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Original article

Intravascular hemolysis in patients with mitral regurgitation: Evaluation by erythrocyte creatine

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ABSTRACT

Background: Intravascular hemolysis has been reported in patients with cardiac valve prostheses, but intravascular hemolysis in patients with mitral regurgitation with native valve has not been evaluated in detail. We designed a study to elucidate the impact of regurgitation flow on intravascular hemolysis in patients with primary mitral regurgitation by measuring erythrocyte creatine.
Methods: Erythrocyte creatine was enzymatically assayed in 29 patients with moderate to severe primary mitral regurgitation and 12 age-matched healthy volunteers. The size and characteristics of mitral regurgitation were determined by color Doppler echocardiography.
Results: Erythrocyte creatine was significantly higher in patients with eccentric jet ($n = 17$, 2.64 ± 0.77 μmol/g Hb) than that of central jet ($n = 12$, 1.68 ± 0.13 μmol/g Hb) and control subjects (1.39 ± 0.25 μmol/g Hb). Patients with eccentric jet had a significantly lower erythrocyte count and hemoglobin (385 ± 58 x10^4/μL and 116 ± 19 g/l) compared to those with central jet ($450 \pm 47 \times 10^4$/μL and 137 ± 14 g/l) and control subjects ($433 \pm 31 \times 10^4$/μL and 134 ± 19 g/l). There were no significant differences in age, estimated glomerular filtration rate, pulmonary artery systolic pressure, left atrial size and left ventricular end-diastolic dimension between patients with eccentric jet and central jet.
Conclusions: Intravascular hemolysis associated with subclincal anemia in patients with eccentric jet was due to the destruction of erythrocyte by collision of the eccentric jet to the atrial wall.

Introduction

Intravascular hemolysis has been reported in heart valve replacement with mechanical prostheses, stenotic valve diseases, hypertrophic cardiomyopathy, and pulmonary hypertension [1–9]. Even in cases of subclinical anemia, intravascular hemolysis due to destruction of erythrocytes remains to be evaluated. Red blood cell life span, measured by ^{51}Cr-labeling, has been used as a gold standard test to estimate the severity of intravascular hemolysis [1–3], but the ^{51}Cr-labeling method requires a prolonged examination period with series of blood drawn from the patient. In contrast to the ^{51}Cr-labeling method, measurement of erythrocyte creatine is simple, rapid, and economically favorable to determine the erythrocyte age by a single blood sample measurement [4,7–12]. Intravascular hemolysis has been reported in patients with mitral regurgitation after incomplete mitral valve repair [13–16], but intravascular hemolysis in patients with mitral regurgitation with native valve has not been evaluated in detail. Accordingly, we measured erythrocyte creatine in patients with primary mitral regurgitation and examined the relation between the degree of intravascular hemolysis and mitral regurgitation jet morphology.

Materials and methods

Patients

Twenty nine patients with moderate to severe primary mitral regurgitation (due to an abnormality of the mitral valve apparatus)

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in the absence of aortic valve disease, mitral stenosis, left ventricular outflow obstruction (hypertrophic cardiomyopathy, sigmoid septum), or history of myocardial infarction were enrolled in this study. There were 12 men and 17 women aged 51–82 years. To avoid possible interference with other pathogenic processes including abnormality in production or destruction of erythrocytes, patients with rheumatologic, immunologic, hepatic, and renal diseases, or apparent recent bleeding were excluded. Twelve age- and gender-matched healthy volunteers with no history of cardiovascular disease, and normal physical findings, electrocardiogram, and chest radiogram were used as a control group. This study complies with the Declaration of Helsinki and written informed consent was obtained from all subjects. This study was approved by the Ethical Review Board of Kochi Medical School.

Measurements

Blood samples were collected from the antecubital vein within 24 h of the echocardiography. Blood samples were checked for hemolysis during blood preparations (blood sampling and after centrifugation). Red blood cells, hemoglobin, hematocrit, and platelets were measured using a Sysmex SE 9000 (Sysmex, Kobe, Japan). Reticulocyte count was performed with a Sysmex R-3000 (Sysmex). Erythrocyte creatine was assayed enzymatically in accordance with previous reports [4,17,18]. Measured data are expressed as micromole per gram of hemoglobin (μmol/g hemoglobin). Serum lactate dehydrogenase was measured by the Japanese Society of Clinical Chemistry method and serum creatinine was measured by the creatinase-HMMS method using a JCA-BM2250 analyzer (Japan Electron Optics Laboratory, Tokyo, Japan) and estimated glomerular filtration rate was calculated according to the equation previously described in Japanese population [19].

Echocardiography

Transthoracic echocardiography was performed using a Philips ultrasound iE 33 phased-array sector scanner (Philips, Bothell, WA, USA) or a Sequoia 512 (Siemens, Erlangen, Germany) by an experienced echocardiographer. Echocardiographic images were taken with patients in the left lateral decubitus position, and two-dimensional and M-mode echocardiographic measurements were carried out according to the guidelines of the American Society of Echocardiography [20,21]. Left atrial and left ventricular dimensions were measured from two-dimensional images in the parasternal long-axis view. Color Doppler flow imaging was performed from parasternal, apical, and subcostal windows using multiple imaging planes. Mitral and tricuspid regurgitations were graded as mild, moderate, or severe based on the size and characteristics of the jet on color Doppler flow imaging [22]. Classification of mitral regurgitation jets was performed by initial direction of the jet immediately behind the point of coaptation of the mitral leaflets. Jets were classified into two types based on their spatial velocity distribution within the left atrium (Fig. 1). Eccentric jet was defined as one directed eccentrically occurring immediately upon its exit from the regurgitant orifice, directed and struck either the roof, inferior, anterior, lateral, or septal atrial wall and remained in close contact with these surfaces. The central jet was defined as initial direction of the jet directed into the main cavity of the left atrium and expansion of the jet further down in the atrium. Doppler recordings of the tricuspid regurgitation were performed in the apical four-chamber view. The tricuspid regurgitation jet was recorded by means of color and continuous-wave Doppler, and the maximum velocity was used to calculate the transtricuspid pressure gradient by means of the modified Bernoulli equation [23]. Pulmonary artery systolic pressure was calculated as the sum of the transtricuspid pressure gradient in conjunction with an echocardiographic estimation of right atrial pressure. Echocardiographic estimation of right atrial pressure was performed based on inferior vena cava size and collapsibility, according to the previously established criteria [24]. Peak flow velocity at the aortic valve was obtained by continuous-wave Doppler echocardiography. All echocardiograms were reviewed and analyzed by an experienced cardiologist with special expertise in echocardiography.

Statistical analysis

The measured values are expressed as mean ± standard deviation. Statistical analysis between the two groups was performed by the Student's *t*-test or the Mann–Whitney *U*-test for continuous variables and the chi-square analysis or the Fisher exact test for categorical variables. Statistical analysis among the three groups was performed by one-way layout analysis of variance followed by the Wilcoxon test when a significant difference was found. Correlation between two variables was determined by linear regression analysis. Receiver-operating characteristic curve analysis was used to determine the discriminating cut-off value of erythrocyte creatine. Results were considered to be significant at $p < 0.05$.

Results

All of the blood samples were confirmed to be free of hemolysis before the measurement. No patient showed fragmentation of erythrocytes on blood smear or bilirubinemia. There were no

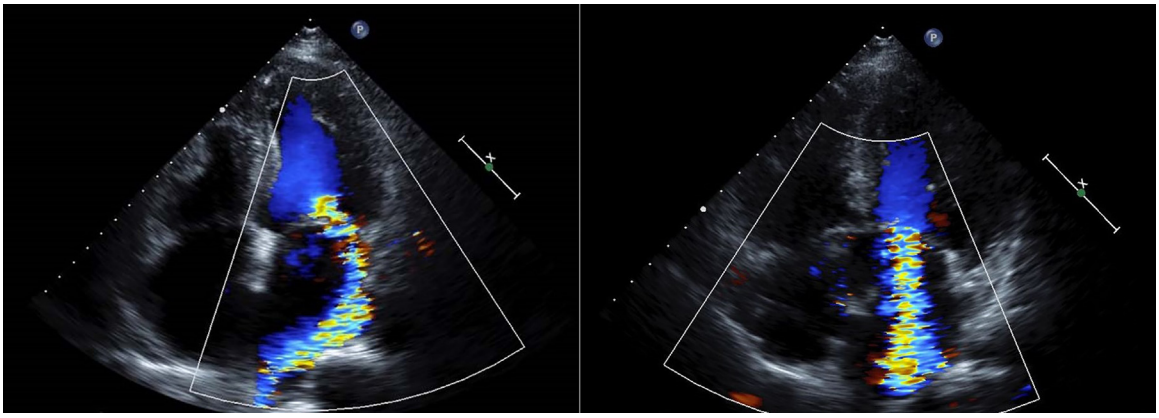

Fig. 1. Left panel: eccentric jet. Right panel: central jet.

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