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Research Brief

Association of the serum apelin, but not ghrelin, with the presence and severity of coronary artery disease

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ABSTRACT

Increasing evidence suggests that apelin and ghrelin may participate in atherogenesis. We sought to investigate whether the serum levels of apelin and ghrelin are significantly different in patients with coronary artery disease (CAD) compared to patients with nonsignificant coronary stenosis and determine the correlation between these adipokines and the severity of coronary stenosis.

The study population included 31 stable CAD patients, 38 unstable CAD patients, and 39 non-CAD subjects. Serum levels of apelin and ghrelin, fasting blood glucose, lipid parameters, hs-CRP and hematological indices were determined in all groups using routine standard laboratory procedures. Serum apelin levels were significantly lower in patient with unstable CAD (0.354 ± 0.063 ng/mL) compared to stable CAD patients (0.401 ± 0.045 ng/mL, $p = 0.003$) and non-CAD subjects (0.415 ± 0.055 ng/mL, $p<0.001$). In addition, serum apelin levels were inversely correlated with the severity of coronary stenosis in CAD patients ($p<0.05$). However, there was no significant difference in ghrelin levels among the 3 groups.

This data may suggest that the presence of unstable CAD may be associated with lower serum apelin which may indicate the potential role of this peptide in the progression and destabilization of coronary plaques.

1. Introduction

Atherosclerosis, the main cause of coronary artery disease (CAD), is a chronic inflammatory disorder with long asymptomatic period for years and usually manifests as acute coronary events with occlusion of coronaries. Apelin is an adipokine produced by various cells, including adipocytes, endothelial cells, vascular smooth muscle cells, and cardiomyocytes.[1,2] It has been recently shown that apelin is involved in important cardiovascular functions, inducing arterial vasodilatation, lowering arterial blood pressure, and improvement of cardiac output.[3,4]

Human ghrelin, a 28-amino acid peptide hormone, is secreted primarily by the gastric mucosa. Although ghrelin is not produced by adipose tissue, its metabolic and cardiovascular functions are closely related to those of adipokines.[5,6] Ghrelin has been demonstrated to inhibit proatherogenic inflammatory mechanisms in experimental models of atherosclerosis.[7]

Recent studies have also demonstrated that red blood cell distribution width (RDW) is an independent predictor of CAD and is associated with other known atherosclerosis predictive factors.[8,9] Elevated RDW level was found to be associated with inflammatory biomarkers in patients with CAD, and apelin and ghrelin was shown to inhibit expression of pro-inflammatory cytokines.[10,11]

The aim of this study was first to assess whether serum levels of apelin and ghrelin are significantly different in patients with stable CAD and unstable CAD compared with non-CAD subjects. In addition, we sought to evaluate the relationship of RDW with apelin and ghrelin levels in the patients, given the existence of mechanistic relationships between them in atherosclerosis pathogenesis.

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2. Methods

2.1. Patients population

The study group included 108 consecutive patients (including 39 non-CAD subjects, 31 stable and 38 unstable CAD patients) who underwent coronary angiography for evaluation of chest pain at the Department of Cardiology (Sahid Beheshti Hospital, Kashan, Iran) between May 2018 to September 2018.

Unstable CAD patients had acceleratory angina or angina at rest along with transient ST-segment shifts, and/or T-wave inversion on ECG, but normal serum troponin levels, while stable patients only had chest pain during physical exertion, which was relieved by rest, and the pattern or frequency of their chest pain duration was constant. Patients with a history of diabetes, hematologic diseases, cancer, or infective or inflammatory diseases were excluded. In addition, patients with recent myocardial infarction (<6 months) which was identified based on symptoms, signs of electrocardiography, and myocardial ischemia markers (creatine kinase (CK-MB) and troponin T) were not entered into the study. Hypertension was defined as blood pressure >140/90 mm Hg or use of antihypertensive agents. Hyperlipidemia was defined as total cholesterol >200 mg/dl, LDL-cholesterol >130 mg/dl and triglycerides >180 mg/dl or current use of lipid lowering drugs.

The severity of coronary stenosis was scored based on coronary angiography data and a numerical score from 0 to 21 was allocated to each patient as described previously.[12] The investigation was conducted based on the Declaration of Helsinki principals. The study protocol was approved by the ethics committee of Kashan University of Medical Sciences (IR.KAUMS.MEDNT.REC.1396.25) and written informed consent was obtained from each participant. This study was supported by grants from Kashan University of Medical Sciences (grant number 96082).

2.2. Biochemical and hematological analysis

Fasting blood glucose (FBG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC) and triglycerides (TG) were determined by enzymatic methods and hs-CRP was determined by immunoturbidometric method using an automated analyzer (Hitachi 902, Kyoto, Japan) and standard commercial laboratory kits (Parsazmun Co., Tehran, Iran). Apelin and ghrelin levels were measured using commercially available ELISA kits (ZellBio GmbH, Wurttemberg, Germany) according to the manufacturer's instructions.

2.3. Statistical analysis

All the statistical analyses were performed using the SPSS for Windows™, version 16 software package (SPSS Inc., Chicago, IL, USA). Comparisons of normally distributed continuous variables between groups were performed by one-way analysis of variance

Table 1
Anthropometric, biochemical, and clinical data in CAD patients and non-CAD subjects.

Variable measured	Non-CAD (n = 39)	Stable (n = 31)	Unstable (n = 38)	P-Value		
				S vs.N	U vs.N	S vs.U
Demographic data						
Age (years)	59.4 ± 11.1	64.4 ± 10.4	62.1 ± 13.4	0.21	0.61	0.71
Men/women (n)	17/22	15/16	23/15	0.33	0.10	0.31
Body mass index (kg/m2)	27.3 ± 5.2	26.3 ± 4.2	25.7 ± 4.1	0.64	0.32	0.89
Waist hip ratio	0.90 ± 0.05	0.92 ± 0.05	0.92 ± 0.05	0.48	0.36	0.99
Systolic pressure (mm Hg)	127.9 ± 17.3	131.3 ± 14.9	126.4 ± 15.3	0.68	0.91	0.44
Diastolic pressure (mm Hg)	79.3 ± 9.9	79.7 ± 7.5	79.3 ± 12.9	0.99	1.00	0.99
Risk factors						
Hypertension, n (%)	21 (53.8%)	19 (61.3%)	19 (50%)	0.53	0.74	0.35
Hyperlipidemia, n (%)	20 (51.3%)	10 (32.3%)	14 (36.8%)	0.11	0.20	0.69
Family history of CAD, n (%)	12 (30.8%)	5 (16.1%)	7 (18.4%)	0.16	0.21	0.96
Smoking, n (%)	3(7.7%)	4 (12.9%)	3 (7.9%)	0.47	0.97	0.80
Full blood count analysis						
Erythrocyte count ($\times 10^6/\mu L$)	4.88 ± 0.50	4.91 ± 0.47	4.63 ± 0.62	0.97	0.13	0.10
Haematocrit (%)	40.80 ± 4.21	42.01 ± 3.39	40.19 ± 4.27	0.46	0.80	0.18
Hemoglobin (g/dL)	13.86 ± 1.63	14.31 ± 1.29	13.45 ± 1.57	0.47	0.49	0.07
MCV (fl)	85.69 ± 4.18	86.02 ± 4.11	86.94 ± 5.18	0.95	0.48	0.70
RDW (%)	13.3 ± 0.5	13.8 ± 0.7	13.5 ± 0.5	0.001*	0.39	0.06
White blood cell count ($\times 10^3$)	6.20 ± 1.42	7.10 ± 2.10	6.72 ± 1.78	0.11	0.43	0.68
ESR (mm/h)	9 ± 1	10 ± 2	16 ± 4	0.95	0.01	0.004
Medications						
Aspirin, n (%)	32 (82%)	22 (71%)	24 (63.2%)	0.273	0.06	0.49
Nitrates, n (%)	20 (51.3%)	20 (64.5%)	14 (36.8%)	0.27	0.20	0.02†
ACE inhibitors	17 (43.6%)	12 (38.7%)	10 (26.3%)	0.68	0.11	0.27
Clopidogrel, n (%)	13 (33.33%)	10 (32.3%)	9 (23.7%)	0.92	0.35	0.43
Statins, n (%)	28 (71.8%)	17 (54.8%)	19 (50.0%)	0.14	0.06	0.69
Biochemistry						
Fasting blood glucose (mg/dl)	94.3 ± 13.0	90.1 ± 18.4	94.4 ± 16.3	0.53	1.0	0.53
Triglycerides (mg/dl)	141.4 ± 42.3	158.5 ± 80.6	139.3 ± 63.0	0.64	1.0	0.63
				0.8	0.1	0.04
Total cholesterol (mg/dl)	183.3 ± 36.3	189.1 ± 43.0	161.8 ± 40.5	0.83	0.07	0.02*
HDL cholesterol (mg/dl)	59.0 ± 11.3	60.0 ± 10.2	49.6 ± 12.1	0.93	0.002*	0.001*
LDL cholesterol (mg/dl)	97.4 ± 20.2	103.9 ± 24.3	84.9 ± 25.5	0.51	0.07	0.005*
hs-CRP mg/L	1.4 (1–3.9)	1.4 (0.9–3.6)	1 (0.4–1.7)	0.82	0.07	0.09

Values are expressed as mean ± SD or median and interquartile range for continuous variables, and as number of patients and % for categorical variables. *For ANOVA with posthoc Scheffe analysis; †for chi-square test. A $p < 0.05$ is statistically significant. ACE: Angiotensin Converting Enzyme; CAD: Coronary Artery Disease; HDL: High Density Lipoprotein; hsCRP: high-sensitivity C-Reactive Protein; LDL: Low Density Lipoprotein; MCV: Mean Corpuscular Volume; RDW: Red blood cell Distribution Width; S vs.N: Stable CAD patients versus non-CAD subjects; S vs.U: Stable CAD patients versus unstable CAD patients; U vs.N: Unstable CAD patients versus non-CAD subjects.

(ANOVA) with post hoc Scheffe analysis. Categorical variables were compared using the chi-square test. The correlation between variables was evaluated using the Pearson's correlation coefficient.

3. Results

Results of the anthropometric, biochemical, and clinical variables in the study groups are summarized in Table 1. No significant differences were observed in age, sex, body mass index (BMI), waist hip ratio (WHR), hypertension, smoking and other cardiovascular risk factors among the 3 groups.

Serum total cholesterol, LDL-C and HDL-C levels were significantly lower in unstable CAD patients than in stable CAD patients. Unstable CAD patients also had significantly lower levels of total cholesterol in comparison with non-CAD subjects. Erythrocyte sedimentation rate (ESR) levels were significantly higher in unstable CAD patients than in stable CAD patients and non-CAD subjects. We also found significantly increased level of RDW in patients with stable CAD compared with non-CAD subjects. Serum apelin levels were significantly lower in unstable CAD patients (0.354 ± 0.063 ng/mL) than in stable CAD patients (0.401 ± 0.045 ng/mL, p = 0.003) and non-CAD subjects (0.415 ± 0.055 ng/mL, p<0.001). However, no significant difference

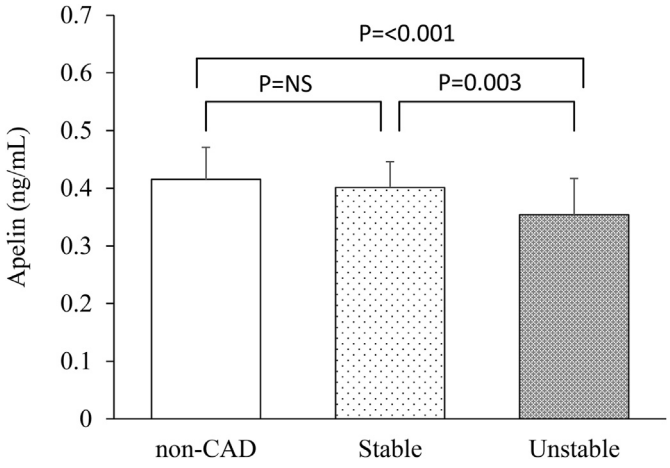

Fig. 1. Serum apelin levels in CAD patients and non-CAD subjects. Values are mean ± SD. A p < 0.05 is statistically significant. CAD, coronary artery disease; NS, nonsignificant.

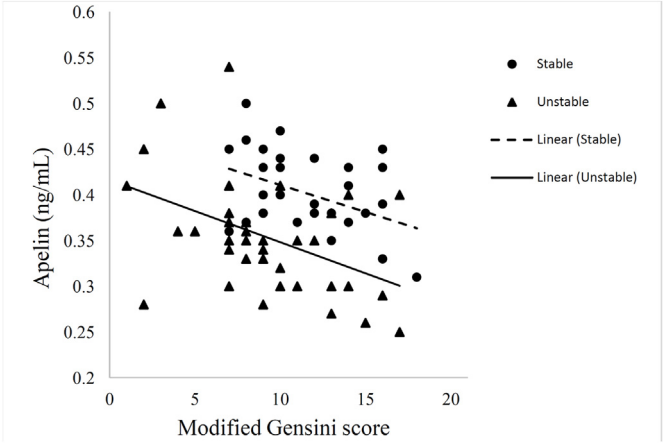

Fig. 2. Correlation of coronary stenosis scores with apelin in all CAD patients.

Table 2
Multivariate analysis between cardiovascular risk factors and serum apelin in the study participants.

	β	t	P value
Age	0.133	1.444	0.152
Sex	0.018	0.176	0.860
BMI	0.039	0.396	0.693
WHR	−0. 020	−0.203	0.840
Smoking	0.030	0.308	0.759
Hyperlipidemia	−0.057	−0.590	0.556
Hypertension	−0.056	−0.590	0.556
FBG	−0.142	−1.683	0.096
Total cholesterol	−0.259	−1.299	0.197
LDL cholesterol	0.271	1.198	0.234
HDL cholesterol	0.450	4.661	<0.001
Statins	0.085	0.924	0.358
Angiography score	−0.294	4.793	<0.001
Significance (ANOVA)			<0.001

ANOVA: analysis of variance; BMI: Body Mass Index; FBG: Fasting Blood Glucose; HDL: High Density Lipoprotein; hsCRP: high-sensitivity C-Reactive Protein; LDL: Low Density Lipoprotein; RDW: Red blood cell Distribution Width; WHR: Waist-to Hip Ratio. Values are expressed as mean ± SD or median and interquartile range for continuous variables.

was observed in the serum levels of ghrelin among the 3 groups (Fig. 1). Serum apelin was positively correlated with the HDL-C in all groups (Stable: r = 0.409, p = 0.022; Unstable: 0.399, p = 0.013; non-CAD: 0.375, p = 0.019). In all CAD patients (stable and unstable, n = 69), serum apelin levels were negatively correlated with the severity of coronary stenosis (Fig. 2). By multivariate analysis, only HDL cholesterol and CAD score remained associated with the serum apelin (Table 2). Serum levels of apelin and ghrelin were not significantly correlated with RDW levels in the whole study population.

4. Discussion

The main finding of the current study was that the circulating levels of apelin were significantly lower in patients with unstable CAD than in stable CAD patients and non-CAD patients. It has been reported that apelin promotes cellular cholesterol efflux and reduces macrophage-derived foam cell formation, indicating a potential antiatherogenic function of this peptide.[13] Kadoglou et al have demonstrated lower apelin and ghrelin levels in patients with CAD as compared to healthy controls, and that apelin is independently associated with CAD severity and ACS incidence.[14] However, our results showed that the serum levels of ghrelin were not significantly associated with the severity of coronary artery stenosis. We also found no significant differences in ghrelin levels among the 3 groups. Previous studies have shown that plasma ghrelin levels were significantly lower in both stable and ACS groups when compared to that of the control group, but did not differ between the patients with stable CAD and ACS.[14,15]

In the present study, a significant positive correlation was found between serum apelin and HDL-C levels. There is evidence that apelin may increase HDL-C by increasing expression of ATP-binding cassette transporter A1 (ABCA1), a major regulator of cholesterol efflux and HDL-C metabolism.[13]

Another important finding of the present study was that patients with stable CAD had a significantly higher level of RDW compared with unstable CAD patients and non-CAD groups. Previous studies have demonstrated that apelin and ghrelin acts as an anti-inflammatory factor via inhibiting macrophage inflammation and the expression of pro-inflammatory cytokines such as TNF-α and IL-6 in humans.[10,11] We hypothesized that higher levels of apelin and ghrelin might be associated with lower RDW in patients

with CAD, but no correlation was found between these two adipokines and RDW.

Several potential limitations of our study should be noted. First, the subjects in our study are exclusively limited to Iranian patients, so that conclusions should be drawn cautiously. Second, this study has a relatively small sample size, and therefore, studies with a larger sample size are needed to confirm our findings. Finally, Innovative imaging technologies, such as intravascular ultrasound (IVUS) may provide accurate information on atherosclerotic lesions, but no subjects in our study were evaluated by IVUS.

5. Conclusion

The present study suggests that serum apelin is inversely associated with the presence and severity of unstable CAD which may indicate its potential role in the progression and destabilization of coronary plaques.

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