Acta Cardiol Sin 2018;34:259–266
Original Article
doi: 10.6515/ACS.201805_34(3).20180207A

Valvular Heart Disease

The Relationship between Serum Apelin Levels and the Severity of Calcific Aortic Stenosis

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Background: Apelin, an endogenous peptide, has recently gained attention due to its positive inotropic effects in heart failure physiopathology. We investigated the relationship between serum apelin levels and the severity of calcific aortic stenosis (AS).

Methods: A total of 68 consecutive patients diagnosed with calcific AS and a control group of 32 subjects were included in the study. The subjects were divided into three group as follows: the control group, the mild-moderate AS group and the severe AS group. Blood samples were obtained from all of the subjects, which were used for biochemical comparisons of apelin 36 and high-sensitive C-reactive protein (hsCRP) levels.

Results: Plasma apelin 36 levels were significantly lower in the patients with severe AS [490 (247-1074) pg/ml] compared to both the mild-moderate AS [209 (97-453) pg/ml] and control [660 (378-1200) pg/ml] groups ($p < 0.001$). Correlation analysis between the left ventricular mass index and apelin concentrations revealed a significant negative correlation between the two parameters ($p < 0.001$, $r = -0.478$).

Conclusions: Our study demonstrated decreased apelin levels and increased hsCRP concentrations in patients with severe calcific AS. Our findings may help to clarify the exact pathophysiologic role of apelin in cardiovascular diseases.

Key Words: Aortic stenosis • Apelin • Apelin 36 • Left ventricular hypertrophy

INTRODUCTION

Aortic valve stenosis (AS) is the most common valvular heart disease in developed countries,[1] with a 1-year mortality rate up to 14% even if interventional treatment is performed.[2] Both the management of the disease and the accompanying structural diseases make it difficult to manage AS.[3,4] Although several pathophysiological factors have been proposed regarding the initiation and progression of AS, there is an obvious resemblance between AS and atherosclerosis due to similar histopathological findings.[3] In addition, both entities share the same etiologic risk factors including hypertension, smoking and hypercholesterolemia.[4] Furthermore, Palta et al. reported that the presence of hypercholesterolemia, smoking, increased serum creatinine, and calcium concentrations accelerate the progression of stenosis similar to atherosclerosis.[5] Aortic valve sclerosis is closely related to endothelial dysfunction.[6] Kadoglou and coworkers[7] reported increased hsCRP levels and decreased apelin levels in patients with both stable and unstable coronary artery disease and acute coronary syndrome compared to control subjects.[8] Apelin, first isolated from bovine stomach as a 36 amino acid peptide, is the only known ligand of the human orphan G-

Received: April 2, 2017 Accepted: February 7, 2018
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protein coupled receptor (APJ).[9] Although shorter C-terminal sequences elicit biological activity, apelin-36 and 13 are known to be physiologically active.[10] In addition to adipose tissue, brain, lung, and liver, apelin mRNA is richly synthesized in the cardiovascular system, mostly in the right atrium and coronary arteries.[11] Data suggest that apelin may have important regulatory effects on cardiac contraction, blood pressure, angioneogenesis, and fluid balance in addition to inhibiting apoptosis. Apelin levels were also found to be decreased in various severe cardiovascular diseases except for obesity and mild compensated heart failure, in which apelin levels were higher compared to controls.[12] Furthermore, a recent study reported higher plasma apelin levels and lower apelin/APJ myocardial expression in left ventricular hypertrophy due to chronic pressure overload in patients with AS.[13] A direct correlation has also been demonstrated between plasma apelin and left ventricular mass index, a reliable indicator of left ventricular hypertrophy, and its severity.[13]

To the best of our knowledge, no studies have investigated the relationship between serum apelin 36 levels and the severity of calcific AS. The purpose of this study was to investigate the association between apelin 36 levels and the severity of calcific AS.

MATERIALS AND METHODS

Study population

The present observational study was performed at the Recep Tayyip Erdoğan University Education & Research Hospital between January 2015 and October 2015. A total of 68 consecutive patients diagnosed with calcific AS by 2-D transthoracic echocardiography were included in the study. The patients were categorized into two groups as follows: 34 with mild-moderate AS (18 females, 16 males; mean age: 73.5 ± 9.9 years), and 34 with severe AS (23 females, 11 males; mean age: 76 ± 9.1 years). Thirty-two control subjects with a similar age and gender but without AS (14 females, 16 males; mean age: 73 ± 9.2 years) were also enrolled and gave blood samples which were used for biochemical comparisons of apelin and high-sensitive C-reactive protein (hsCRP) levels. The demographic, clinical and laboratory characteristics of the patients and control subjects were recorded through a systematic review of the patient files on admission. All patients underwent standard transthoracic echocardiography and 12-lead electrocardiography.

The exclusion criteria were as follows: presence of severe coronary artery disease, peripheral artery disease, congestive heart failure, recent acute coronary syndrome, atrial fibrillation, congenital heart disease, myocarditis, pericarditis, cardiomyopathy, rheumatic or congenital aortic stenosis, gout arthritis, use of diuretics, use of uricosuric or hepatotoxic agents, acute or chronic renal dysfunction, presence of prosthetic heart valves, primary or secondary hyperparathyroidism, malignancies, moderate-severe aortic insufficiency, acute or chronic inflammatory disease, and active infection.

This study was conducted in accordance with the Declaration of Helsinki, and approved by the local ethics committee. Fifty-eight consecutive patients between 40-85 years of age were enrolled after providing written informed consent.

Definitions

Hypertension and diabetes mellitus (DM) were diagnosed according to recent guideline definitions. Hypertension was defined as either a systolic arterial pressure greater than 140 mmHg and/or diastolic arterial pressure of 90 mmHg, or if the patient used antihypertensive drugs.[14] DM was confirmed if the patient had a history of DM, or was on a diet, or used antidiabetic drugs, or had a fasting venous blood glucose concentration ≥ 126 mg/dL in previously untreated patients. Hyperlipidemia was defined as fasting total serum cholesterol > 200 mg/dL, low-density lipoprotein (LDL) cholesterol > 130 mg/dL, or serum triglycerides > 180 mg/dL or if the patient used lipid-lowering drugs because of hypercholesterolemia.[15] The height and weight of the subjects were recorded, and body mass index (BMI) was calculated as the ratio of weight in kilograms divided by the square of height in meters. Patients smoking 20 or more cigarettes daily at the time of diagnosis were recognized as active smokers.

Transthoracic echocardiography

All of the study participants underwent comprehensive 2-dimensional, M-mode, conventional Doppler and color Doppler echocardiographic examinations to determine the underlying structural heart disease with a

Philips ie33 echocardiography system using a 2.5- to 3.5-MHz probe by two experienced cardiologists who were blinded to the patients' data. A continuity equation was used to calculate aortic valve area. A continuous wave Doppler scan at the level of the aortic valve was used to measure peak aortic velocity, and time velocity integral was used to calculate the mean aortic gradient. AS severity was graded using recent guidelines as follows:[16] mild, valve area exceeding 1.5 cm^2, transvalvular velocity 2.0 to 2.9 m/s, and mean gradient < 20 mmHg; moderate, valve area of 1.0 to 1.5 cm^2, transvalvular velocity 3.0 to 3.9 m/s, and mean gradient 20 to 39 mmHg; and severe, valve area less than 1.0 cm^2, transvalvular velocity ≥ 4 m/s, and mean gradient ≥ 40 mmHg.

Biochemical measurements

Fasting venous blood samples were acquired via antecubital fossa in the morning hours. Serum was obtained after centrifugation at 3000 rpm at 4 °C for 15 minutes, and stored at -80 °C until being used for analysis of apelin 36 levels. Fasting blood glucose, postprandial glucose, creatinine, total cholesterol, triglycerides, and high-density lipoprotein cholesterol concentrations were measured routinely using an Abbott Diagnostics C8000i (Abbott, Germany) autoanalyzer in the biochemistry laboratory of our hospital with commercial kits. LDL cholesterol was assayed using Friedwald's formula for samples with triglycerides ≤ 400 mg/dL.[17] Hematological parameters were obtained using a Coulter LH 780 Hematology Analyzer (Beckman Coulter Ireland Inc., Mervue, Galway, Ireland).

Serum apelin 36 levels were measured using a commercially available kit. After collection of blood for apelin 36 measurements, the serum of all patients was immediately obtained by centrifugation, transferred into cryotubes and stored at -80 °C until assay. Serum apelin 36 was measured using enzyme-linked immunosorbent assay (ELSIA) method (AP36 test kit, Cloud-Clone Corp., Houston, USA) according to the manufacturer's protocol. Absorbance of each well was determined at 450 nm with a microtiter plate reader (Multiskan GO, Thermo Scientific, Waltham, MA, USA) in the 5th minute. A standard curve was fitted using Titri ELISA software, and was then used to convert sample absorbance readings to apelin 36 concentration. The serum apelin 36 minimum detection range was 7.41 pg/ mL. Serum hsCRP levels were measured using the nephelometric method on a UniCel DxC 800 System (Beckman Coulter Inc., USA). The reference range was 0-0.77 mg/dL.

Statistical analysis

Continuous variables were expressed as means ± standard deviations or median (minimum-maximum) values, and categorical variables were presented as percentages. The One-Sample Kolmogorov-Smirnov test was used to evaluate the distribution of continuous variables. Continuous variables were compared between two groups using the Student's t test or Mann-Whitney U test. The Kruskal-Wallis test was used to compare plasma apelin and hsCRP levels among groups. Categorical variables were compared appropriately with the chi-square or Fisher's exact tests. Correlations between variables were tested using the Pearson correlation test for normally distributed variables and Spearman correlation test for non-normally distributed variables. All analyses were performed using SPSS software, version 16.0 (SPSS Inc., Chicago, Illinois, USA). A p value < 0.05 was considered to be significant.

RESULTS

The mild to moderate AS group consisted of 34 patients (18 females, 16 males; mean age 72 ± 8.5 years), and the severe AS group also included 34 patients (23 females, 11 males; mean age 73.7 ± 6.8). The control group consisted of 32 patients (17 females, 15 males; mean age 70.7 ± 7.2 years). Baseline demographic, clinical characteristics and echocardiographic parameters of the AS patients by group are presented in Table 1. The groups were similar in terms of age and BMI, and the presence of hypertension, hyperlipidemia, smoking, and DM.

Plasma apelin 36 levels were significantly lower in the patients with severe AS compared to those with mild-moderate AS and the control subjects (p < 0.001) (Figure 1). We also found significantly higher hsCRP concentrations in the patients with severe AS compared to the other groups (p < 0.001). Even though serum apelin levels were significantly lower in the mild-moderate AS group compared to the control group [490 (247-1074) vs. 660 (378-1200) pg/ml], the lowest apelin concentra-

Table 1. Clinical characteristics of the study patients

	Patients with aortic valve stenosis		Control group (n = 32)
	Mild-moderate AS (n = 34)	Severe AS (n = 34)	
Age, yr	72 ± 8.5	73.7 ± 6.8	70.7 ± 7.2
Sex, male/female	16/18	11/23	17/15
BMI, kg/m^2	28.5 ± 5.3	26.7 ± 2.1	30 ± 4.2
Body surface area, m^2	1.89 ± 0.1	1.76 ± 0.1	1.8 ± 0.1
Aortic valve area, cm^2	1.52 ± 0.2$^{\#}$	0.78 ± 0.1$^{\#}$	
Mean pressure gradient, mmHg	25.9 ± 6$^{\#}$	49.2 ± 11.9$^{\#}$	
Peak pressure gradient, mmHg	45.9 ± 9.4$^{\#}$	78.5 ± 17.8$^{\#}$	
LV mass index, g/m^2	121 (69-350)*	137 (13-185)*	89 (66-117)
LV ejection fraction, %	61.7 ± 3.7	60.8 ± 3.5	62.4 ± 3.4
Hypertension %	41.2	35.3	37.5
Diabetes mellitus, %	23.5	20.6	12.5
Smoking, %	11.8	14.7	9.4
Hyperlipidemia, %	37.5	41.2	46.9
Medication			
Beta-blockers, %	76.5	79.4	18.8
Statin, %	32.4	50	21.9
ACEi-ARB, %	44.1	52.9	37.5

ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; LV, left ventricular; Significant difference for patients with vs. without heart failure; * $p \leq 0.05$; $^{\#}$ $p < 0.001$.

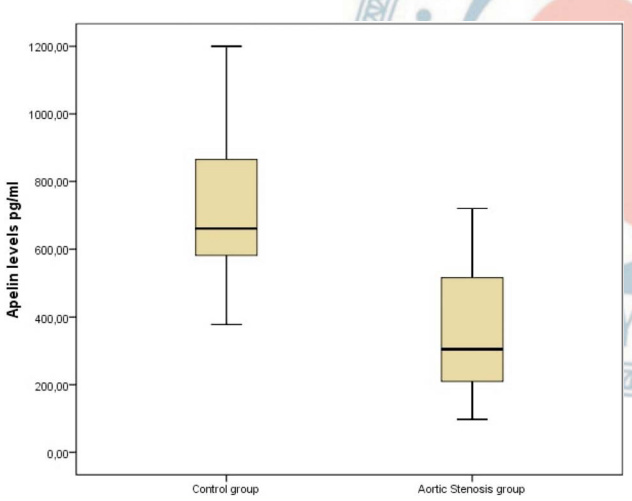

Figure 1. *Serum apelin concentrations according to aortic stenosis and control group.*

tions were observed in the severe AS group [209 (97-453) pg/ml] (Table 2). Spearman's rank test showed a statistically significant negative correlation between serum apelin 36 levels and mean aortic gradient in the patients with AS (r = -0.637, p < 0.001, Figure 2). There was also a significant negative correlation between the left ventricular mass index and apelin 36 concentrations (p < 0.001, r = -0,478), whereas aortic valve area and apelin 36 had a statistically significant positive association (p < 0.001, r = 0.646).

DISCUSSION

In our study we revealed a strong correlation between various parameters denoting the severity of calcific AS and plasma apelin 36 levels. We demonstrated lower apelin 36 levels in patients with severe AS compared to patients with mild-moderate AS and normal control subjects. Additionally, consistent with previous reports, hsCRP levels were correlated with the severity of AS.

Apelin levels have been reported to be lower in patients with various cardiovascular diseases but higher in those with obesity and compensated mild heart failure compared to controls.[9] Recent studies have shown lower apelin levels in patients with significant coronary atherosclerosis.[7,8] Since there are several shared risk factors for calcific AS and atherosclerosis, lower levels of apelin in both situations may help to reveal the pathophysiologic processes of calcific AS.

Preclinical studies have demonstrated that apelin

Table 2. Plasma apelin and hsCRP concentrations in patients with AS and the control subjects

	Patients with AS		Control group (n = 32)	p value
	Mild-moderate AS (n = 34)	Severe AS (n = 34)		
Apelin, ng/ml	490 (247-1074)	209 (97-453)	660 (378-1200)	< 0.001
hs-CRP, mg/L	0.40 (0.10-1.20)	0.75 (0.30-2.10)	0.20 (0.1-0.54)	< 0.001

Data are medians with range in parentheses. p values are from Kruskal-Wallis tests across the three groups.
AS, aortic stenosis; hs-CRP, high-sensitive C-reactive protein.

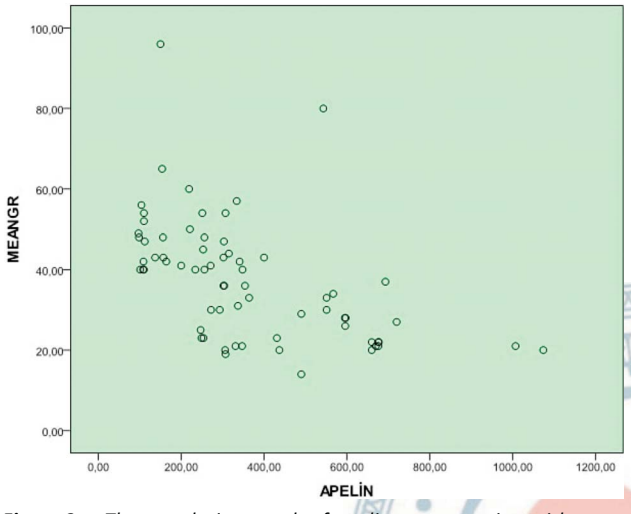

Figure 2. *The correlation graph of apelin concentration with mean aortic gradient.*

signaling exerts major effects on both vascular tone and cardiac contractility. Apelin has been identified as an endogenous circulating peptide with potent positive inotropic activity.[18] Apelin signaling may also be involved in the regulation of blood pressure, cardiac contractile function, fluid balance, angiogenesis and inhibition of apoptosis. Moreover, apelin has been shown to increase cardiac contractility in vivo and cause a rapid fall in both arterial blood pressure and systemic venous tone with corresponding reductions in left ventricular afterload and preload in rodents.[19-23]

Japp and coworkers found that apelin exerts direct coronary vasodilation and positive inotropic effects in humans.[24] In addition, apelin decreased both peak and end-diastolic left ventricular pressures. The authors also revealed that systemic apelin administration caused a reduction in peripheral vascular resistance accompanied by an increase in cardiac output and heart rate.[24]

Previous investigations have demonstrated either decreased or unaltered levels of plasma apelin in patients with heart failure compared to control subjects.[25-30] In contrast, Chen et al. reported that plasma apelin was increased mostly in patients with mild to moderate heart failure with symptoms and left ventricular dysfunction, whereas apelin levels declined with the transition from moderate to severe heart failure.[31] Thus, previous data suggest that a failing cardiac muscle could increase production of this powerful inotropic peptide as a compensatory mechanism to augment inotropic capacity and maintain cardiac output. Our finding of decreased apelin levels in severe AS may be consistent with these observations. However, it must be remembered that our study group included patients with normal ejection fraction, and the patients with mild-moderate AS tended to have lower apelin levels compared to the healthy controls. A recent study demonstrated significantly lower apelin levels in hypertensive patients with left ventricular hypertrophy compared to those without hypertophy.[25] In addition, the in vitro part of their study revealed a potent inhibitory effect of apelin to hypertrophic stimuli.[25] Similarly, we demonstrated decreased apelin concentrations in both the mild-moderate AS and severe AS groups, however apelin levels were significantly lower in the patients with severe AS compared to those with mild-moderate AS. In contrast to the findings of Chen and coworkers, the decreased apelin levels in our patients with mild-moderate AS and normal ejection fraction may have been due to higher left ventricular mass index (hypertrophy). Aortic valve calcification is an active process which depends on osteoblastic differentiation of aortic valve interstitial cells. Yuan and coworkers demonstrated that apelin attenuates the osteoblastic differentiation of aortic valve interstitial cells.[32] Therefore, the inverse correlation between plasma apelin and degree of aortic stenosis in our study group may reflect a low inhibitory effect of apelin culminating in more severe AS, using a different mechanism than heart failure.

A recent study demonstrated reduced circulating apelin levels in patients with essential hypertension, and found that lower plasma apelin was independently asso-

ciated with an increasing degree of left ventricular systolic and diastolic function impairment.[33] In contrast, Helske et al. reported increased serum apelin 12 levels in patients with severe aortic stenosis, and also found different apelin concentrations in the aortic root and coronary sinus of their patients.[34] However the control group was considerably younger than the patients in that study. A possible explanation for this contradictory finding either may be due to different concentrations of apelin 36 and 12 levels in the same patient subsets or by differences in ages between the control group and patient group. The demographic characteristics of our patient group and control subjects were similar, which strengthens our results.

Japp et al. raised the idea that the loss of apelin may be deleterious in left ventricular pressure overload, and they suggested that the acute administration of apelin may cause peripheral and coronary vasodilatation and increased cardiac output. This finding supports the idea that restoration of cardiac apelin stores could be beneficial in heart failure.[24] The combined strong inotropic effect and afterload reduction with vasodilatation and diuresis suggests that apelin could serve as a therapeutic option.[24,35-38] The administration of apelin has also been shown to exert a positive inotropic effect in rats with experimental right ventricular failure.[33] According to these data, we think that severe calcific AS patients may also benefit from the cardiovascular effects of apelin. This benefit may be achieved in different ways such as slowing disease progression, lessening medication, decreasing hospitalizations for decompensated heart failure, or prolonging the time to valve replacement.

Limitations

Our study has several limitations. The major limitation is the relatively small number of patients. Since the patients with mild-moderate AS had left ventricular hypertrophy and we did not include a control group with left ventricular hypertrophy, we could not define apelin levels in this population independently of left ventricular hypertrophy. We did not perform myocardial strain imaging in order to understand whether or not the decrease in apelin in severe AS was due to subclinical systolic dysfunction. Since our study was cross-sectional and observational, our results cannot implicate causality. Follow-up of patients within a time frame would have given the chance to identify changes in valvular gradients with respect to apelin concentration. Moreover, since we only measured apelin 36 and not the other bioactive forms of apelin, our results only apply to apelin 36.

CONCLUSIONS

Our study demonstrated decreased apelin levels and increased hsCRP concentrations in patients with severe calcific AS. We believe that patients with calcific AS and preserved ejection fraction have lower apelin concentrations. Our findings may help to clarify the exact pathophysiologic role of apelin in cardiovascular diseases.

AUTHOR CONTRIBUTIONS

Duman H, Hamur H, Bahçeci I conceived and designed the study. Duman H, Hamur were responsible for data collection. Hamur H, Duman H, Dilek AR were responsible for data analysis and interpretation. Hamur H, Duman H were responsible for the statistical analyses. Duman H, Demirelli S, Durakoğlugil ME, Erdogan T, Şatıroğlu Ö were responsible for manuscript writing and literature search. All authors approved the final version of the text.

DECLARATION OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The authors received no financial support for the research, authorship, and/or publication of this article.

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