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Basic Science

Anti-Platelet Factor 4/Heparin Antibody Plays a Significant Role in Progression of Arterial Stiffness among Hemodialysis Patients

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Background: Arterial stiffness is a determinant of cardiovascular disease in end stage renal disease. Hemodialysis patients may develop anti-platelet factor 4/heparin antibody (PF4-H Ab) because of heparin treatment in dialysis. We tested whether PF4-H Ab was associated with progression of arterial stiffness in a 3-year follow-up.

Methods: We enrolled 74 hemodialysis patients and studied their clinical, biochemical and arterial stiffness measurement with brachial-ankle pulse wave velocity (baPWV) over 3 years. Baseline and changes in baPWV after 3 years (ΔbaPWV) were collected and compared with related clinical and biochemical parameters. PF4-H Ab was evaluated by the enzyme-linked immunosorbent assay and titer ≥ 0.4 was defined to have PF4-H Ab.

Results: We found a positive PF4-H Ab status in 25 of 74 patients. Mean baPWV was 16.1 ± 3.8 (m/s) at baseline and 17.6 ± 4.0 (m/s) after 3 years. Mean ΔbaPWV was 3.4 ± 2.2 (m/s) in the PF4-H Ab positive group, and 0.6 ± 1.2 (m/s) in the PF4-H Ab negative group. Baseline baPWV was only significantly associated with age ($\beta = 0.49$, $p < 0.01$). ΔbaPWV was significantly different between the PF4-H Ab positive and negative groups ($p < 0.01$). In multivariate regression analysis, only PF4-H Ab was positively associated with ΔbaPWV ($\beta = 0.71$, $p < 0.01$).

Conclusions: Our study concluded that PF4-H Ab was associated with progression of arterial stiffness in hemodialysis patients.

Key Words: Anti-platelet factor 4-heparin antibody • Arterial stiffness • Brachial-ankle pulse wave velocity • Hemodialysis

INTRODUCTION

Arteriosclerosis increases arterial stiffness, which is an independent predictor of cardiovascular mortality.[1,2] Brachial-to-ankle pulse wave velocity (baPWV) is a non-invasive measure of arterial stiffness and reflects the stiffness of both the central and peripheral arteries.[3] Yamashina et al. reported that baPWV is associated with Framingham Risk Score and Pocock's score.[4] Additionally, baPWV is also found to be related to the 10-year risk of developing coronary heart disease and cerebral ischemic small vessel disease in elderly hypertensive patients.[5,6]

Hemodialysis patients usually receive heparin to prevent clotting in the extra-corporeal circuit. Heparin may induce anti-platelet factor 4/heparin antibody (PF4-H Ab), which causes shedding of microparticles derived from platelets and endothelial cells.[7,8] Microparticles have been found to play a significant role in endothelial and vascular dysfunction and cardiovascular mortality in end stage renal disease.[9,10] The prevalence of PF4-H Ab in hemodialysis patients is approximately 2.3-17.9%.[11-13] Several reports showed PF4-H Ab to be associated with

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cardiovascular events and mortality in hemodialysis patients,[14,15] although other studies did not confirm this.[16]

PF4-H Ab was found to induce endothelial damage and release of endothelial microparticles.[8,17] Endothelium plays a prominent role in the remodeling process of arterial stiffness.[18,19] We have found that PF4-H Ab is associated with progression of peripheral artery disease of the legs among hemodialysis patients.[20] In this study, we used post-hoc analysis to evaluate the association between PF4-H Ab and arterial stiffness measured by baPWV in hemodialysis patients.

MATERIALS AND METHODS

Study design

We initially investigated the relation among risk factors and baPWV at the study's onset (baseline baPWV), and then, re-evaluated the factors associated with the changes of baPWV from the same patient population after 3 years (ΔbaPWV). PF4-H Ab was screening from May to June, 2009 as in our previous report.[21] baPWV measurements were routinely performed every year since 2009.

Ethics

This study was approved by our Institutional Ethics Committee (Tainan Sinlau Hospital), and patient informed consent was obtained in accordance with the Declaration of Helsinki.

Patient samples

We recruited patients undergoing hemodialysis 3 times a week for 3 months or longer at the Division of Nephrology and Dialysis of Tainan Sinlau Hospital. The following patients were excluded: acute or chronic infection, malignancy, severe disease of liver, or drug abuse. All patients were treated with unfractionated or lower molecule weight heparin during hemodialysis; the length of dialysis was 4 hours. Blood samples from the monthly routine were collected immediately before each dialysis session directly from the fistula. The complete blood count (CBC) and laboratory blood test were routinely assessed. The intact PTH (i-PTH) was routinely assessed every 6 months. Body mass index (BMI) was assessed and calculated as weight divided by height in meters squared before a dialysis session at study entry. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded at every session of hemodialysis. Mean arterial pressure (MAP) was calculated as follows: MAP = DBP + (SBP – DBP)/3. The blood pressure was controlled to below 140/90 mmHg by increasing ultrafiltration or anti-hypertensive drugs including angiotensin-converting enzyme inhibitors, calcium channel blockers, or beta-blockers given in monotherapy or in combinations. Hyperlipidemia was defined as a fasting serum total cholesterol level > 220 mg/dl, or an low-density lipoprotein cholesterol level > 100 mg/dl, or a triglyceride level > 150 mg/dl. Thereafter, the lipid-lowering drug therapy (such as statins) was used.

Enzyme-linked immunosorbent assay (ELISA) for determination of anti-PF4/heparin Ab

Commercial PF4-heparin ELISA kit (PF4 Enhanced™, Cat. No. X-HAT45, GTI, Waukesha, WI, USA), which detects IgG, IgM and IgA directed against PF4 bound to polyvinyl sulfonate, was used to determine PF4-H Ab according to our previous report.[21] Then, the optic density (OD) at 405 nm was determined. Each assay included a known positive (PF4-H Ab positive) and negative control (PF4-H Ab negative) run in parallel in an identical manner. The cut-off value for a positive test result was an $OD_{405\ nm} > 0.400$.[22]

Measurements of baPWV

baPWV measurement was obtained according to our previous report[23] and by using PWV/ABI-form (VaSera VS-1000; Fukuda Denshi, Tokyo, Japan).

Statistical analysis

Descriptive values were shown as means ± standard deviation (SD). Differences between groups were analyzed using unpaired Student's t test for continuous variables. Univariate regression analysis was performed to examine the factors related to baseline baPWV and changes of baPWV after 3 years. Then, variables with $p < 0.05$ and selected biochemical and clinical variables were entered into the multivariate regression analysis. A p value < 0.05 was considered statistically significant for all analysis, and SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis.

RESULTS

There were 74 patients enrolled in this study, of which 46 were female. The mean age was 60 ± 11 years, and mean duration of hemodialysis was approximately 98 ± 62 months. The number of patients in hemodialysis for < 2 years was 4, for 2-5 years was 16, for 5-10 years was 31, and for > 10 years was 23. There were 14 patients with DM, and 23 patients were smokers. PF4-H Ab positive status was 25 patients. Mean baPWV was 16.1 ± 3.8 (m/s) at baseline and 17.6 ± 4.0 (m/s) after 3 years. Mean ΔbaPWV was 1.5 ± 2.1 (m/s) in all patients, 3.4 ± 2.2 (m/s) in PF4-H Ab positive patients, and 0.6 ± 1.2 (m/s) in PF4-H Ab negative patients. The clinical characteristics of study patients are summarized in Table 1. In Table 2, it is shown that baseline studied parameters were not different between the positive and negative PF4-H Ab groups. Data in Table 3 showed that PF4-H Ab was not associated with the clinical and laboratory factors.

Factors correlated to baseline baPWV at study entry

Baseline baPWV was not different between the positive and negative PF4-H Ab groups (15.0 ± 3.5 m/s vs. 16.7 ± 3.8 m/s, p = 0.07; Table 2). In univariate regression analysis, baseline baPWV was related only to age, systolic blood pressure, diastolic blood pressure and mean arterial pressure (r = 0.47, p < 0.01; r = 0.36, p < 0.01; r = 0.27, p = 0.02; r = 0.32, p = 0.01; respectively, data not shown). Mean arterial pressure was found to be collinear with systolic blood pressure and diastolic blood pressure. After age, systolic blood pressure and diastolic blood pressure were entered into multivariate regression analysis, but only age remained with a significant role in baseline baPWV (β = 0.49, p < 0.01; data not shown).

Factors correlated to change in baPWV over 3 years (ΔbaPWV)

As shown in Figure 1, ΔbaPWV was significantly different between the PF4-H Ab positive and negative groups (3.4 ± 2.2 m/s vs. 0.6 ± 1.2 m/s, p < 0.01). In univariate regression analysis, only PF4-H Ab was positively associated with ΔbaPWV (r = 0.64, p < 0.01) (Table 4). After age, baPWV, BMI and PF4-H Ab were entered into multivariate regression analysis, PF4-H Ab remained a significant role in baseline ΔbaPWV (β = 0.71, p < 0.01; Table 5).

DISCUSSION

The traditional risk factors for arterial stiffness and cardiovascular disease are age, cholesterol, triglyceride, diabetes mellitus, smoking and blood pressure; but these

Table 1. Characteristics of the 74 patients

Parameters	At inclusion	Time-average or 3-year after	Difference
Age (year)	60.3 ± 11.2		
Gender (F/M)	46/28		
HD(mo), (range)	98.4 ± 62.1 (16-230)		
DM history	14		
Smoking history	23		
PF4-H Ab positive	25		
BMI (kg/m^2)	21.9 ± 3.1		
Cholesterol (mg/dL)	180.4 ± 40.2	186.5 ± 27.9*	
Triglyceride (mg/dL)	166.4 ± 135.8	169.3 ± 94.0*	
Calcium (mg/dL)	9.6 ± 0.5	9.4 ± 0.3*	
Phosphorus (mg/dL)	4.9 ± 1.6	5.0 ± 0.9*	
Ca x P	47.0 ± 15.0	45.4 ± 8.8*	
Intact PTH (pg/mL)	334.0 ± 373.9	345.6 ± 329.1*	
Platelet (×10^3/ul)	175.6 ± 58.7	161.4 ± 41.6*	
SBP (mmHg)	127.1 ± 18.3		
DBP (mmHg)	73.0 ± 10.0		
MAP (mmHg)	91.0 ± 12.3		
Anti-H/T drugs (%)	40.2%		
baPWV (m/s)	16.1 ± 3.8	17.6 ± 4.0	
baPWV (Ab+) (m/s)	15.0 ± 3.5	18.4 ± 3.6	
baPWV (Ab-) (m/s)	16.7 ± 3.8	17.3 ± 4.1	
ΔbaPWV (m/s)			1.5 ± 2.1
ΔbaPWV (Ab+) (m/s)			3.4 ± 2.2
ΔbaPWV (Ab-) (m/s)			0.6 ± 1.2

anti-H/T drugs, anti-hypertensive drugs; baPWV, brachial-to-ankle pulse wave velocity; baPWV (Ab+), baPWV in PF4-H Ab positive patients; baPWV (Ab-), baPWV in PF4-H Ab negative patients; BMI, body mass index; Ca, calcium; DBP, diastolic blood pressure; DM, diabetes mellitus; HD, hemodialysis duration; Intact PTH, intact parathyroid hormone; MAP, mean arterial pressure; P, phosphorus; PF4-H ab, anti-platelet factor 4/heparin antibody; SBP, systolic blood pressure; ΔbaPWV, difference between 3-year and baseline baPWV; ΔbaPWV (Ab+), difference between 3-year and baseline baPWV in PF4-H Ab positivepatients; ΔbaPWV (Ab-), difference between 3-year and baseline baPWV in PF4-H Ab negative patients.
* 3 year time-averaged values.

Table 2. Baseline characteristics between PF4-H Ab positive and negative patients

Parameters	PF4-H Ab (+) (n = 25)	PF4-H Ab (-) (n = 49)	p
Age (year)	60.6 ± 10.1	60.1 ± 11.9	0.85
Gender (F/M)	31/18	15/10	0.79
HD (mo)	101.2 ± 59.6	97.0 ± 63.8	0.79
DM history	4	10	0.65
Smoking history	6	17	0.35
BMI (kg/m^2)	21.1 ± 2.8	22.5 ± 3.5	0.18
Cholesterol (mg/dL)	188.2 ± 50.1	176.4 ± 34.0	0.24
Triglyceride (mg/dL)	156.9 ± 73.6	171.2 ± 158.9	0.67
Calcium (mg/dL)	9.7 ± 0.7	9.6 ± 0.4	0.39
Phosphorus (mg/dL)	4.8 ± 1.3	5.0 ± 1.7	0.64
Ca x P	46.5 ± 13.5	47.4 ± 15.8	0.79
Intact PTH (pg/mL)	356.0 ± 397.3	322.8 ± 365.2	0.72
Platelet (×10^3/ul)	178.8 ± 67.7	173.9 ± 54.2	0.74
SBP (mmHg)	127.3 ± 19.1	127.0 ± 18.1	0.72
DBP (mmHg)	73.0 ± 11.4	73.0 ± 9.3	0.22
MAP (mmHg)	91.1 ± 13.6	91.0 ± 11.7	0.39
baPWV (m/s)	15.0 ± 3.5	16.7 ± 3.8	0.07

BMI, body mass index; DBP, diastolic blood pressure; HD, hemodialysis duration; Intact PTH, intact parathyroid hormone; MAP, mean arterial pressure; SBP, systolic blood pressure.

Table 3. Correlation between PF4-H Ab and clinical parameters at entrance

Parameter	PF4-H Ab	
	r	p
Age (year)	0.02	0.85
Gender (F/M)	0.03	0.79
HD (mo)	0.03	0.79
DM history	-0.05	0.65
Smoking history	-0.11	0.35
Cholesterol (mg/dL)	0.14	0.24
Triglyceride (mg/dL)	-0.05	0.67
Calcium (mg/dL)	0.10	0.39
Phosphorus (mg/dL)	-0.06	0.64
Ca x P	-0.03	0.79
Intact PTH (pg/mL)	0.04	0.72
Platelet (×10^3/ul)	0.04	0.74
baPWV (m/s)	-0.22	0.07

r, Pearson's correlation coefficient; * p value < 0.05 was considered statistically significant.
HD, hemodialysis duration; Intact PTH, intact parathyroid hormone; PF4-H Ab, anti-PF4/heparin Ab.

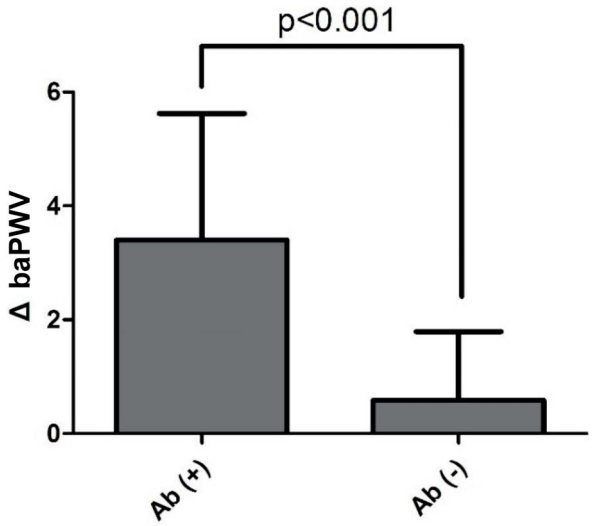

Figure 1. *Change in baPWV over 3 years (ΔbaPWV) was significantly different between PF4-H Ab positive and negative groups. * p value < 0.05 was considered statistically significant.*

Table 4. Correlation between ΔbaPWV and clinical parameters

Parameter	Dependent variable: ΔbaPWV	
	r	p
Age	-0.02	0.90
Gender (F/M)	0.10	0.41
HD (mo)	-0.06	0.64
DM history	-0.11	0.36
Smoking history	-0.03	0.83
BMI (kg/m^2)	-0.34	0.06
Cholesterol (mg/dL)$^{\#}$	-0.01	0.96
Triglyceride (mg/dL)$^{\#}$	-0.12	0.30
Calcium (mg/dL)$^{\#}$	0.16	0.18
Phosphorus (mg/dL)$^{\#}$	-0.08	0.49
Ca x P$^{\#}$	-0.02	0.85
Intact PTH (pg/mL)$^{\#}$	0.02	0.84
PF4-H Ab	0.64	< 0.01*
baPWV (m/s)	-0.18	0.12
SBP (mmHg)	0.01	0.94
DBP (mmHg)	-0.02	0.90
MAP (mmHg)	-0.00	0.98

r, Pearson's correlation coefficient; * p value < 0.05 was considered statistically significant. $^{\#}$ 3 year time-averaged values.
BMI, body mass index; DBP, diastolic blood pressure; HD, hemodialysis duration; Intact PTH, intact parathyroid hormone; MAP, mean arterial pressure; SBP, systolic blood pressure; ΔbaPWV, difference between 3-year and baseline baPWV.

factors do not entirely account for the high number of cardiovascular events in dialysis patients.[24] In this study, analyzing the effect of chronic exposure to PF4-H Ab on arterial stiffness revealed a significant difference in

Table 5. Multivariate regression analysis of factors associated with ΔbaPWV

	Standardized β coefficient	p
PF4-H Ab	0.71	< 0.01*
baPWV	-0.13	0.33
BMI	-0.14	0.30
Age	0.03	0.60

* p value < 0.05 was considered statistically significant.
BMI, body mass index.

ΔbaPWV between the PF4-H Ab-positive and PF4-H Ab-negative groups. In regression analysis, ΔbaPWV was positively correlated with PF4-H Ab. However, baseline baPWV was not significantly different between the PF4-H Ab positive and negative groups. Because aging, systolic blood pressure, diastolic blood pressure and mean arterial pressure were significant in baseline baPWV, PF4-H Ab did not have a significant role in baseline baPWV. Then, in the different chronicity of exposure to various risk factors (numbers of patients in hemodialysis duration < 2 years: 4, 2-5 years: 16, 5-10 years: 31, > 10 years: 23), this may lead to the conclusion that PF4-H Ab does not play a significant role in baseline baPWV. Therefore, ΔbaPWV is used to evaluate the effect of PF4-H Ab on arterial stiffness to decrease the confounding effects of aging, systolic blood pressure, diastolic blood pressure, mean arterial pressure and the different chronicity of exposure on arterial stiffness.

No larger scale studies to date have demonstrated whether PF4-H Ab titers can disappear spontaneously in patients who continue to be exposed to heparin on long-term dialysis. The mean time between initiation of hemodialysis and development of PF4-H Ab in a UK survey was 61 days (range 5-390 days).[25] The authors considered that long-term use of heparin in hemodialysis patients did not directly trigger the onset of PF4-H Ab. Several studies showed that PF4-H Ab was not associated with dialysis duration.[26-28] In our present study, we found PF4-H Ab not to be associated with dialysis duration at study entry. From the above results, we can consider that the development of PF4-H Ab is within 13 months after hemodialysis begins, PF4-H Ab may not disappear spontaneously in hemodialysis patients. Therefore, PF4-H Ab was measured only at study entry.

baPWV has been reported to be associated with systolic blood pressure, diastolic blood pressure and mean arterial pressure in a survey of 12517 Japanese subjects.[29] Also, Im et al. reported that baPWV is positively correlated with systolic blood pressure and diastolic blood pressure in healthy adolescents.[30] However, Chuang et al. reported that baPWV is only significantly related to systolic blood pressure, but not diastolic blood pressure in community-dwelling adults.[5] Our study showed that systolic blood pressure, diastolic blood pressure and mean arterial pressure were positively related to baseline baPWV. However, when analyzed in combination with age, systolic blood pressure, diastolic blood pressure and mean arterial pressure did not have significant roles in baseline baPWV. This finding indicated that blood pressure was not a significant factor in baseline baPWV in hemodialysis patients.

Regarding factors associated with baPWV, Nomura and associates have demonstrate that baPWV is positively associated with age, body mass index and serum levels of total cholesterol and fasting glucose in young Japanese males.[31] baPWV is also significantly related to sex, uric acid, triglyceride, hemoglobin A_1C and smoking status.[29,32,33] Our results indicated that age is positively related to baseline baPWV, but did not correlate to ΔbaPWV. Our results also revealed that BMI did not associate with baPWV and ΔbaPWV. This result was different from the report of Nomura et al.,[31] because the effect of fluid retention on BMI and an altered relationship between fat and lean tissue in dialysis patients.[34] Then, no other factors related to baseline baPWV and ΔbaPWV were in the present study. This indicated that aging was not a significant factor relating to progression of arterial stiffness in hemodialysis patients.

This study has several limitations. The number of patients in this study was only 74. Thus, a lower statistical power may exist. In this study, the positive rate of PF4-H Ab (33.8%) was higher than other reports.[11-13] We chose our study subject from a group of patients who remained to receive hemodialysis in our hemodialysis unit in the 3-year study period. Initially, we enrolled 96 patients in this study. Among these patients, 6 died and 16 left our hemodialysis unit and went to other hemodialysis units in the 3-year study period. Therefore, we missed 22 enrolling patients that might have resulted in a higher positive rate of PF4-H Ab in our study. Then, the baPWV value might be underestimated in patients with advanced peripheral artery disease (ankle-brachial

index < 0.9) because arterial occlusion retards baPWV.[35] Due to the retrospective nature of this study; however, the results could not infer causality.

CONCLUSIONS

In summary, this study showed that PF4-H Ab positive patients were at a high risk for progression of arterial stiffness among hemodialysis patients.

DISCLOSURE

All authors report no disclosures.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest.

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