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Original Research

The Association of Plasma Apelin Levels with Plaque Vulnerability

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Abstract

Objectives: Apelin is a recently discovered peptide that is expressed in many tissues particularly in the cardiovascular system and exerts several actions, most of which are vasodilatory and positive inotropic effects. Based on this, an apelin deficiency is believed to play a significant role in the development of hypertension and heart failure. However, the association of apelin with the pathogenesis of atherosclerosis and especially plaque vulnerability remains unestablished. Thus, to contribute to the literature, in this study, we sought to determine the association of apelin concerning plaque vulnerability in the setting of the acute coronary syndrome.

Methods: In this study, we prospectively enrolled a total of 80 patients; 40 with acute coronary syndrome and 40 patients with stable chronic ischemic heart disease. Plasma apelin levels were measured in all patients along with other routine biochemical parameters, and all patients underwent a transthoracic echocardiographic examination.

Results: Plasma apelin levels were significantly lower in patients with the acute coronary syndrome (221.2±66.7 vs 254.3±77.9 p=0.04). However, there was no correlation between plasma apelin levels and serum inflammatory markers or coronary artery disease severity.

Conclusion: Low plasma apelin levels may create a tendency towards vulnerable plaque and acute coronary syndrome.

Keywords: Acute coronary syndrome; apelin; atherosclerosis; plaque vulnerability.

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Apelin, which is an endogenous ligand for G protein-coupled receptor (APJ), is expressed in many tissues throughout the body, particularly in the cardiovascular system and the heart.[1] There is evidence showing that apelin has a significant role in the homeostasis of the cardiovascular system.[2] This apelin-APJ interaction counterbalances the renin-angiotensin-aldosterone pathway via a nitric-oxide (NO) mediated mechanism leading to hypotension and vasodilatation.[3] Small scale studies conducted in humans revealed that apelin levels tend to be lower even in prehypertensive states and continue to decrease as hypertension progresses.[4, 5] Furthermore, it also exerts a positive inotropic effect on the heart and studies in heart failure patients showed lower levels compared to controls.[6] However, we should note that the role of apelin in the pathogenesis of atherosclerosis and the plaque vulnerability is not fully elucidated in the relevant literature, which remained under-researched.

Atherosclerosis is a chronic disorder that is characterized

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by inflammation, plaque formation, stenosis, and ultimately, acute abrupt vessel closure.[7] The hallmark of this pathogenesis is inflammation, which is shown both within the vessel wall and in the circulation. Therefore, therapies targeting this inflammatory pathway are of paramount importance. In this context, apelin, which is a member of the adipokine family has a putative atheroprotective role based on experimental data that showed inhibition of inflammation within the vascular wall and atherosclerosis progression.[8] Based on the data, we wanted to investigate plasma apelin levels in patients who have an acute coronary syndrome (ACS) which represents a clinical situation where plaque vulnerability, and thus inflammation are the main pillars of the pathogenesis and compare it with stable chronic ischemic heart disease patients.

Methods

In this prospective study, a total of 80 patients; 40 with the acute coronary syndrome who were admitted to coronary care unit between March and June 2017 and 40 patients with stable chronic ischemic heart disease were enrolled. The diagnosis of the acute coronary syndrome was based on the presence of at least two of the following: dynamic ECG changes consistent with ischemia, elevated cardiac biomarkers and clinical symptoms suggestive of ischemia. The diagnosis of chronic ischemic heart disease was established after evaluating the patients' prior coronary angiograms if available. Thus, patients who did not undergo coronary angiography were excluded from this study. We also excluded patients with chronic inflammatory disorders, chronic renal failure (eGFR<60 ml/min), hepatic failure, malignancy, pulmonary embolism. Demographics and clinical characteristics of patients including age, gender, smoking habits, lipid profile, diabetes, hypertension, inflammatory markers and number of diseased vessels and echocardiograms were all collected. This study was approved by our hospital's Ethics Committee and conducted in accordance with the principles stated in the Declaration of Helsinki. Informed consent was obtained from all patients before enrollment.

Apelin Measurement

Blood samples were drawn from the antecubital vein after 8-hour fasting and within the first 24-hour period of admission in ACS patients. All samples were collected into the lavender vacutainer tubes containing EDTA. After rocking the tubes a few times for adequate anti-coagulation, blood was transferred to centrifuge tubes containing aprotinin. After that, blood samples were centrifuged at 1600 g for 15 minutes at 4 C, and isolated plasma was stored -80 C for later biochemical analysis. Plasma concentrations of human apelin-12 were assayed using commercially available enzyme immunoassay kits (Phonix Pharmaceuticals, Belmont, CA, catalog number: EK-057-23). The intra-assay and inter-assay variations were less than 10% and 15% respectively. The lowest detection limit was 0.07 ng/ml.

Echocardiographic Assessment

All transthoracic echocardiographic examinations were performed based on the criteria of the American Society of Echocardiography guidelines.[9] The patients were examined in the left lateral and supine positions with two dimensional, M-mode, pulsed, and continuous Doppler echocardiography. For all measurements, the average of at least five cardiac cycles was used. Left atrium (LA) diameter, diastolic interventricular septum, left ventricular (LV) posterior wall thickness, LV end-systolic and end-diastolic dimensions and right ventricle outflow diameter were recorded from the parasternal short and long-axis views. The left ventricular ejection fraction was calculated using the bi-apical Simpson's rule.

Statistical Analysis

Continuous variables were expressed as means±SD, and categorical variables were presented as numbers and percentages. The Kolmogorov-Smirnov test was used to assess the distribution of continuous variables. Independent sample t-test was used to compare measurements of continuous variables and chi-square test for categorical variables. Pearson rank correlation coefficient was used to analyze the relationship between two numerical variables. A p-value of <0.05 was considered statistically significant. The statistical analysis was performed using IBM SPSS Statistics for Windows, Version 20 (IBM Corp., Armonk, NY, USA).

Results

Overall 40 patients with ACS (age 55.9±11.0, male 31 [77.5%]) and 40 with chronic ischemic heart disease (age 57.9±8.2, male 28 [70.0%]) were included in the study. Clinical characteristics and the laboratory values are presented in Table 1. There was no difference between the two groups in terms of body mass index, hypertension, diabetes and the number of diseased vessels. Patients in the ACS group had a significantly higher smoking rate (27 [69.2%] vs 16 [40.0%] p<0.01) and leukocyte counts (10.3±2.8 vs 8.0±2.2 p<0.01). There was no statistical difference between the groups in terms of renal function, lipid parameters and fasting plasma blood glucose levels.

Plasma apelin levels were statistically lower in ACS patients compared to patients with chronic ischemic heart disease (221.2±66.7 vs 254.3±77.9 p=0.04, Fig. 1). However, there was no correlation between plasma apelin levels with in-

Table 1. Clinical and laboratory parameters of the study patients

	Patients with ACS (n=40)	Patients with chronic IHD (n=40)	p
Apelin (pg/ml)	221.2±66.7	254.3±77.9	0.04
Age	55.9±11.0	57.9±8.2	0.34
BMI (kg/m^2)	27.6±4.3	29.7±6.2	0.07
Gender(male)	31 (77.5%)	28 (70.0%)	0.44
Diabetes mellitus	9 (22.5%)	13 (32.5%)	0.31
Hypertension	11 (27.5%)	17 (42.5%)	0.16
Smoking	27 (69.2%)	16 (40.0%)	<0.01
3-vessel disease	10 (25%)	14 (35%)	0.32
Leukocyte (x103)	10.3±2.8	8.0±2.2	<0.01
Hemoglobin (gr/dl)	14.9±5.8	13.8±5.0	0.36
Platelet (x103)	214 (182-266)	237 (193-273)	0.85
Glucose (mg/dl)	112 (92-151)	100 (92-113)	0.32
Total cholesterol (mg/dl)	167 (138-219)	175 (141-206)	0.45
LDL cholesterol (mg/dl)	111 (68-137)	101 (78-131)	0.49
HDL cholesterol (mg/dl)	39 (31-44)	38 (32-42)	0.42
Triglyceride (mg/dl)	135 (99-188)	152 (110-215)	0.24
Creatinine (mg/dl)	0.8±0.2	0.9±0.2	0.29
ALT (mg/dl)	20 (16-27)	19 (16-23)	0.06
AST (mg/dl)	26 (20-66)	18 (16-23)	<0.01
Admission CRP (mg/dl)	3.3 (1.5-8.0)	-	N/A
Maximum CRP (mg/dl)	29.4 (14.5-46.4)	-	N/A
Admission Troponin I (ng/mL)	0.8 (0.1-4.9)	-	N/A
Maximum Troponin I (ng/mL)	26.8 (10.0-58.7)	-	N/A

ACS: acute coronary syndrome; IHD: ischemic heart disease; BMI: body mass index; LDL: low-density lipoprotein; HDL: high-density lipoprotein; ALT: alanin transaminase; AST: aspartate aminotransferase.

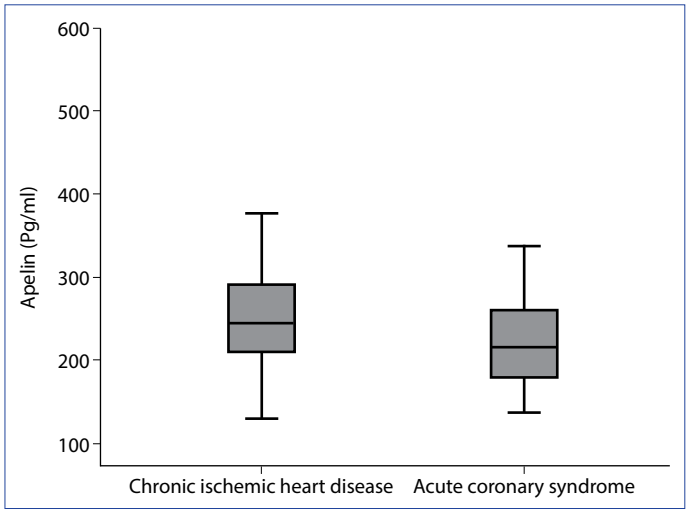

Figure 1. Shows plasma apelin levels in patients with acute coronary syndrome and stable chronic ischemic heart disease.

flammatory markers, such as c reactive protein (CRP) and leukocyte count (p=0.23 and p=0.60 respectively) and apelin levels, were not affected by the number of diseased vessels (p=0.87, Fig. 2).

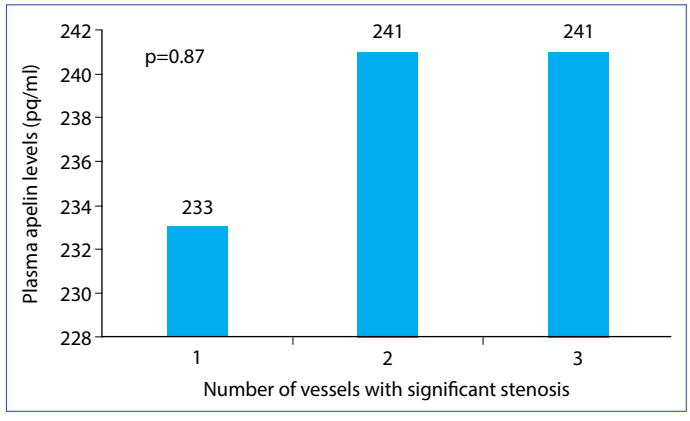

Figure 2. Depicts mean plasma apelin levels according to the severity of coronary artery disease.

Echocardiographic findings are presented in Table 2. Left ventricular ejection fraction was statistically lower in ACS patients (59±9.1 vs 55±7.9 p=0.01), and the left atrium was slightly enlarged in patients with stable chronic ischemic heart disease (3.7±0.4 vs 4.0±0.6 p=0.01). There was no statistical difference in other parameters.

Table 2. Echocardiographic findings of the study patients

	Patients with ACS (n=40)	Patients with chronic IHD (n=40)	p
Left atrial size, cm	3.7±0.4	4.0±0.6	0.01
LV end-diastolic diameter, cm	4.8±0.5	4.9±0.5	0.55
LV end-systolic diameter, cm	3.2±0.5	3.1±0.6	0.67
IST, cm	1.1±0.1	1.0±0.1	0.06
PW, cm	1.0±0.1	1.0±0.1	0.09
LVEF, %	50±9.1	55±7.9	0.01
Right ventricle, cm	2.7±0.3	2.7±0.3	0.39
Pulmonary artery pressure, mmHg	24.0±6.4	26.4±10.2	0.22

ACS: acute coronary syndrome; IHD: ischemic heart disease; IST: interventricular septum thickness; PW: posterior wall; LVEF: left ventricular ejection fraction.

Discussion

In our study, we found that plasma apelin levels were lower in patients with ACS compared to chronic stable ischemic heart disease patients. However, neither plasma apelin levels had a significant correlation with the severity of coronary artery disease nor with the degree of inflammation based on C reactive protein or leukocyte counts.

There is mounting evidence showing that apelin plays a critical role in the homeostasis of the cardiovascular system. It is widely expressed in many tissues, such as heart, large conduit vessels, and particularly in the endothelium and exerts significant vasodilatory and inotropic effects. These vasodilatory effects are mediated through NO pathway and the apelin-APJ complex acts as an antagonist to the angiotensin II, which is a very strong vasoconstrictor.[10] Thus, studies conducted so far in patients with heart failure and hypertension repeatedly demonstrated lower levels, which supports the hypothesis that apelin deficiency might lead to heart failure and hypertension.

Despite its protective effects on the cardiovascular system, the role of apelin in the pathogenesis of atherosclerosis is less established. Animal studies had conflicting results. The study led by Hashimoto et al.[11] found a reduced atherosclerotic burden in apelin receptor-deficient mice and speculated that the apelin-APJ system was linked to oxidative stress. On the contrary, Leeper et al.[8] reported that apelin reduced vascular wall inflammation by inhibiting macrophages. Human studies found lower apelin levels in patients with established coronary artery disease as compared to controls. Based on that, apelin was regarded as an atheroprotective peptide.

One recent study conducted by Zhou et al.[12] investigated acute myocardial infarction patients with intravascular ultrasound and compared findings and plasma apelin levels with healthy controls. In their study, they demonstrated that apelin levels tend to be lower in patients with a ruptured plaque than in healthy controls. Our study was different from its design in which we compared ACS patients with chronic ischemic heart disease patients and still found lower apelin levels in ACS patients. This finding may be related to plaque vulnerability rather than established atherosclerosis. However, the lack of correlation with the degree of acute inflammation, which was assessed by serum CRP and leukocyte levels may be a result of a complex interaction between apelin and vulnerable plaque rather than just simple inflammation. In addition to that, although we found lower apelin levels in patients with single-vessel disease as compared to other multi-vessel disease patients, this difference was not statistically significant.

One important factor that needs to be taken into consideration is the difference in left ventricular ejection fractions between the groups which potentially might have affected our results.

Systolic heart failure is particularly associated with lower apelin levels. On the other hand, in our study, the difference in left ventricular ejection fractions between the two groups was slight, with no statistical significance (LVEF 50% vs 55% respectively p=0.01). In addition, none of the patients developed clinical signs of heart failure. Therefore the difference in left ventricle ejection fraction values should not have a significant effect on apelin levels. Nevertheless, some studies showed that plasma apelin levels tend to decrease after an acute coronary syndrome.[13] For example, Kuklinska et al.[14] showed that apelin levels keep decreasing during the first five days after myocardial infarction, and Weir et al.[15] reported that this time might be up to 24 weeks after myocardial infarction. The reason for this gradual decrease was not clear. Thus, we believe that apelin may have a role in plaque vulnerability, and low plasma levels may expose the patients to cardiac events. In order to establish whether apelin is a bystander or an active player for plaque destabilization, further research is needed.

Limitations

Although prospective, this was a single-center study with a relatively small number of patients. The association of apelin with plaque vulnerability could be strengthened by adding other markers that are thought to involve in this process, such as matrix metalloproteinases. Imaging techniques, particularly intravascular ultrasound or optical coherence tomography, would help define the plaque structure and the underlying cause. However, this study may be assessed as a hypothesis-generating pilot study.

Conclusion

Low plasma apelin levels may create a tendency towards vulnerable plaque and acute coronary syndrome. To further define this cause and effect relationship, further studies are needed.

Disclosures

Ethics Committee Approval: This study was approved by our hospital's Ethics Committee and conducted in accordance with the principles stated in the Declaration of Helsinki.

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Conflict of Interest: None declared.

Authorship Contributions: Concept – K.K., H.K.; Design – K.K., H.K., K.O.K.; Supervision – K.O.K.; Materials – N.Ç.; Data collection &/or processing – N.Ç., K.K., S.S.Y., G.Ç., H.K.; Analysis and/or interpretation – G.Ç.; Literature search – K.K., E.B.K.; Writing – K.K., E.B.K.; Critical review – K.O.K.

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