Journal of Cardiology 67 (2016) 274–278

Contents lists available at ScienceDirect

Journal of Cardiology

journal homepage: www.elsevier.com/locate/jjcc

Original article

Erythrocyte creatine as a marker of intravascular hemolysis due to left ventricular outflow tract obstruction in hypertrophic cardiomyopathy

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ARTICLE INFO

Article history:
Received 4 February 2015
Received in revised form 28 April 2015
Accepted 11 May 2015
Available online 4 August 2015

Keywords:
Hypertrophic cardiomyopathy
Biomarker
Erythrocyte creatine
Hemolysis
Left ventricular outflow tract obstruction

ABSTRACT

Background: Erythrocyte creatine, a marker of erythrocyte age that increases with shortening of erythrocyte survival, has been reported to be a quantitative and reliable marker for intravascular hemolysis. We hypothesized that hemolysis could also occur due to intraventricular obstruction in patients with hypertrophic cardiomyopathy (HCM). The purpose of this study was to examine the presence of subclinical hemolysis and the relation between intravascular hemolysis and intraventricular pressure gradient (IVPG).

Methods and results: We measured erythrocyte creatine in 92 HCM patients. Twelve patients had left ventricular outflow tract obstruction (LVOTO), 4 had midventricular obstruction (MVO), and the remaining 76 were non-obstructive. Erythrocyte creatine levels ranged from 0.92 to 4.36 μmol/g hemoglobin. Higher levels of erythrocyte creatine were associated with higher IVPG ($r = 0.437$, $p < 0.001$). If erythrocyte creatine levels are high (≥1.8 μmol/g hemoglobin), subclinical hemolysis is considered to be present. Half of LVOTO patients and no MVO patients showed high erythrocyte creatine levels. Although non-obstructive patients did not show significant intraventricular obstruction at rest, some showed high erythrocyte creatine levels. When LVOT-PG was measured during the strain phase of the Valsalva maneuver in 20 non-obstructive patients, 7 of those 20 patients showed LVOTO. In the 20 patients, there was no relation between erythrocyte creatine levels and LVOT-PG before the Valsalva maneuver ($r = 0.125$, $p = 0.600$), whereas there was a significant correlation between erythrocyte creatine and LVOT-PG provoked by the Valsalva maneuver ($r = 0.695$, $p = 0.001$).

Conclusions: There is biochemical evidence of subclinical hemolysis in patients with HCM, and this hemolysis seems to be associated with LVOTO provoked by daily physical activities.

Introduction

Heart valve replacement with mechanical prostheses is known to induce mild but chronic intravascular hemolysis [1,2]. There have been some reports that clinically significant anemia was caused by intravascular hemolysis in patients with a malfunctioning prosthesis [3,4]. Although several markers including reticulocyte count, schistocyte count, and serum levels of hemoglobin, indirect bilirubin, lactate dehydrogenase (LDH), and haptoglobin have been used for estimating the severity of intravascular hemolysis, red blood cell lifespan is the gold standard test of hemolysis [2,5–12]. We previously reported that erythrocyte creatine, a marker of erythrocyte age that increases with shortening of red blood cell survival, was a quantitative and reliable marker for intravascular hemolysis in patients with prosthetic valves [13]. This study found that erythrocyte creatine was sensitive to detect mild and subclinical hemolysis, and that it showed a better correlation with total peak flow velocity than did LDH and haptoglobin in the patients studied [13].

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http://dx.doi.org/10.1016/j.jjcc.2015.05.005
0914-5087/

Previously, we presented a case report of hemolytic anemia in a hypertrophic cardiomyopathy (HCM) patient with a marked left ventricular (LV) outflow tract obstruction [14]. Although this is a rare case of HCM, we hypothesized that subclinical hemolysis might occur due to intraventricular obstruction in patients with HCM. Therefore, in the present study, we measured erythrocyte creatine in HCM patients and examined the presence of subclinical hemolysis and the relation between intravascular hemolysis and intraventricular pressure gradient (IVPG).

Methods

Subjects

We measured erythrocyte creatine in 110 consecutive patients with HCM at Kochi Medical School Hospital. To avoid possible interference with other pathogenic processes including abnormality in production or destruction of erythrocytes, patients with hematologic, immunologic, hepatic, renal diseases, and organic valvular abnormalities were excluded. Finally, the study group consisted of 92 HCM patients. The diagnosis of HCM was based on echocardiographic demonstration of unexplained LV hypertrophy (LVH), i.e. maximum LV wall thickness $\geq$ 15 mm, in the absence of systemic hypertension or other cardiac diseases (such as aortic stenosis or storage disease) that could produce hypertrophy of such magnitude. Informed consent was obtained from all patients in accordance with the guidelines of the Ethics Committee on Medical Research of Kochi Medical School.

Clinical evaluation

Evaluation of patients included medical history, clinical examination, 12-lead electrocardiography, and M-mode, 2-D, and Doppler echocardiography. The severity and distribution of LVH were assessed in the parasternal short-axis plane at mitral valve and papillary muscle levels. LV end-diastolic diameter (LVEDD) and end-systolic diameter (LVESD) were measured from M-mode and 2-D images obtained from parasternal long-axis views, and fractional shortening [%FS = (LVEDD − LVESD)/LVEDD × 100] was calculated. LV outflow tract gradient was calculated from continuous-wave Doppler using the simplified Bernoulli equation.

Based on morphologic and hemodynamic assessments by echocardiography, we divided the patients into the following five groups: (1) hypertrophic obstructive cardiomyopathy (HOCM), defined as the presence of basal LV outflow tract obstruction (gradient $\geq$ 30 mmHg at rest), (2) midventricular obstruction (MVO), defined as the presence of systolic LV cavity obliteration at the midventricle creating midventricular obstruction with a peak systolic gradient $\geq$ 30 mmHg at rest, (3) end-stage phase of HCM (end-stage HCM), defined as LV systolic dysfunction of global ejection fraction (EF) $<$ 50% (global EF was determined from apical two- and four-chamber views. Concomitant coronary artery disease was excluded by coronary angiography and/or myocardial scintigraphy), (4) apical HCM, defined as hypertrophy confined to the LV apex, and (5) HNCM: non-obstructive HCM other than end-stage HCM and apical HCM.

Measurements of erythrocyte creatine

Erythrocyte creatine was assayed enzymatically in accordance with a previous report [15]. In brief, the plasma and buffy coat were aspirated from the blood sample after centrifugation. After lysis and deproteinization of packed erythrocytes, the supernatant was obtained by centrifugation and filtration. Creatine concentration in the supernatant was measured with the enzymatic assay method involving creatine amidinohydrolase, sarcosine oxidase, and peroxidase. Measured data were expressed as micromoles per gram of hemoglobin.

Statistical analysis

All data are expressed as mean ± SD or frequency (percentage). Differences in the means of continuous variables were assessed using a Student's *t*-test. Pearson's chi-square test was used for comparisons between non-continuous variables, and Fisher's exact test was used when expected frequency was lower than 5. B-type natriuretic peptide values were subjected to logarithmic transformation for statistical analysis. Statistical analysis was performed using SPSS (version 14.0J) statistical software (SPSS Japan Inc., Tokyo, Japan).

Results

Patient characteristics

Clinical characteristics of the patients in the present study are summarized in Table 1. Two thirds of the patients were men and the majority were in New York Heart Association class I or II. Erythrocyte creatine levels ranged from 0.92 to 4.36 μmol/g hemoglobin.

Erythrocyte creatine and IVPG

We correlated erythrocyte creatine with other accepted measurements of hemolysis with linear regression. Erythrocyte creatine levels correlated significantly with LDH levels ($r = 0.449$, $p < 0.001$) and hemoglobin levels ($r = -0.656$, $p < 0.001$), but not with haptoglobin, bilirubin, and reticulocyte counts.

Fig. 1 shows that there was a significant correlation between erythrocyte creatine levels and IVPG in 92 HCM patients ($r = 0.437$, $p < 0.001$). Levels of LDH and hemoglobin were also correlated with IVPG ($r = 0.242$, $p = 0.020$ and $r = -0.427$, $p < 0.001$, respectively). But there were no correlations between levels of haptoglobin, bilirubin, or reticulocyte counts and IVPG in these patients.

In our previous examination, if erythrocyte creatine levels were greater than or equal to 1.8 μmol/g hemoglobin, subclinical hemolysis was considered to be present [13]. When the patients were divided into two groups based on erythrocyte creatine levels of 1.8 μmol/g hemoglobin, patients with abnormally high erythrocyte creatine levels (≥1.8 μmol/g hemoglobin) showed significantly higher B-type natriuretic peptide values and tended to have higher IVPG (Table 2). Next, we examined the prevalence of

Table 1
Clinical characteristics in 92 patients with HCM.

Age, years	61.4 ± 5.0
Gender: men, *n* (%)	61 (66%)
Erythrocyte creatine, μmol/g hemoglobin	1.69 ± 0.59
Atrial fibrillation, *n* (%)	8 (9%)
NYHA functional class, *n* (%)	
I	57 (62%)
II	31 (34%)
III	4 (4%)
Subtypes, *n* (%)	
HOCM	12 (13%)
MVO	4 (4%)
HNCM	61 (66%)
End-stage HCM	3 (3%)
Apical HCM	12 (13%)

Data are shown as mean ± SD or number (percent).
HCM, hypertrophic cardiomyopathy; NYHA, New York Heart Association functional class; HOCM, hypertrophic obstructive cardiomyopathy; MVO, midventricular obstruction; HNCM, hypertrophic non-obstructive cardiomyopathy.