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Anti-Heparin-Platelet Factor 4 Antibody is a Risk Factor for Vascular Access Obstruction in Patients Undergoing Hemodialysis

Since heparin is an anticoagulant commonly used in hemodialysis and the patients on hemodialysis are repeatedly exposed to heparin, heparin may be the cause of the development of heparin-dependent antibodies and thrombotic complications in patients on hemodialysis. The purpose of this study was to determine the prevalence and the clinical significance of the antibodies against heparin-platelet factor 4 complexes as determined by enzyme immunoassay in patients on maintenance hemodialysis. The prevalence of anti-heparin-platelet factor 4 antibodies was higher in hemodialysis patients than in normal subjects (8.8 vs 0.0%, $p<0.05$). The number of past episodes of vascular access obstruction per year was significantly higher in the anti-heparin-platelet factor 4 antibody positive group than antibody negative group. Anti-heparin-platelet factor 4 antibody positive patients experienced more frequent vascular access obstructions than control subjects. In conclusion, anti-heparin-platelet factor 4 antibody might be a risk factor for vascular access obstructions in patients with end-stage renal disease on maintenance hemodialysis.

Key Words : *Anti-Heparin-Platelet Factor 4 Antibody; Renal Dialysis; Vascular Access Obstruction; Kidney Failure, Chronic*

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INTRODUCTION

The wide use of heparin led to the increasing recognition of untoward complications including heparin-induced thrombocytopenia/thrombosis. This phenomenon, which is characterized by a sharp drop in the platelet count and subsequent platelet activation and aggregation after heparin treatment (1, 2), is caused by the binding of heparin-IgG immune complexes to platelets by the Fc portion of the IgG molecule, and the binding of antibodies to platelets is markedly enhanced by the presence of heparin (3, 4).

Since heparin is an anticoagulant most often used during hemodialysis and patients with end-stage renal disease on maintenance hemodialysis are repeatedly exposed to heparin, one might expect a higher frequency of heparin-dependent antibodies in hemodialysis patients. Since these antibodies are associated with thromboses (5) and thrombotic complications such as vascular access obstructions are common in patients on maintenance hemodialysis, one might also expect the association between heparin-dependent antibodies and vascular access obstructions in hemodialysis patients. However, the incidence of anti-heparin-platelet factor 4 antibodies and the association between anti-heparin-platelet factor 4 antibodies and vascular access obstructions in hemodialysis patients are not well known so far.

The purpose of this study was to determine the prevalence of anti-heparin-platelet factor 4 antibodies and to evaluate the risk of vascular access obstruction imposed by these antibodies in patients on maintenance hemodialysis.

MATERIALS AND METHODS

A total of 91 end-stage renal disease patients on maintenance hemodialysis (41 men, 50 women, mean age 51.0±13.1 yr) and 40 healthy subjects (20 men, 20 women, mean age 47.9 ±13.5 yr) were included in this study. We excluded patients who had never used heparin during hemodialysis for various medical reasons. Blood samples were obtained prior to the hemodialysis procedure. Informed consent was obtained from each subject.

All end-stage renal disease patients were undergoing regular hemodialysis 8 to 12 hr weekly using a cellulose acetate hollow-fiber dialyser (surface area 1.2 m^2, Gambro, Stockholm, Sweden) at a hemodialysis unit of the Soonchunhyang University Chonan Hospital, Chonan, Korea.

The anti-heparin-platelet factor 4 antibodies were measured by enzyme immunoassay according to the instructions of the manufacturer (Asserachrom HPIA, Diagnostica Stago, Paris, France). The microtiter plate was covered by heparin-platelet factor 4 complexes. Bound antibodies were discovered through a mixture of anti-human IgG, IgA, and IgM. Absorbance was measured at 492 nm, and the cut-off was defined as mean+ 2S.D. The lower limit was confirmed in our laboratory by test-

ing 20 normal control serum samples. Plasminogen activator inhibitor-1 (Asserachrom PAI-1, Diagnostica Stago, Paris, France) and tissue plasminogen activator (Asserachrom tPA, Diagnostica Stago, Paris, France) were also determined quantitatively by enzyme-linked immunosorbent assay (6, 7).

Data were presented as mean±S.D. for continuous variables and as frequency (%) for categorical variables. *p*-value was calculated by Student's t-test for continuous variables or by Fisher's exact test for categorical variables. A value of $p<0.05$ was considered statistically significant and all statistical analysis was performed using SPSS for Windows (version 10.0, Chicago, Illinois, U.S.A.).

RESULTS

In 91 end-stage renal failure patients, the mean duration of hemodialysis was 4.1±3.4 (range; 0.4 to 15.2) yr. The causes of renal failure were diabetes mellitus (n=30), hypertension (n=26), polycystic kidney disease (n=4), chronic glomerulonephritis (n=13), and unknown (n=18). Normalized protein catabolic rate was 1.1±0.3. Mean systolic and diastolic blood pressures were 141.1±17.3 and 85.3±10.7 mmHg, respectively. Seventy-six patients (83.5%) were on hypertensive medications. The serum albumin level was 37±4 g/L and hematocrit was 0.26±0.03. Serum calcium and phosphorus were 2.3±0.3 mmol/L and 1.6±0.6 mmol/L, respectively. Twenty-six patients (28.6%) had experienced vascular access obstruction at least once. All of the patients had the arteriovenous fistula except only one patient who had the atreriovenous graft.

Eight patients were positive for anti-heparin-platelet factor 4 antibodies. The prevalence of anti-heparin-platelet factor 4 antibodies was higher in hemodialysis patients than in normal subjects (8.8 vs 0.0%, $p<0.05$). The mean age was 50.6±7.2 yr, and the mean duration of hemodialysis was 63.3±55.8 months. Four patients experienced vascular access obstruction more than once. None of them experienced a significant decline of their platelet count after the initiation of heparin exposure during hemodialysis treatment. Three patients also had liver cirrhosis (Hepatitis B-associated in two patients and alcoholic in one patient). The mean levels of plasminogen activator inhibitor-1 and tissue plasminogen activator were 18.06 ±8.48 and 1.67±0.58 ng/mL, respectively, in patients positive for anti-heparin-platelet factor 4 antibodies.

Table 1. Comparisons between anti-heparin-PF4 antibodies positive and negative groups of patients with end-stage renal failure on maintenance hemodialysis

Variable	Positive (n=8)	Negative (n=83)	*p*-value
Age (yr)	50.6±7.2	51.0±13.5	0.94
Male (%)	25.0	47.0	0.23
Duration of HD (yr)	5.4±4.7	4.1±3.3	0.30
No. with a past history of VAO (%)	50.0	26.5	0.16
No. of episodes of VAO (/yr)	0.49±1.19	0.13±0.30	0.03
Systolic BP (mmHg)	142.5±27.6	144.3±16.3	0.79
Diastolic BP (mmHg)	80.0±10.7	85.9±10.7	0.14
Platelet final (x10^9/L)	186.9±78.0	193.4±73.2	0.83
Hematocrit	0.27±0.05	0.26±0.03	0.72
Calcium (mmol/L)	2.4±0.3	2.3±0.2	0.77
Phosphorus (mmol/L)	1.5±0.6	1.6±0.6	0.53
PAI-1 (ng/mL)	18.1±8.5	15.1±5.4	0.17
t-PA (ng/mL)	1.67±0.58	1.55±2.58	0.90

HD, hemodialysis; VAO, vascular access obstruction; BP, blood pressure; PAI-1, plasminogen activator inhibitor-1; t-PA, tissue plasminogen activator. Data were presented as mean±S.D. for continuous variables and as frequency (%) for categorical variables.

The number of past episodes of vascular access obstruction per year was significantly higher in the anti-heparin-platelet factor 4 antibody positive group than in antibody negative group (Table 1, $p<0.05$). There were no other significant differences in age, sex, duration of hemodialysis, prevalence of patients with a past history of vascular access obstruction, systolic and diastolic blood pressures, platelet count at the last follow-up, hematocrit, calcium, phosphorus, plasminogen activator inhibitor-1, or tissue plasminogen activator levels between anti-heparin-platelet factor 4 antibody positive and negative groups of hemodialysis patients (Table 1). There was no significant difference in Kt/V, normalized protein catabolic rate, mean dose of heparin used, or past history of cerebrovascular accident between anti-heparin-platelet factor 4 antibody positive and negative groups.

DISCUSSION

This study demonstrates that anti-heparin-platelet factor 4 antibodies were more prevalent in end-stage renal disease patients who had been using heparin for prolonged periods than in the control subjects. Anti-heparin-platelet factor 4 antibody positive patients experienced more frequent vascular access obstructions than antibody negative patients. Therefore, anti-heparin-platelet factor 4 antibody may be a risk factor for vascular access obstructions in patients undergoing hemodialysis.

The incidence of anti-heparin-platelet factor 4 antibodies induced by heparin in patients with normal renal function varied from 2.7% to 34% in various clinical settings (8, 9). Because heparin is used in almost all hemodialysis patients, the prevalence of these heparin-dependent antibodies might be higher in patients on hemodialysis than in the general population who have never used heparin. Our study showed that there was a higher prevalence of anti-heparin-platelet factor 4 antibodies in hemodialysis patients and this supports that heparin can induce formation of these heparin-dependent antibodies. The frequency of anti-heparin-platelet factor 4 antibody formation increased progressively with the duration of heparin treatment (9). Recent reports have shown that during prolonged heparin treatment 30-60% of patients with nor-

mal renal function form anti-heparin-platelet factor 4 antibodies after open heart surgery or vascular surgery (9, 10). Since heparin is also used for a prolonged duration in maintenance hemodialysis patients, the prevalence of these heparin-dependent antibodies might be higher in patients on hemodialysis than in patients on a short term heparin treatment. Surprisingly, however, a longer duration of heparin treatment did not lead to as high a percentage of anti-heparin-platelet factor 4 antibodies in chronic hemodialysis patients as expected. These results might be caused by uremia-related obtundation of the immune response in patients with end-stage renal failure. However, this hypothesis was not fully confirmed in this study.

Other studies have shown that vascular access obstruction contributes substantially to the morbidity, mortality, and high costs associated with hemodialysis treatment (11). Several risk factors of vascular access obstruction including diabetes mellitus, hypotension, hypoalbuminemia, anticardiolipin antibodies, increased serum levels of lipoprotein (a), and fibronectin have been reported (11, 12), however, a significant percentage of vascular access obstruction remains unexplained. A hypercoagulable state caused by alterations of coagulation and fibrolytic factors frequently occurs in renal failure (13). Anticoagulation with heparin is intended to reduce these complications during the hemodialysis procedure. However, paradoxically, the administration of heparin can lead to heparin-induced thromboembolic complications caused by the formation of anti-heparin-platelet factor 4 antibodies (14), therefore, heparin use in chronic dialysis patients might be a risk factor causing vascular access obstruction. Since the anti-heparin-platelet factor antibody increases the number of past episodes of vascular access obstruction in this study, we might say that heparin dependent anti-heparin-platelet factor 4 antibody is a risk factor for vascular access obstructions in patients receiving regular hemodialysis.

How antibodies can be generated and why only part of them are pathogenic are not yet clear. However, the presence of the IgG isotype at a high concentration and the clinical context which induces accumulation and activation of platelets at pathological sites, are suggested to be important factors for disease occurrence (15).

Several methods to detect the heparin-dependent antibodies have evolved. The most recent tests for heparin-dependent antibodies are based on the recognition that the antibodies are directed against the heparin-platelet factor 4 complex by enzyme immunoassay (3, 16). Since this method was more sensitive and easier to perform than conventional platelet function assays such as platelet aggregation, serotonin release, and flow cytometry (17), and immunoassays and functional assays agree in about 80% of cases (18), we applied the method to determine the anti-heparin-platelet factor 4 antibodies in this study. These antibodies, however, are generated only in part of heparin-treated patients, and still only a subgroup of them develop vascular access obstruction.

In conclusion, the prevalence of anti-heparin-platelet factor 4 antibodies was higher in patients who had used heparin as an anticoagulant during hemodialysis than a control subjects and the number of past episodes of vascular access obstruction was significantly higher in the anti-heparin-platelet factor 4 antibody positive patient group than in the antibody negative patient group. Therefore, we suggest that the anti-heparin-platelet factor 4 antibody is a risk factor for vascular access obstructions in patients with end-stage renal disease on maintenance hemodialysis.

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