as a lacunar infarct. This common form of subcortical infarction is defined by Fisher [13] as small, deep cerebral infarctions caused by occlusion of small penetrating cerebral arteries.

The significance of increased Lp(a) levels in HD patients with SCI has not been adequately investigated. We hypothesized that increased Lp(a) levels are associated with SCI in HD patients. To test our hypothesis, we compared magnetic resonance imaging (MRI) findings and metabolic profiles between Japanese HD patients with SCI

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and those without SCI and determined the independent predictors of SCI in these patients.

2. Subjects and methods

2.1. Patients

A total of 84 patients, who were maintained on HD from September 2006 to August 2007 at Oita Red Cross Hospital, were included in the study. Patients with complications considered critical were excluded from the study, including 8 patients with atrial fibrillation, 7 patients with a history of symptomatic stroke, 5 patients with transient ischemic attacks, and 2 patients with malignancies. Therefore, 62 of the original 84 patients, 35 men and 27 women (mean age, 61 ± 7 years), were selected for this study.

The primary renal disease causing chronic renal failure included diabetic nephropathy in 25 patients, chronic glomerulonephritits in 21 patients, chronic nephrosclerosis in 14 patients, and lupus nephritis in 2 patients. All subjects gave their written informed consent to participate in the study. The study protocol was approved by the ethics committee of the Oita Red Cross Hospital.

2.2. Risk factors

To evaluate potential risk factors, we checked for the presence or absence of hypertension, diabetes mellitus, dyslipidemia, ischemic heart disease (IHD), and smoking history. *Essential hypertension* was defined as systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg, or subjects on antihypertensive drugs regimen [14]. There were 25 of 34 with-SCI patients and 20 of 28 without-SCI patients who met this criterion. All of these patients were being treated with calcium channel antagonists, *β*-blockers, angiotensin-converting enzyme inhibitors, and/or angiotensin II receptor blockers, either alone or in combination. *Diabetes mellitus* was recognized in patients using insulin or oral hypoglycemic agents and in patients whose fasting glucose concentration was greater than 126 mg/dL. *Dyslipidemia* was defined as fasting triglycerides ≥200 mg/dL, high-density lipoprotein cholesterol (HDL-C) <45 mg/dL for women and <35 mg/dL for men [14], or patients who were receiving medical treatment of hyperlipidemia. *Ischemic heart disease* was considered in patients who experienced angina, history of myocardial infarction, prior coronary artery bypass surgery, or percutaneous coronary intervention. *Smoking history* encompassed current cigarette smokers.

Blood samples were taken from the arterial line before HD treatment sessions. Serum from blood samples for these assays was separated and stored at −20°C until tested. Serum Lp(a) concentration was measured by a latex agglutination method with an anti–human Lp(a) monoclonal antibody using a commercial kit (Lp[a] Latex Daiichi) from Daiichi Pure Chemicals (Tokyo, Japan) [15].

The detection antibody is directed to a nonrepeating epitope present in apo(a) K-IV type 9, making this assay insensitive to apo(a) size. The Lp(a) concentrations are expressed in nanomoles per liter. The analytic coefficient of variation of Lp(a), based on 5 duplicate samples of varying Lp(a) concentrations (12-120 nmol/L) in each enzyme-linked immunosorbent assay plate, ranged from 4.0% to 6.7%.

2.3. Hemodialysis method

Hemodialysis in these patients was performed using a 4F catheter micropuncture set or a 4F Kumpe access catheter (Cook Group, Bloomington, IN). This method has been described in a previous study [16]. In all cases, access to the graft or fistula was initially obtained with a 19-gauge needle. Hemodialysis patients received regular dialysis 3 times per week using a high-flux cellulose-triacetate dialyzer membrane in sessions lasting 4 hours. The dialysate flow rate was 500 mL/min, and the blood flow ranged from 120 to 200 mL/min. Dry weight was determined for each patient from the post-HD cardiothoracic ratio. During the HD session, we also noted several clinical observations, such as the presence of muscle cramps, general fatigue, thirst, or hypotension. All patients were maintained at their set dry weight. No differences in dialysis methods were observed between the 2 groups.

2.4. Evaluation of SCI

All participating patients underwent cranial MRI scan of the brain. T_1- and T_2-weighted axial images of 5-mm–thick slices were collected with a 1.5-T field on proton density (Visart EX; Toshiba, Tokyo, Japan). The head position was oriented in the scanner and stabilized during the scanning procedure using a head support. To establish slice orientation, the first scanning sequence was a T_1-weighted sagittal series (repetition time [TR], 500 nanoseconds; echo time [TE], 15 milliseconds; matrix, 256 × 256) to define the orbitomeatal line. We oriented the second series of T_1-weighted (TR, 500 milliseconds; TE, 15 milliseconds; thickness, 8 mm; gap, 1.5 mm; matrix, 256 × 256) and T_2-weighted (TR, 4000 milliseconds; TE, 120 milliseconds; thickness, 8 mm; gap, 1.5 mm; matrix, 320 × 320) axial images parallel to the orbitomeatal line. Fourteen slices were inspected at each examination. The accuracy of this method has previously been validated [17].

Lacunar infarcts were identified by the presence of hyperintense areas on T_2-weighted images (5 mm < diameter < 15 mm), whereas they were visible as low-signal intensities on T_1-weighted images. As described by Braffman et al [18], lesions <5 mm were not counted as infarctions to exclude enlarged periventricular spaces. Two neurologists blinded to the subjects' names, characteristics, and clinical status interpreted the MRI images of the subjects that had been randomly stored.

2.5. Statistical analysis

All data were summarized as the mean ± SD. Differences between the 2 groups were examined for continuous variables

Table 1
Clinical characteristics of patients

	SCI (−)	SCI (+)	*P* value
Age (y)	60 ± 6	61 ± 7	NS
Sex (men/women)	16/12	19/15	NS
Body mass index (kg/m^2)	22.1 ± 1.6	22.7 ± 2.4	NS
Dialysis duration (y)	1.9 ± 1.3	1.8 ± 1.5	NS
Diabetes mellitus (%)	57	62	NS
Hypertension (%)	71	74	NS
Dyslipidemia (%)	46	53	NS
Smoking habit (%)	18	44	.0187
IHD (%)	18	41	.0475
Drug use (%)			
Sulfonylurea	31	29	NS
α-Glucosidase inhibitors	25	26	NS
Insulin	11	12	NS
Statin	39	44	NS
Calcium channel antagonists	61	68	NS
ACE inhibitors	14	18	NS
Angiotensin receptor blocker	29	32	NS
β-Blocker	18	21	NS
Hematocrit (%)	30.9 ± 3.6	29.6 ± 2.9	NS
Total cholesterol (mg/dL)	155 ± 56	174 ± 65	NS
Triglyceride (mg/dL)	104 ± 35	123 ± 46	NS
HDL-C (mg/dL)	48 ± 14	36 ± 11	.0004
Fasting plasma glucose (mg/dL)	125 ± 24	129 ± 27	NS
HbA_{1c} (%)	6.3 ± 1.1	6.5 ± 1.4	NS
Uric acid (mg/dL)	6.9 ± 1.5	8.1 ± 2.1	.0443

Data are presented as mean ± SD. ACE indicates angiotensin-converting enzyme; NS, not significant.

using the Student *t* test and for categorical variables with the χ^2 test. Logistic regression analysis was used to assess the influence of explanatory variables on SCI, in which sex, hypertension, diabetes mellitus, dyslipidemia, IHD, and smoking were represented by dummy variables (1 = male, 0 = female; 1 = present, 0 = absent). If x_i ($i = 1,2,\ldots,I$) were the explanatory variables and Y was the dichotomous response variable, such that $Y = 0$ represented the without-SCI group and $Y = 1$ represented the with-SCI group, the conditional probability of $Y = 1$ in the logistic regression model given the explanatory variables was given as:

$$\Pr(Y = 1|x_i, i = 1, 2, \ldots, I) = \frac{\exp\left(\sum_{i=1}^{I} \beta_i x_i\right)}{1 + \exp\left(\sum_{i=1}^{I} \beta_i x_i\right)}$$

A forward stepwise logistical regression was selected using a cutoff level of 0.05 for significance. Differences were considered statistically significant at $P < .05$.

3. Results

As demonstrated in Table 1, the mean age was similar between the with-SCI group and without-SCI group ($P = .8042$). No significant differences were observed between the 2 groups with respect to sex, body mass index, or HD duration ($P = .7873$, $P = .3353$, and $P = .9503$, respectively). The percentages of patients with diabetes, hypertension, and dyslipidemia were similar between the 2 groups (57% vs 62%, 71% vs 74%, and 46% vs 53%, respectively). However, the with-SCI group had a higher percentage of smokers and IHD than the without-SCI group (44% vs 18% and 41% vs 18%, respectively).

There were no significant differences in hematocrit, fasting plasma glucose concentration, or hemoglobin (Hb) A_{1c} levels between the 2 groups ($P = .1088$, $P = .5299$, and $P = .4431$, respectively). With regard to lipid metabolism, serum HDL-C was lower in the with-SCI group than in the without-SCI group ($P = .0004$), whereas serum total cholesterol and triglyceride levels were not significantly different between the groups ($P = .2339$ and $P = .874$, respectively). Serum uric acid level was higher in the with-SCI group than without-SCI group ($P = .0443$). The Lp(a) levels were higher in the with-SCI group than in the without-SCI group (33.0 ± 10.9 vs 17.9 ± 8.3 ng/mL, $P < .0001$).

By univariate logistic regression analysis, the risk of SCI was increased with smoking (odds ratio [OR], 3.63; 95% confidence interval [CI], 1.12-11.8; $P = .0323$), decreased HDL-C (OR, 0.93; 95% CI, 0.88-0.97; $P = .0015$), elevated uric acid (OR, 1.31; 95% CI, 1.00-1.71; $P = .0483$), and elevated Lp(a) (OR, 1.23; 95% CI, 1.09-1.38; $P < .0001$) (Table 2).

Multiple logistic regression analysis included the following independent variables in the model: smoking, HDL-C, uric acid, and Lp(a) as categorical variables. Multivariate logistic regression analysis identified plasma Lp(a) in HD patients as a significant indicator associated with SCI (OR, 1.23; 95% CI, 1.09-1.38; $P < .0001$).

Table 2
Univariate logistic regression analysis of HD patients with SCI as the dependent variable

	SCI		
	OR	95% CI	*P* value
Age	1.01	0.94-1.09	NS
Sex	0.95	0.35-2.61	NS
Diabetes mellitus	0.90	0.32-2.53	NS
Hypertension	1.30	0.42-4.07	NS
Dyslipidemia	1.29	0.48-3.54	NS
Smoking habit	3.63	1.12-11.8	.0323
IHD	3.22	0.98-10.5	NS
Hematocrit	0.88	0.74-1.03	NS
Total cholesterol	1.01	0.99-1.01	NS
Triglyceride	1.01	0.99-1.02	NS
HDL-C	0.93	0.88-0.97	.0015
Fasting plasma glucose	1.01	0.99-1.03	NS
HbA_{1c}	1.18	0.78-1.78	NS
Uric acid	1.31	1.00-1.71	.0483
Lp(a)	1.23	1.12-1.38	<.0001

Significant predictors of SCI were explored among 6 parameters: sex (female= 0, male = 1), diabetes mellitus (absent = 0, present = 1), hypertension (absent = 0, present = 1), dyslipidemia (absent = 0, present = 1), smoking habit (absent = 0, present = 1), and IHD (absent = 0, present = 1).

4. Discussion

In this study, the measurement of metabolic parameters revealed that serum HDL-C levels were lower and Lp(a) levels were higher in the with-SCI group than in the without-SCI group. There was a significant association on increased Lp(a) levels and SCI in Japanese HD patients.

Silent cerebral infarction is an important risk factor for stroke. Several reports have examined SCI in the general population. According to Kobayashi et al [12], MRI studies revealed an incidence of SCI of 10.6% in 993 neurologically normal adults without a history of cerebrovascular disease (mean age, 58 years). The National Institute for Longevity Sciences Longitudinal Study of Aging predicted an incidence of SCI of 10.3% (mean age, 59 years) [17]. The prevalence of SCI in HD patients is approximately 5 times greater than that seen in the normal population (mean ages, 54 and 56 years, respectively) [4,5]. This incidence of SCI is similar to that seen in HD patients (34 of 62 [54.8%]) in this study.

Prospective study of 5888 community-dwelling adults in the United States reveals that the mean Lp(a) level is 4.2 mg/dL [19], and the mean Lp(a) level of the Framingham heart study is 7.1 mg/dL [20]. However, Lp(a) levels are elevated in dialysis patients [10,11]. Ohashi et al [10] reported that in a study of 268 patients, the mean level of Lp(a) was 29.3 mg/dL in patients with cardiovascular events compared with the mean level of 19.5 mg/dL in patients without cardiovascular events. Krosenberg et al [11] demonstrated that the mean Lp(a) levels was 23.4 mg/dL in HD patients. In the present study of Japanese HD patients, the mean Lp(a) is 26.2 mg/dL. In addition, the mean levels of Lp(a) were 33.0 and 17.9 mg/dL in patients in the with-SCI group (n = 34) and without-SCI group (n = 28), respectively. Therefore, these previous reports are in agreement with our study. Although the precise mechanisms responsible for the high levels of Lp(a) in HD patients are still unclear, further studies are recommended to explain this view.

The underlying mechanism of the links between serum Lp(a) and SCI remains to be elucidated. In our opinion, several mechanisms could explain this observation. First, it has been suggested that Lp(a) plays a part in the initiation, progression, and subsequent rupture of atherosclerotic plaque [21,22]. Second, because of the structural homology of apoprotein (a) and plasminogen, Lp(a) may compete with, bind, and inhibit the thrombolytic activity of tissue plasminogen. Lipoprotein (a) could therefore have a thrombogenic effect through interference with intrinsic fibrinolysis [21-23]. Third, Lp(a) has been associated with endothelial dysfunction [24]. Fourth, Lp(a) activates monocytes, colocalizes with plaque macrophages, stimulates smooth-muscle cells, and could induce inflammation [25]. Thus, Lp(a) not only appears to be a risk factor for arterial sclerosis, but possibly acts as a pathophysiological modulator of other causes of endothelial dysfunctions.

There are several limitations to this study. Firstly, this study included a relatively small number of patients and did not include age-matched control subjects. Secondly, there is a limitation associated with the interpretation of data in a cross-sectional study. In this case, the long-term consistency of plasma levels of Lp(a) needs to be demonstrated.

Elevated Lp(a) levels may be increased as a result of underlying ischemia. The elevation in the context of this study represents an epiphenomenon. A prospective longitudinal study will be required to address the issues and to identify factors determining the plasma level of Lp(a) in relation to the development of stroke in HD patients.

In conclusion, our study indicates that chronic renal failure treated by HD increases the prevalence of SCI. In addition, elevated Lp(a) levels may be an epiphenomenon significantly associated with SCI in HD patients.

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