Relationship of urocortin-2 with systolic and diastolic functions and coronary artery disease: an observational study

Ürocortin-2 ile sistolik, diyastolik fonksiyonlar ve koroner arter hastalığının ilişkisi: Gözlemsel bir çalışma

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ABSTRACT

Objective: The urocortin (Ucn) hormones have many important roles in the cardiovascular system. Apart from systolic dysfunction (SD), there is no sufficient data on the relationship between serum Ucn-2 and diastolic dysfunction (DD), or coronary artery disease (CAD). We investigated serum Ucn-2 levels in SD, DD, and CAD.

Methods: In this observational cross-sectional study, study population was enrolled among outpatients who underwent coronary angiography with the pre-diagnosis of CAD. By examining the echocardiography 86 subjects were selected to study after coronary angiography. The subjects distributed over three groups to investigate the relationship between serum Ucn-2 and SD according to their ejection fraction (EF): subjects with moderate to severe SD (Group A, EF=33.6%), subjects with mild to moderate SD (Group B, EF=46.1%), and those without SD (Group C, EF=64.5%). Apart from these groups, the serum Ucn-2 levels were compared between subjects with and without DD (EF≥45%), and also compared between subjects with and without CAD (EF≥55%). Statistical analyses were performed using one-way ANOVA, Kruskal-Wallis, Chi-square, Mann-Whitney U, Spearman correlation and multiple regression analyses tests.

Results: Serum Ucn-2 levels were decreased in Group A and were increased in Group B compared to Group C (9.4±3.4, 12.8±3.6 vs. 10.4±3.9 pg/mL, respectively, p=0.003). Unlike SD; there was no significant difference in serum Ucn-2 levels between subjects with and without DD (11.4±4.1 vs 11.7±3.9 pg/mL, p=0.8) or CAD (10.7±4.7 vs 10.2±3.2 pg/mL, p=0.7).

Conclusion: Ucn-2 is elevated in mild to moderate SD. But, DD (impaired relaxation pattern), or CAD (without myocardial infarction) seems to have no effect on Ucn-2 hormone levels. (Anadolu Kardiyol Derg 2012; 12: 115-20)

Key words: Urocortin 2, left ventricular systolic and diastolic dysfunction, coronary artery disease, regression analysis

ÖZET

Amaç: Ürocortin (Ucn) hormonları kardiyovasküler sistemde önemli rol oynamaktadır. Sistolik işlev bozukluğu (SD) dışında, diyalostik işlev bozukluğu (DD) veya koroner arter hastalığı (KAH) serum Ucn-2 ile ilişkisine dair yeterli veri bulunmamaktadır. Bu çalışmamızda SD, DD ve KAH’da serum Ucn-2 düzeyini araştırdık.


Bulgular: Grup C’ye göre, Ucn-2 düzeyinin Grup A’da azaldığı ve Grup B’de arttığı saptanmıştır (Grup A, EF=33.6%, Grup B, EF=46.1% ve Grup C, EF=64.5%). Ayrıca, DD sahip olan ve olmayanlar (EF≥45%) arasında ve KAH’ı olan ve olmayanlar (EF≥55%) arasında da Ucn-2 düzeyi karşılaştırıldığında istatistiksel olarak anlamlı bir fark saptanmıştır. (Anadolu Kardiyol Derg 2012; 12: 115-20)

Anahtar kelimeler: Ürocortin-2, sol ventrikül sistolik ve diyalostik işlev bozukluğu, koroner arter hastalığı, regresyon analizi

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Introduction

In recent studies, novel corticotropin releasing factor (CRF)-related peptides, named Ucn-1, Ucn-2, and Ucn-3 have been identified (1-3). The actions of these peptides are mediated by binding to two G-coupled receptors; CRF receptor type 1 and 2 (CRF-R1, CRF-R2) (1-4). CRF-R1 is found predominantly in the pituitary and brain regions, whereas the CRF-R2 is more localized in the heart chambers and peripheral vascular system (1-6). Although Ucn-1 binds to both receptors, Ucn-2 and Ucn-3 show more affinity to CRF2 receptor (2, 3). Kimura et al. (7) reported that urocortins and CRF2 receptor were detected in all chambers of human hearts, especially in the left ventricle.

The available evidence from animal models and human clinical studies indicate that urocortins have many important pathophysiological and regulatory roles in the cardiovascular system. These effects of urocortins can be summarized as follows; a dose-dependent increase in heart rate, cardiac output, coronary blood flow (8), coronary vasodilatation and positive inotropic effect (9), protection against ischemic and reperfusion injury in ventricular myocytes, a diminution in free radical damage following myocardial infarction (10, 11), stimulation of cardiac natriuretic peptide secretion, decrease in left atrial pressure and peripheral vascular resistance (12), decrease in blood pressure, reduction of serum catecholamine levels in hyperadrenergic hypertension (13).

It has been known that heart failure (HF) is a complex syndrome, characterized by the progressive activation of a series of neuroendocrine hormones; such as norepinephrine, angiotensin II, aldosterone, and endothelin. Acting locally and systemically these hormones cause unfavorable effects in HF process. Endothelin and natriuretic peptides are predictors for prognosis and left ventricle dysfunction in HF (14). In a blood sample practically measurement of such determinants may contribute to the improvement of quick diagnosis of HF and could allow to detect the staging of HF. The published results show that Ucn is a diagnostic marker and a potentially attractive therapeutic target for HF (13, 15-17). Relatively few studies (18-20) have been performed related to the role of Ucn hormone in humans HF, and there are no sufficiently investigations regarding relation of serum Ucn levels with diastolic dysfunction (DD) or CAD.

The aim of this study was to investigate serum Ucn-2 levels in subjects with systolic dysfunction (SD), DD and CAD.

Methods

Study design and sample size

This observational cross-sectional study included 86 subjects (62±10 years, 36 women). Demographic characteristics and known to be traditional risk factors for CAD were inquired (Table 1). Different study groups were composed to investigate serum Ucn-2 levels in 86 subjects. The appropriate sample size for the study was assessed by power analysis, with 27 patients in each group, a power of 80% would be achieved ($\alpha$=0.05, power=80%, n=27 in each group).

Study population

The study subjects enrolled to study between April and June 2010 at the İnönü University. The study population were selected among hemodynamically stable outpatients who consecutively underwent coronary angiography with the pre-diagnosis of CAD. Study population had anginal symptoms or ischemic findings in laboratory tests. They had SD, DD or normal ventricular functions and coronary angiography was performed without considering left ventricle functions. The patients with SD and DD had NYHA 1, 2 functional class and many patients with ejection fraction (EF) ≤40% were under HF treatment. Angiograms were examined by an experienced interventional cardiologist, who was blinded to indication for angiography. After coronary angiography, by examining the transthoracic echocardiography and eliminating according to exclusion criteria, 86 subjects were selected finally. To investigate between serum Ucn-2 levels and SD, DD and CAD different study groups were composed and three separate comparison were carried out; (1) between subjects with DD and CAD (Group B; subjects with moderate to severe SD, EF ≤40%, n=27 / Group C; subjects with mild to moderate SD, EF=40-55%, n=29) and without DD (Group B; EF ≥55%, n=30), (2) between subjects with DD (EF ≥45%, n=34) and without DD (EF ≥45%, n=14), and (3) between subjects with CAD (EF ≥55%, n=14) and without CAD (EF ≥55%, n=16).

Exclusion criteria were as follows; (1) patients with NYHA functional class 3 or 4, (2) concomitant liver, thyroid or kidney diseases, (3) acute coronary syndrome status (patients were excluded which requiring emergency angiographic evaluation or primary percutaneous intervention due to acute coronary syndrome), (4) severe valvular disease, (5) severe chronic obstructive pulmonary disease.

All subjects gave written informed consent and the study was approved by the Local Ethics Committee.

Study variables

The clinical variables including age, gender, body mass index, and major risk factors for CAD and laboratory (echocardiographic) variables are summarized in Table 1.

The outcome variable of study was serum Ucn-2. Predictor variables were: SD-defined as above: mild-moderate, moderate-severe SD and absence of SD; DD-defined as presence or absence of DD (see below definitions) and presence or absence of CAD. The patients with ≥50% occlusion in at least one coronary artery on angiogram were accepted as those with CAD.

Echocardiography

The EF values were determined using transthoracic echocardiographic measurement (ATL HDI-5000 Bothell, Washington, USA), after coronary angiography, during bed rest, within 6 hours. The SD grading on the echocardiogram was calculated a
quantitative, apical biplane modified Simpson’s method and EF predictive values of Group A, B and C were classified in accordance with recommendations of American Society of Echocardiography (ASE) (21). The diastolic dysfunction parameters; ratio of transmitral flow velocities during early and late filling (E/A ratio), ratio of transmitral flow velocity to mitral annular velocity during early filling (E/Em ratio), deceleration time (DT), and isovolumetric relaxation time (IRVT) were recorded by Doppler echocardiography. We determined the presence of diastolic dysfunction and grading according to guidelines from the ASE (22). Diastolic dysfunction was assessed using transmitral Doppler inflow velocity patterns and Doppler tissue imaging (average of septal and lateral mitral annulus Doppler signals). Normal diastolic pattern was defined; E/A=0.75-1.5, DT=150-220 ms, IVRT<100 ms, E/Em ≤8. Grade 1 diastolic dysfunction was defined as impaired relaxation; E/A <0.8, DT>200 ms, IVRT≥100 ms, E/Em ≤8. Grade 2 DD was defined as a pseudonormal filling pattern; E/A=0.8-1.5, DT=160-200 ms, IVRT=60-100, E/Em=9-12. Grade 3 diastolic dysfunction was defined as a restrictive filling pattern; E/A ≥2, DT<160ms, IVRT≤60, E/Em>13 (22, 23).

Ucn-2 assay

Venous blood samples for Ucn-2 assays were taken in serum separator tubes from the resting subjects after echocardiographic examination. All blood samples were centrifuged at 3000 rpm for 10 min. The sera were separated and stored as two aliquots at -80°C in the polypropylene tubes, until the assay of Ucn-2. Quantitative analysis of Ucn-2 in serum samples of subjects was detected by using Human Urocortin II ELISA Kit (detection range: 6.25-400 pg/mL) which was maintained from Cusabio Biotech Co. Ltd, China (Lot No:C04051522). The principle of that assay depends on competitive ELISA method. The Ucn level was

| Table 1. Clinical characteristics of the groups |
|----------------|----------------|----------------|----------------|----------------|
| **Variables** | **Group A** (n=27) | **Group B** (n=29) | **Group C** (n=30) | **χ² or F** | **p** |
| Age, year | 69 (52-83) | 64 (30-80) | 58 (35-79) | 8.2 | 0.001 |
| Gender, female, n (%) | 11 (40) | 14 (48) | 11 (36) | 0.8 | 0.7 |
| Body mass index, kg/m² | 25 (21-28) | 27 (22-31) | 27 (18-33) | 3.1 | 0.049 |
| Hypertension, n (%) | 14 (51) | 18 (62) | 18 (60) | 0.6 | 0.5 |
| Diabetes mellitus, n (%) | 9 (33) | 12 (41) | 9 (30) | 0.8 | 0.7 |
| Hyperlipidemia | 11 (40) | 9 (31) | 16 (53) | 3.0 | 0.3 |
| Smoking, n (%) | 15 (55) | 19 (65) | 11 (36) | 5.0 | 0.5 |
| Glucose, mg/dL | 118 (74-286) | 102 (65-307) | 106 (79-311) | 1.2 | 0.5 |
| LDL cholesterol, mg/dL | 104 (23-189) | 100 (54-169) | 117 (63-209) | 0.6 | 0.5 |
| Triglyceride, mg/dL | 148 (64-339) | 152 (56-360) | 171 (42-397) | 0.2 | 0.8 |
| Ucn-2, pg/mL | 8.9 (4.2-16.6) | 12.7 (4.9-18.6) | 11.0 (4.1-17.7) | 6.2 | 0.003 |
| CAD | 21 (77) | 23 (79) | 14 (46) | 9.0 | 0.01 |
| **Echocardiographic variables** | | | | | |
| LVEF, % | 34 (25-40) | 45 (42-54) | 64 (55-73) | 75.5 | <0.001 |
| LVEDD, mm | 56 (46-69) | 48 (42-65) | 45 (38-57) | 37.5 | <0.001 |
| LVESD, mm | 42 (36-49) | 35 (30-47) | 29 (26-38) | 58.7 | <0.001 |
| E-wave velocity, cm/s | 59 (20-76) | 55 (20-90) | 57 (32-69) | 4.9 | 0.01 |
| A-wave velocity, cm/s | 59 (20-76) | 58 (25-76) | 69 (30-120) | 8.3 | <0.001 |
| E/A ratio | 0.7 (0.3-2.3) | 0.8 (0.4-2.5) | 0.7 (0.4-1.7) | 1.5 | 0.4 |
| Em velocity, cm/s | 7.0 (3.7-9.8) | 6.5 (3.8-11.2) | 8.6 (4.3-17.8) | 12.6 | 0.002 |
| E/Em ratio | 6.9 (3.3-12.6) | 7.4 (4.0-12.6) | 6.6 (3.1-14.3) | 0.9 | 0.6 |
| Deceleration time, ms | 225 (70-330) | 195 (90-320) | 200 (100-315) | 1.2 | 0.2 |
| IVRT, ms | 105 (50-150) | 110 (75-145) | 92 (55-140) | 3.7 | 0.02 |

Data are presented as median (min-max) and number (percentage) values
*one-way ANOVA, Kruskal-Wallis, or Chi-square tests
post hoc paired comparisons of Tukey or Mann-Whitney U test: *between Group A and B, *between Group A and C, *between Group B and C; p<0.05
A - transmitral flow velocity during late filling, CAD - coronary artery disease, E - transmitral flow velocity during early filling, Em - mitral annular flow velocity during early filling, IVRT - isovolumetric relaxation time, LDL - low-density lipoprotein, LVEDD - left ventricular end-diastolic diameter, LVEF - left ventricular ejection fraction, LVESD - left ventricular end-systolic diameter, Ucn-2 - urocortin-2
measured according to the manufacturer’s protocol. Optic density values were analyzed according to the values of standards.

**Statistical analysis**

Data were analyzed on SPSS version 15.0 for Windows statistical package (SPSS, Chicago, IL, USA). The parameters were evaluated for normality and the distribution and homogeneity of variances were verified. The parameters satisfying these conditions were examined with one-way ANOVA, otherwise Kruskal-Wallis test was performed to compare the difference between three groups. Post-hoc Tukey test or Mann-Whitney U test was used to determine the differences of paired groups analysis in Group A, B and C. The differences in proportions among the three groups were analyzed using Chi-square test. Multiple regression analysis was used to evaluate the effects of all independent factors that potentially affect the serum Ucn 2.

**Results**

**Demographic and clinical characteristics of the population and Ucn-2**

Clinically and hemodynamically stable (before and after coronary angiography) subjects were selected. Demographic parameters were not different among groups and more than 50% of the subjects had hypertension and nearly half had other coronary risk factors (Table 1). We did not find any significant relationship between serum Ucn-2 levels and other parameters (age, gender, coronary artery risk factors, CAD and left ventricular dimensions) among the groups; there was no or weak relationship, Spearman’s rank correlation test, r=close to zero. Although we observed differences in age, BMI and CAD parameters between groups (Table 1), no relation detected in a multiple regression analysis after adjustment for age (p=0.3), BMI (p=0.5), and CAD (p=0.8) with Ucn 2. Because our study not included the patients with NYHA functional class 3 or 4 or acute dyspneic attack, we could not investigate the relationship between Ucn-2 levels and NYHA functional class.

**SD, DD and Ucn-2 (Table 1).**

Comparison of Ucn-2 levels in SD groups demonstrated a decrease in Group A, an increase in Group B compared to normal EF Group (Group C) and serum Ucn-2 levels were 9.4, 12.8, 10.4 pg/mL, respectively (p=0.003) (Fig. 1).

We also compared the serum Ucn-2 levels between subjects with and without DD. The patients with DD were in Group B and C (EF ≥45%, mean EF=57±9, n=34). Twenty-five subjects had Grade 1 (73%) and, 9 subjects had Grade 2 DD (none had Grade 3). Those without diastolic abnormalities were 14 patients (EF ≥45%, mean EF=61±8%). No significant association or correlation were observed in Ucn-2 levels, compared between subjects with DD and without DD (11.4±4.1 vs 11.7±3.9 pg/mL; p=0.8, r=-0.04), or compared subjects with Grade 1 DD and Grade 2 DD (11.5±4.1 vs 11.1±4.6; p=0.9).

**Discussion**

In our study, we detected elevated serum Ucn-2 in mild to moderate systolic dysfunction (Fig. 1). But, DD (impaired relaxation pattern), or CAD (without myocardial infarction) did not affect the serum Ucn-2 hormone levels.

Over the recent few decades there have been striking advances in understanding of the pathogenesis of HF at molecular basis. Neurohormonal activation rise early after the onset of the deterioration of hemodynamic conditions. B-type natriuretic peptides are well-known peptide hormones, have a considerable role in the diagnosis of HF. Several novel cardiac neurohormonal biomarkers (chromogranin A, apelin, galectin-3, adrenomedullin, ST2, adiponectin, etc.) have been identified in HF (24-26), they
are still under investigation because of bringing potential diagnostic and therapeutic notion (role) in HF. The Ucn hormone is a group of peptides acting on specific G-coupled CRF receptors. Ucn-2 shows a greater affinity to CRF-R2 and it is found predominantly in the heart chambers and peripheral vascular system (1-3). The available evidence from several animal models and human clinical studies is considered that the urocortins have many pathophysiological and regulatory roles in the cardiovascular system (8-13). Also Ucn-2 infusion has improved cardiac output and EF and reduced systemic vascular resistance and a cardiac work in human cases of HF (15).

Relatively few human studies are available in patients with HF on Ucn hormone. The researchers performed the experimental or clinical studies in relation to Ucn family (Ucn, Ucn-1, Ucn-2, and Ucn-3) (8-17). Ng et al. (18) are the first researchers to explore the elevated levels of serum Ucn in human systolic HF, especially in early stages of HF, when compared with healthy controls. They suggested that Ucn system has an up-regulatory role, might be cardioprotective in the early stages of HF. On the other hand, Wright et al. (19) reported elevated plasma Ucn-1 hormone, and Gruson et al. (20) also reported increased plasma Ucn hormone levels in HF in proportion with the degree of cardiac dysfunction according to NYHA functional class I to IV. Unlike these studies, our study excluded the patients with NYHA 3 and 4, and clinically decompensated HF cases. We investigated levels of serum Ucn-2 hormone in outpatients with moderate to severe depressed left ventricular function (Group A; mean EF=33, 25-39%), mild to moderate depressed systolic function (Group B; mean EF=40, 40-54%), and normal systolic function (Group C; mean EF=64, 55-70%). Elevated serum Ucn-2 levels found in Group B (Fig. 1). Similar to Ng et al. (21)'s study, we also observed that Ucn-2 levels were not elevated in severe left ventricular dysfunction (differently they tested serum Ucn hormone, instead of Ucn-2). Our results indicated that levels of circulating Ucn-2 changed in left ventricular dysfunction, and there was no or a weak linear correlation between Ucn-2 levels and age or other cardiovascular risk factors using multiple regression analysis. The mechanism of stimulating the secretion of Ucn-2 hormone in early HF is not clear. The previous studies indicated that an activated serum Ucn hormones were related to anti-inflammatory response (reducing the interleukin and tumor necrotizing factor secretion), in exaggerated hyperadrenergic state, or as a cardioprotective role of Ucn (Ucn-1, Ucn-2 isofoms and Ucn) in HF process (12, 13, 27, 28). The reason for the lack of the serum Ucn-2 response in the presence of moderate to severe left ventricular dysfunction (Group A) may be due to withdrawal of anti-inflammatory and cardioprotective response in HF process.

In the light of current studies, it is difficult to say which Ucn hormone or isoform is predictor for systolic HF or threshold level of EF. Regarding the specificity of the assay; unlike Ucn-1, Ucn-2 is highly selective for the CRF₂ receptor and does not show affin-
Conflict of interest: None declared.


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