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Plasma biomarkers of endothelial dysfunction in patients with hypertrophic cardiomyopathy

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Abstract:
Impairment of endothelium-dependent coronary vasodilatation has been reported in hypertrophic cardiomyopathy (HCM). The aim of our study was to evaluate whether HCM patients have increased circulating blood markers of endothelial dysfunction.
We compared 29 HCM patients with sinus rhythm, including 11 with the left ventricular outflow tract (LVOT) obstruction (gradient ≥ 30 mmHg), versus 29 age- and sex-matched controls without cardiovascular diseases. Plasma levels of the following endothelial biomarkers were determined: soluble thrombomodulin (sTM), von Willebrand factor (vWF), tissue factor pathway inhibitor (TFPI), asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDAM) and L-arginine to ADMA (Arg/ADMA) ratio.
Both sTM (49.1 ± 9.9 *vs.* 39.1 ± 4.8 ng/ml, $p < 0.00001$) and TFPI (18.6 ± 2.5 *vs.* 16.2 ± 1.7 ng/ml, $p < 0.0001$) were elevated in HCM patients compared with controls, whereas vWF levels were similar in both groups (105.8 ± 11.6 *vs.* 102.2 ± 10.9 U/dl, $p > 0.05$). Among markers related to the nitric oxide pathways, we observed elevations of both ADMA (0.57 ± 0.08 *vs.* 0.44 ± 0.04 μmol/l, $p < 0.0001$) and SDMA (0.43 ± 0.05 *vs.* 0.34 ± 0.04 μmol/l, $p < 0.0001$) and decrease in the Arg/ADMA ratio (118.1 ± 18.2 *vs.* 144.3 ± 22.1, $p < 0.0001$) in HCM patients. The obstructive HCM subgroup displayed higher values of ADMA, SDMA and sTM compared with the non-obstructive HCM subgroup.
HCM patients show specific features of endothelial dysfunction detectable in peripheral blood, involving increased sTM and TFPI, but not vWF, along with increased ADMA levels.

Key words:
hypertrophic cardiomyopathy, ADMA, endothelium

Abbreviations: ADMA – asymmetric dimethylarginine, Arg – L-arginine, HCM – hypertrophic cardiomyopathy, LVOT – left ventricular outflow tract, SDMA – symmetric dimethylarginine, sTM – soluble trombomodulin, TFPI – tissue factor pathway inhibitor, vWF – von Willebrand factor

Introduction

Patients with hypertrophic cardiomyopathy (HCM) have been shown to have abnormal intramural coro-

nary arteries and subendocardial arterioles with thickened walls and narrowed lumens [14, 20]. The intima, including the endothelium layer, is hypertrophied and endothelial cells are structurally abnormal in HCM [14, 20, 21, 23], which provides a morphological substrate for functional impairment of the endothelium. In HCM patients, endothelium-dependent coronary vasodilatation (tested invasively by acetylcholine or noninvasively by cold pressor test or pacing stimulation) has been found to be impaired both in conductance and resistance vessels [7, 8, 13, 16]. In some HCM patients, acetylcholine can even induce not only a marked vasoconstriction, but also vasospasm with subtotal or even total occlusion of the epicardial coronary artery [13, 16]. However, it is unclear whether such endothelial dysfunction might be reflected by elevated levels of its circulating markers. Thus, the aim of the present study was to assess plasma levels of endothelium-released biomarkers in HCM.

Materials and Methods

Study population

We compared 29 HCM patients (16 males and 13 females, mean age 47 ± 12 years with sinus rhythm versus 29 age- and sex-matched controls without cardiovascular diseases). The diagnosis of hypertrophic cardiomyopathy was based on typical clinical, electrocardiographic and echocardiographic features. Four patients had NYHA class I, 19 patients were in class II and 6 patients were in class III. In the HCM group, 9 patients had a history of syncope, 14 had nonsustained ventricular tachycardia in Holter monitoring, and 16 patients had a family history of HCM. Patients were treated with verapamil (n = 10) or beta-blockers (n = 11) and 8 patients who were referred to the initial evaluation received no medication.

Left ventricular outflow tract gradient in Doppler echocardiography ≥ 30 mmHg (as a marker of significant obstruction) was present in 11 patients.

Like in the study of Thaman et al. [25], only patients with a typical chest pain or risk factors for coronary artery disease underwent coronary angiography. Accordingly, in 10 patients coronary angiography was performed and normal coronary arteries were detected.

The control group included 29 subjects matched for age, sex (15 males and 14 females, mean age 51 ± 14 years), who were recruited from hospital staff. Echocardiographic evaluation in the control group revealed no abnormalities of cardiac structure and function. The septal thickness was ≤ 11 mm in all control subjects.

All patients and control subjects gave their informed consent. The Jagiellonian University Ethical Committee approved the study.

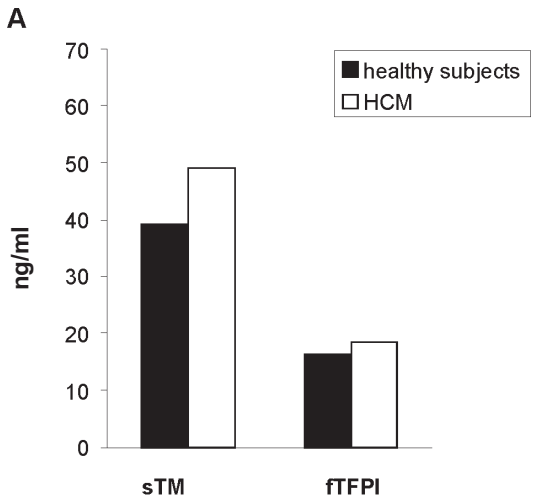
Blood was taken immediately after echocardiographic evaluation. Blood samples were taken with minimal stasis between 8 and 9 a.m. after an overnight fast. Plasma samples were centrifuged at 2000 × g for 20 min and stored in aliquots at –80°C until analysis. Using commercially available ELISA assays according to the manufacturers' instructions, we measured plasma levels of the following endothelial biomarkers: soluble thrombomodulin (sTM; Diagnostica Stago), von Willebrand factor (vWF; Dade Behring), and total tissue factor pathway inhibitor (TFPI; Diagnostica Stago). According to the methodology by Teerlink et al. [24], asymetric dimethylaginine (ADMA), symmetric dimethylarginine (SDMA) and arginine were measured by high-performance liquid chromatography. In addition, L-arginine (Arg) to ADMA (Arg/ADMA) ratio was calculated.

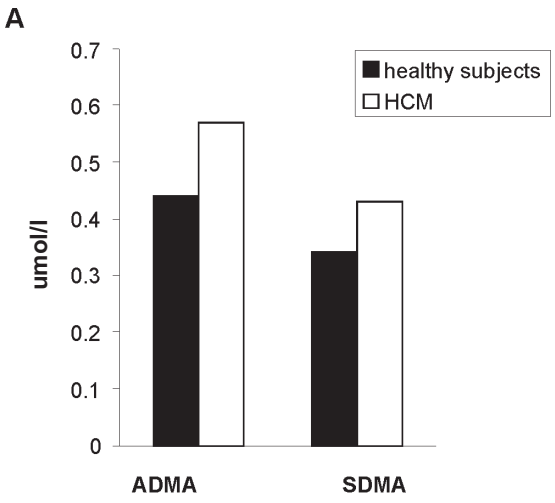
Statistical analysis

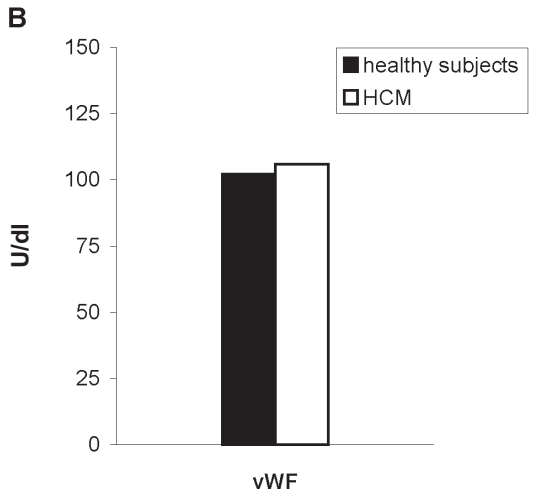
Data are expressed as the mean ± SD. The Kolmogorov-Smirnov test was used to determine normal distribution. Intergroup comparison of data was done using Student's *t*-test for independent variables with normal distribution. A p value of < 0.05 was considered significant.

Results

In echocardiographic assessment, in the entire HCM group both LV contractility and cavity size were normal. In all HCM patients, the ventricular septum thickness was markedly increased to 24 ± 5 mm, whereas the thickness of the posterior wall was within a normal range of 11 ± 2 mm. The septum/posterior wall thickness ratio was 2.2 ± 0.4. The above-mentioned parameters did not differ between obstructive and nonobstructive subgroups.







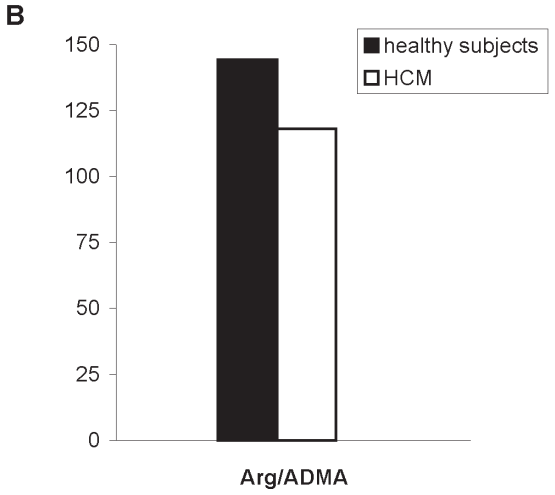


Fig. 1. The comparison of hemostatic biomarkers of endothelial dysfunction between patients with hypertrophic cardiomyopathy and healthy subjects. **(A)** sTM: 49.1 ± 9.9 and 39.1 ± 4.8 ng/ml, $p < 0.00001$; fTFPI: 18.6 ± 2.5 and 16.2 ± 1.7 ng/ml, $p < 0.0001$, respectively; **(B)** vWF 105.8 ± 11.6 and 102.2 ± 10.9 U/dl, $p > 0.05$, respectively

Fig. 2. The comparison of NO pathway biomarkers of endothelial dysfunction between patients with hypertrophic cardiomyopathy and healthy subjects. **(A)** ADMA: 0.57 ± 0.08 and 0.44 ± 0.04 µmol/l, $p < 0.0001$; SDMA: 0.43 ± 0.05 and 0.34 ± 0.04 µmol/l, $p < 0.0001$, respectively; **(B)** Arg/ADMA: 118.1 ± 18.2 and 144.3 ± 22.1, $p < 0.0001$, respectively

Evaluation of endothelial dysfunction markers revealed that both sTM and TFPI were elevated in HCM whereas vWF level was comparable in HCM and controls (Fig. 1A, B). Markers of the nitric oxide (NO) pathway showed changes (elevation of both ADMA and SDMA and decrease in Arg/ADMA ratio, see Figure 2A, B). To evaluate whether the LVOT obstruction (manifested by gradient) might affect endothelial markers in HCM patients, the HCM patients were divided into 2 subgroups: with the obstructive form (n = 11) and without obstruction (n = 18). The obstructive subgroup displayed higher values of ADMA, SDMA and sTM, while other markers of endothelial dysfunction were similar in both subgroups (Fig. 3A, B, and Fig. 4A, B). The difference of Arg/ADMA ratio was close to the level of statistical significance ($p = 0.09$).

Discussion

In HCM coronary endothelium is morphologically and functionally abnormal, and in some patients an unexpected response, i.e. occlusion of the coronary

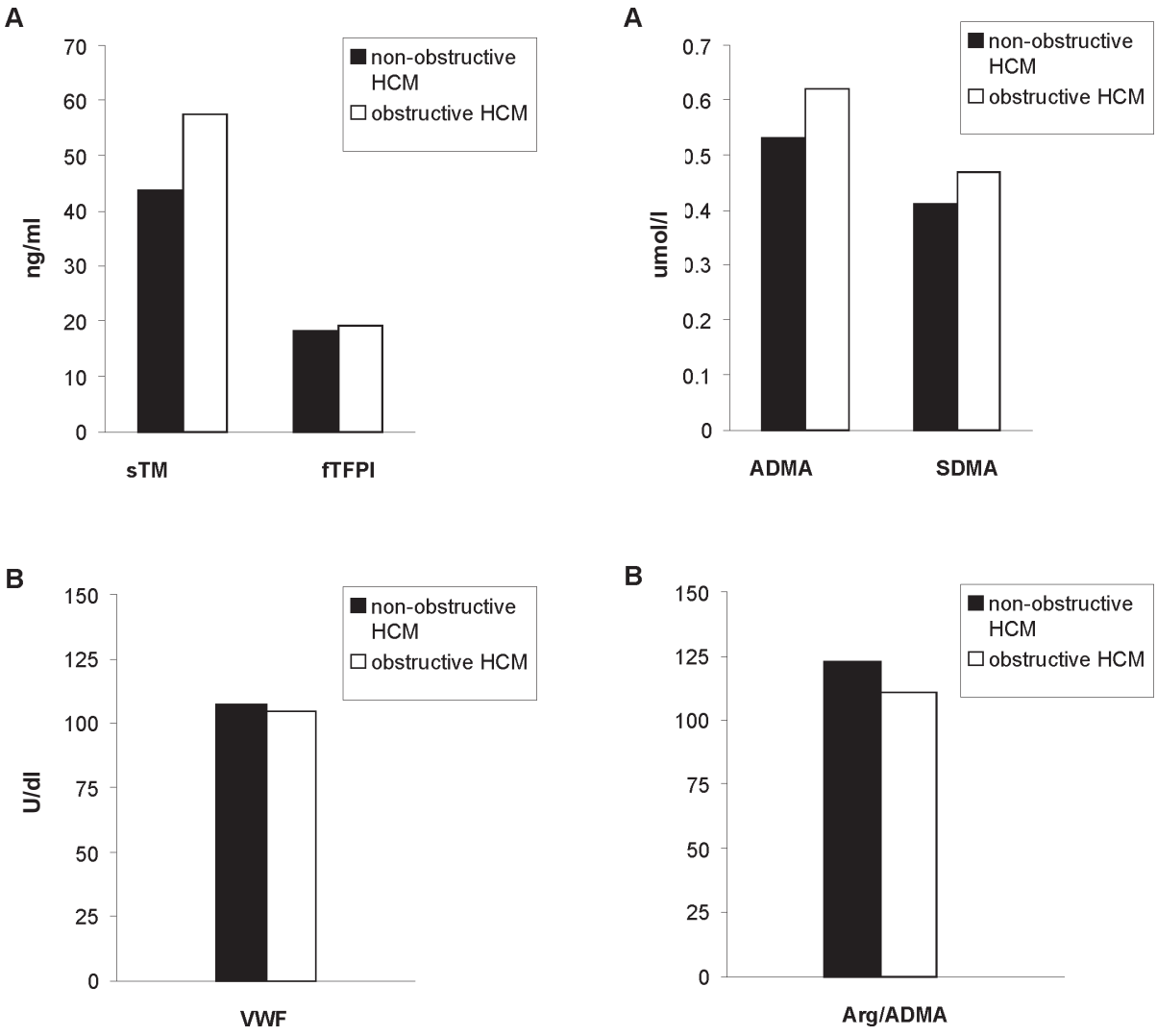


Fig. 3. The comparison of hemostatic biomarkers of endothelial dysfunction between patients with non-obstructive and obstructive hypertrophic cardiomyopathy. **(A)** sTM: 43.8 ± 7.7 and 57.6 ± 7.0 ng/ml, $p < 0.00005$; fTFPI: 18.2 ± 2.2 and 19.3 ± 2.8 ng/ml, $p = 0.24$, respectively; **(B)** vWF 106.7 ± 11.8 and 104.4 ± 11.6 U/dl, $p = 0.61$, respectively

Fig. 4. The comparison of NO pathway biomarkers of endothelial dysfunction between patients with non-obstructive and obstructive hypertrophic cardiomyopathy. **(A)** ADMA: 0.53 ± 0.07 and 0.62 ± 0.06 µmol/l, $p < 0.0008$; SDMA: 0.41 ± 0.04 and 0.47 ± 0.05 µmol/l, $p < 0.001$, respectively; **(B)** Arg/ADMA: 122.5 ± 19.2 and 110.8 ± 14.6, $p = 0.09$, respectively

artery after acetylcholine was reported [13, 16]. In contrast, peripheral circulation revealed only dysfunction in forearm resistance vessels [9, 11, 12, 19] while conductance arteries were normal both functionally (preserved flow-mediated vasodilatation [9]) and morphologically (unchanged intima-media thickness [17]). It might be hypothesized that limited coronary endothelial dysfunction in a genetically determined heart disease may be manifested by the increased levels of endothelial biomarkers assessed in peripheral venous blood. Another plausible explanation of our findings may be the concept that endothelial dysfunction is a generalized, though likely microvascular defect in HCM. It is substantiated by altered markers in circulating blood, but not by normal flow-mediated vasodilatation of the brachial artery. In the articles of Imaizumi et al. [11] and Pedrinelli et al. [19], microvascular impairment of flow-mediated vasodilatation in the peripheral circulation was presented.

To our knowledge, there were no comprehensive reports on plasma markers of endothelial dysfunction in HCM. Plasma vWF levels were comparable in HCM patients and healthy controls in the study by Varol et al. [29]. Our current findings corroborated

these results. However, we found that other markers of endothelial dysfunction, such as sTM, TFPI, ADMA, SDMA were elevated compared to healthy individuals, indicating endothelial dysfunction that can be detected in peripheral blood. Interestingly, ADMA and SDMA were particularly elevated in the obstructive form of HCM. Subvalvular obstruction generating gradient is associated with accelerated and turbulent flow, which increases the shear stress. The shear stress increases arginine methyltransferase activity and stimulates production of ADMA in cultured endothelial cells by activating a transcription factor, nuclear factor-κB [18]. Shear stress increases dimethylarginine dimethylaminohydrolase activity suggesting that the increase in ADMA results from the increased protein methylation and occurs despite concomitant stimulation of ADMA metabolism. Thus, augmented shear stress may contribute to the increase in ADMA level observed in hypervolemic states, such as heart failure, renal failure or high-salt diet [1]. Another mechanism may also be involved in the ADMA release in HCM. The activation of renin-angiotensin system or free radicals with reduction of DDAH activity may increase ADMA level.

Decreased availability of NO that is produced by endothelial cells impairs endothelium-dependent vasodilatation [6]. One mechanism that may explain the occurrence of endothelial dysfunction is the presence of elevated blood ADMA levels. Reduced NO availability results from processes related to ADMA, that is a major endogenous NO synthase inhibitor [28] and a competitive inhibitor of the cellular Arg uptake [15]. Elevated ADMA levels are associated with endothelial dysfunction in lipid disorders, coronary artery disease, chronic heart failure, diabetes mellitus and arterial hypertension. Several experimental and recent epidemiological and prospective studies support the suggestion that ADMA is a novel cardiovascular risk factor [1, 22]. However, it is not clear whether circulating ADMA is biologically active and directly induces endothelial dysfunction, or is a marker of its high intracellular content due to some diseases, and is released, indirectly, if at all *in vivo*, affecting the endothelium [5, 27]. On the other hand, a growing body of evidence suggests that, indeed, higher ADMA levels, through reduction in NO signaling, directly induce and mediate endothelial dysfunction, though *in vivo* evidence is not consistent, yet [3, 27].

In humans, the stereoisomer of ADMA, SDMA, does not inhibit NO synthesis. However, SDMA may compete with arginine for cellular uptake thus limiting NO bioavailability. Arg, the substrate of the NO production, affects cardiovascular system [6]. SDMA is formed by protein arginine methyltransferase type II (PRMT II), while ADMA is produced by PRMT I. Both products are produced in similar amounts, however, their levels may differ in some states since ADMA, in contrast to SDMA, is not only eliminated by renal excretion, but also metabolized by dimethylarginine dimetylaminohydrolases (DDAHs) [27]. Selective increase in ADMA has been reported largely when DDAH dysfunction is present or suspected [4]. Importantly, it has been suggested that, like arginine, ADMA, and SDMA share a common pathway for entry into the cell, high SDMA levels may indirectly lead to impaired NO synthesis through competition with arginine for this uptake [26]. To provide better insights into the ADMA/DDAH pathways in HCM patients, we determined Arg, ADMA, and SDMA, as our method introduced by Teerlink et al. [24] offers such an opportunity. According to Bode-Borger et al. [2] determination of ADMA and Arg plasma levels to calculate the Arg/ADMA ratio might be useful, thus, this parameter was included in the current study.

Recently, we reported that LVOT obstruction was associated with enhanced thrombin generation, platelet activation and inflammatory state in HCM patients with sinus rhythm [10]. Further study exploring the relationships between endothelial markers and thrombin, platelet, or inflammatory activity is needed.

Possible clinical implication: Myocardial ischemia is an important complication of HCM. Endothelial dysfunction may be one of the mechanisms contributing to ischemia. Potential links between ischemia and endothelial biomarkers are worth investigating.

In conclusion, HCM patients show specific features of endothelial dysfunction detectable in peripheral blood, namely, sTM and TFPI, but not vWF, are elevated along with increased ADMA levels.

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