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Original article

Impairment of platelet retention rate in patients with severe aortic valve stenosis

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ABSTRACT

Background: Recent reports revealed the presence of acquired von Willebrand syndrome type 2A in patients with aortic valve stenosis (AS). von Willebrand factor (vWF) has been shown to play a vital role in platelet adhesion. Therefore, we measured the platelet retention rates, which reflect platelet adhesion, in patients with severe AS.

Methods: In addition to echocardiography, routine blood screening tests were performed and the platelet retention rates were measured using collagen-coated bead columns in 21 patients with severe AS and in 21 control subjects.

Results: Patients with severe AS showed the maximum aortic valve pressure gradients of 110.9 ± 22.7 mmHg, and effective orifice areas of 0.59 ± 0.20 cm^2. The results of routine blood tests in patients with severe AS were comparable to those of control subjects; however, the platelet retention rates in the AS patients ($7.3 \pm 5.0\%$) were significantly lower than those in control subjects ($30.5 \pm 11.8\%$, $p < 0.001$). A significant negative correlation was observed between maximum aortic valve pressure gradients and platelet retention rates ($r = -0.81$, $p < 0.001$). In 8 patients with severe AS, the platelet retention rates increased from $5.8 \pm 3.6\%$ to $16.0 \pm 2.4\%$ after aortic valve replacement ($p < 0.001$).

Conclusion: These findings suggest that impairment of platelet retention rate is seen in almost all patients with severe AS. Clinicians should be aware of the possibilities of vWF-mediated platelet dysfunction and bleeding tendency in patients with severe AS.

Introduction

Sclerosis, calcification, and stenosis of the aortic valve are the most common acquired valvular lesions in elderly patients. Increased life expectancy has resulted in an increase in the population of elderly people, with a corresponding increase in the number of patients with such degenerative aortic valve diseases. Otto et al. reported that 29% of individuals older than 65 years have aortic valve sclerosis, and 2% of this population has aortic valve stenosis (AS) [1]. The rate of AS progression may be more rapid in elderly patients [2], and some reports demonstrate that hemodialysis, serum calcium, hypertension, and hyperlipidemia among other things are associated with the progression of AS [3,4].

In 1958, Heyde first reported that 10 patients with AS had gastrointestinal bleeding of unknown cause [5]. In the same year, Goldman reviewed 37,423 patients and showed a 3-fold higher incidence of gastrointestinal bleeding than the predicted incidence in patients with AS [6]. The precise mechanism underlying the link between AS and gastrointestinal bleeding tendency was unknown for a long time. Vincentelli et al. demonstrated that the high-molecular-weight von Willebrand multimers were reduced in proportion to the severity of AS, and valve replacement could restore the impaired distribution of the multimers [7]. Nowadays, the relationship between AS, acquired von Willebrand syndrome type 2A (deficiency in high-molecular-weight von Willebrand multimers), and anemia due to gastrointestinal bleeding from intestinal angiodysplasia is known as Heyde syndrome [8]. However, patients with severe AS usually have normal results in routine blood screening tests. Therefore, many clinicians are probably unaware of the abnormal hemostasis in patients with severe AS.

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The interaction between von Willebrand factor (vWF) and platelet glycoprotein (GP) Ib plays a critical role in the initial phase of platelet adhesion, particularly under high shear stress [9]. Until recently, quantification of platelet adhesiveness was performed using glass-bead columns, and the methods for quantitative estimation were developed and modified [10]. The methods principally measured the retention of platelets during passage of whole blood through glass-bead columns. Now, spherical copolymer plastic beads coated with porcine type I collagen have been developed and are being used for the measurement of platelet retention [11]. Because collagen is a major subendothelial component, measurement of platelet adhesion using this assay is expected to reflect physiologic platelet responses more accurately than the glass-bead columns. vWF might affect the platelet retention rates measured using the collagen-coated bead columns. Ideguchi et al. reported that 62% of platelet adhesion to the collagen-coated bead columns was inhibited by the antibody of vWF (MAS534p), which inhibits GPIb binding site of vWF [12]. We previously reported a patient with Heyde syndrome who showed normal results of almost all routine blood screening tests, but a significant decrease in the platelet retention rate measured using this collagen-coated bead column method and the deficiency in high-molecular-weight von Willebrand multimers by gel electrophoresis [13].

Thus, in this study, we examined whether the platelet retention rates in patients with severe AS are lower than those in control subjects and whether platelet retention rates change after aortic valve replacement (AVR). Additionally, we evaluated the relationship between platelet retention rate and aortic valve pressure gradient as an indicator of shear stress.

Methods

Study patients

Between August 2009 and December 2012, 21 consecutive patients (8 men and 13 women; mean ± SD age, 75.2 ± 10.2 years) referred to Shimane University Hospital for evaluation of severe AS were enrolled in the study.

The diagnosis of severity was made according to the European Association of Echocardiography/American Society of Echocardiography guidelines [14]. Thus, severe AS was defined as an aortic valve area less than 1.0/cm^2 or a mean gradient greater than 40 mmHg, as determined by Doppler echocardiography.

In addition, 21 control subjects who visited our hospital for further medical examination of hypertension and electrocardiogram abnormalities (12 men and 9 women; mean ± SD age, 69.0 ± 8.6 years) were enrolled in the study.

The exclusion criteria were personal or family history of genetic bleeding disorder or thrombotic disorder, platelet counts <100,000 or >450,000 mm^{-3}, serum creatinine levels >2.0 mg/dl, acute infectious disease, autoimmune disorders, neoplasm, viral hepatitis, or significant valve diseases other than AS. Further, patients receiving any antiplatelet or anticoagulant treatment and steroids or nonsteroidal anti-inflammatory drugs at the time of evaluation were excluded from the present study.

Baseline characteristics of patients with severe AS and control subjects are shown in Table 1. Patients with severe AS (75.2 ± 10.2 years) were significantly older than control subjects (69.0 ± 8.6 years, $p = 0.037$). There were no significant differences between the groups regarding the prevalence of diabetes mellitus and hyperlipidemia, and the administration of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, β-blockers, and statins. The numbers of patients with hypertension and receiving administration of a Ca antagonist were much higher among the control subjects ($p = 0.012$, 0.015, respectively).

Written informed consent was obtained from each patient and control subjects.

Echocardiographic evaluation

Transthoracic echocardiography was performed using a Philips iE33 echocardiographic system (Andover, MA, USA) to assess the hemodynamic performance of the aortic valve in patients with severe AS and in control subjects on the same day or within a few days of the platelet retention tests. The mean and peak transvalvular pressure gradients were calculated using the modified Bernoulli equation, and the effective orifice area was calculated using the continuity equation [15].

Blood collection and laboratory assays

Blood samples were collected for simultaneous assessment of platelet retention rates, platelet counts, prothrombin time-international normalized ratio (PT-INR), activated partial thromboplastin time (APTT), and fibrinogen levels.

In the present study, we performed the multimers analysis of vWF by gel electrophoresis in 5 patients with severe AS. Additionally, we measured the activities of the vWF-cleaving protease ADAMTS 13 (a disintegrin and metalloproteinase with thrombospondin type 1 motif, member 13) which targets the A2 domain of vWF and specifically cleaves the protein Tyr1605-Met1606 [16], in 5 patients with severe AS.

Fasting-state blood samples were obtained from all patients with severe AS and from control subjects at 10:00 AM. Blood samples were drawn from the antecubital vein under minimal tourniquet pressure using a sterile 21-gauge needle syringe. All parameters were measured by routine laboratory techniques blinded to the origin of the sample.

Measurement of platelet retention rate

In an air conditioned room at a temperature of 25–26 °C, the platelet retention rate was measured in all patients with severe AS and in control subjects according to the methods described by Kaneko et al. [11]. Briefly, co-polymer plastic beads (diameter, 0.4–0.6 mm) coated with porcine type I collagen were packed into a polyvinyl tubing with an internal diameter of 2 mm and length of 80 mm. These Pla-Bead columns are disposable and commercially available, and they are distributed by ISK Co., Ltd., Tokyo, Japan. The whole blood samples were mixed by twirling and 1.5 ml of the sample was drawn into a plastic syringe (2.5 ml) (Terumo, Tokyo, Japan). A collagen-coated bead column was connected to the syringe, and the syringes were placed in the holder of an injection pump. The blood samples in the syringes were passed through the collagen-coated bead columns at a fixed flow rate of 1.5 ml/30 s.

Table 1
Comparison of baseline characteristics of study patients and control subjects.

	Severe AS group ($n = 21$)	Control subjects ($n = 21$)	p-Value
Age (years)	75.2 ± 10.2	69.0 ± 8.6	0.037
Sex (female/male)	13/8	9/12	0.217
Hypertension	8	16	0.013
Diabetes mellitus	3	1	0.293
Hyperlipidemia	8	8	1.000
Medication use (n)			
ACE-I/ARB	7	8	0.747
Ca-blocker	6	11	0.015
β-blocker	1	3	0.293
Statin	8	8	1.000

ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker. AS, aortic valve stenosis.