Asymmetric dimethylarginine and M30 concentrations in heart failure

Kalp yetmezliğinde asimetrik dimetilarjinin ve M30 düzeyleri

Heart failure, adma and M30

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Öz

Amaç: Kardiyovasküler hastalıkların gelişiminde apoptozun önemi vurgulanmaktadır. Caspase cleaved cytokeratin 18 (M30) apoptotik hücre ölümü sırasında salınan bir biyobelirteçtir. Bu çalışmada M30'un kalp yetmezliği hastalarındaki düzeyinin belirlenmesi ve M30'un asimetrik dimetilarjinin (ADMA) ile olan korelasyonun değerlendirilmesi amaçlanmıştır. Gereç ve Yöntem: Çalışmaya 30 kalp yetmezliği hastası ve 30 sağlıklı kontrol dahil edildi ve çalışma populasyonundaki bireylerin serum M30 ve ADMA düzeyleri ölçüldü. Bulgular: Hastalardaki M30 (p=0.01) ve ADMA (p = 0.012) düzeyleri sağlıklı kontrol grubundan daha yüksek bulundu. Hastalarda M30 konsantrasyonu ile ADMA düzeyi arasında pozitif korelasyon saptandı (p < 0.001, r= 0.627) ancak M30 düzeyi ile N terminal pro brain natriuretic peptide (NT-pro-BNP) düzeyi arasında herhangi korelasyon saptanmadı. Tartışma: Çalışmamızdan elde edilen bulgular kalp yetmezliği hastalarında M30'un apoptotik serum biyobelirteci olabileceğini göstermektedir. Ayrıca hastalarda artan ADMA düzeyinin aktive ettiği apoptotik kaskadın kalp yetmezliğinin oluşum mekanizmasında rol oynadığı düşünülmektedir.

Anahtar Kelimeler

Asimetrik Dimetilarjinin; M30; Sitokereatin-18 Peptid; Kalp Yetmezliği; Apoptoz

Abstract

Aim: Apoptosis has been implicated in the development of various cardiovascular diseases. Caspase-cleaved cytokeratin 18 (M30) is released during apoptotic cell death. The concentrations of M30 and the correlation with asymmetric dimethylarginine (ADMA) in heart failure (HF) are not known. The objectives of this study were to determine the possible association between M30 and ADMA and the potential use of M30 as an apoptotic marker in patients with HF. Material and Method: In this study M30 and ADMA concentrations were evaluated in 30 patients with heart failure and 30 healthy control subjects. Results: Increased M30 (p=0.01) and ADMA (p = 0.012) concentrations were found in the patients and a positive correlation was determined between ADMA and M30 in the patient group (p < 0.001, r= 0.627). No correlation was determined between M30, N terminal pro brain natriuretic peptide (NT-pro-BNP), and ejection fraction. Discussion: These results demonstrate that M30 can be used as a novel apoptotic serum marker in patients with heart failure. The apoptotic cascade activated by increased ADMA concentrations can be considered to contribute to the molecular mechanism of HF.

Keywords

Asymmetric Dimethylarginine; M30; Cytokeratin-18 Peptide; Heart Failure; Apoptosis

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Introduction

Cardiovascular diseases are the leading cause of death in developing countries [1]. Heart failure (HF) is a systemic disease in which the heart cannot pump enough blood to meet the body's requirement [2]. The prevalence of the disease is on the rise, with approximately two million new cases of HF diagnosed per year worldwide [3]. Different pathophysiological mechanisms such as oxidative stress, neurohormonal changes, and inflammatory activation lead to myocyte death by promoting apoptosis, necrosis, and autophagic cell death in HF [4,5]. Cytokeratin 18 (CK-18) is an intermediate filament of epithelial cells. CK 18 fragments such as caspase cleaved cytokeratin 18 (M30) are released into the extracellular space due to caspase digestion during apoptosis and serve as markers of apoptosis [6]. The elevation of plasma M30 level is involved in different diseases including colon cancer [7], sepsis [8], and chronic hepatitis B [9]. To the best of our knowledge, no previous study has investigated the changes of M30 concentrations in heart failure.

Asymmetric dimethylarginine (ADMA) is one of three circulating endogenous analogues of L-arginine derived by methylation of arginine residues catalyzed by a family of proteins known as protein arginine methyltransferases [10]. ADMA is an endogenous competitive inhibitor of NO synthase and is eliminated from the body by a combination of renal excretion and metabolism by the dimethylarginine dimethylaminohydrolase (DDAH) enzymes [10,11]. Increased levels of ADMA have been found to be associated with atherosclerosis [12], coronary artery disease [9], peripheral arterial occlusive disease, hypertension [13], and HF [14]. However, the role of ADMA in heart failure has not been well investigated.

The hypothesis of our study is that increased serum ADMA concentrations may trigger the apoptotic process in HF. Therefore, an assessment was made of the ADMA and M30 concentrations and the possible association between these biomarkers in patients with HF. The potential use of M30 as an apoptotic marker in patients with HF was also investigated. This study provided an important opportunity to advance the understanding of the role of ADMA in HF.

Material and Method

Patients and controls

The study included 30 consecutive patients [17 males and 13 females; mean age: 68 ± 12 years (range, 24-83 years)] with chronic HF with reduced ejection fraction (HFrEF) who were hospitalized for acute decompensated HF and 30 healthy control subjects [10 males and 20 females; mean age: 65 ± 13 years (range, 28-78 years)]. HF had been diagnosed in the patients group at least 12 months previously. Data was collected from the patients' records in the Cumhuriyet University Medical Faculty Hospital laboratory information system, including age, gender, levels of CK-MB, triglyceride (TG), total cholesterol (TCHOL), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), N terminal pro brain natriuretic peptide (NT-pro-BNP), prothrombin time (PT), activated partial thrombin time (aPTT), and international normalized ratio (INR). These laboratory values were taken within the admission period. Patients with moderate to severe aortic stenosis, anticipated cardiac transplantation, chronic dialysis, malignancy, high-output HF, age <18 years, or concomitant use of an investigational product or device were excluded from study. For the control group, the exclusion criteria included clinical suspicion of infections (body temperature out of the range 36°C - 38 °C, heart rate > 90 bpm, respiratory rate > 20/minute, white blood count > 12 x 103 mcL or < 4 x 103 mcL), presence of abnormal ejection fraction, liver disease, kidney disease, rheumatic disease, malignancy, pregnancy, and smoking. The HF patients were referred by physicians from Cumhuriyet University Medical Faculty, Department of Cardiology. Overnight fasting blood samples were collected from all participants into red top tubes (Becton Dickinson, UK) during the admission. The serum samples were allowed to clot before centrifugation. After centrifugation at 4°C for 15 minutes at 3500 rpm, the serum was aliquoted and immediately frozen at -20°C. The study protocol was approved by the Ethics Committee of Cumhuriyet University Medical Faculty (Approval number: 2016-04/06).

Biochemical analysis

Caspase cleaved cytokeratin 18 and ADMA concentrations were determined using commercially available ELISA kits. The Complete Blood Count analysis was performed using a hematology system (Mindray BC 6800, China). CK-MB, TG, TCHOL, HDL-C, and LDL-C concentrations were determined by the enzymatic colorimetric method (Beckman Coulter AU5800, USA). Serum NT-pro-BNP was measured using immunoassay (AQT90 flex Radiomater, Denmark). PT and aPTT concentrations were determined using a coagulation system (ACL TOP 700, Italy). Troponın I values were determined using immunochemical method (Beckman Coulter AU5800, USA). Estimated glomerular filtration rate (eGFR) values were calculated according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.

Echocardiographic examination

Echocardiographic examinations were performed by experienced operators. Patients were imaged in the left lateral decubitus position with commercially available systems (Vivid systems, GE Healthcare, Wauwatosa, USA). Left ventricular dimensions, volumes, and ejection fraction (EF) [by modified Simpson's method] were measured according to the European Association of Echocardiography (EAE)/American Society of Echocardiography (ASE) recommendations [15]. LV diastolic functions were evaluated according to EAE/ASE standards [16]. The diagnoses of HFrEF were made according to guidelines [17].

Statistical analysis

Sample size was determined as 30 observations for each group, based on α=0.05and β=0.10. The power of the actual performed test was calculated as 90%. Analyses were conducted using PASS 11.0 (Power Analysis Statistical System) software. The Shapiro-Wilk test was used to determine the distribution characteristics of the variables. The Student t test and Mann-Whitney U test were applied to compare the differences of the parametric and nonparametric variables between the groups, respectively. Spearman correlation coefficients were calculated to evaluate the relationship between M30, ADMA, NT-pro BNP,

ejection fraction (EF), white blood cell count, eGFR, and creatinine. The results were expressed as mean ± SD and median (25th – 75th percentile). A value of $p < 0.05$ was considered statistically significant.

Results

A summary of the laboratory parameters of the patient and control groups is shown in Table 1. Mean NT-pro BNP, troponin I, and CK-MB levels were 2110 (1088.75 – 8095.50) pg/mL, 0.03 (0.01-0.04) ng/mL, and 18.30 (6.67 – 24.25) U/L in the patient group, respectively. The eGFR values were <60 ml/min/1,73 m2 in 9 patients. The median M30 levels were 178.42 (147.2400 to 222.15) and 236.84 (185.78 – 278.51) IU/L in the control and patient groups, respectively (p = 0.01). The median ADMA levels were 14.72 ± 4.67 and 20.03 ± 10.23 µg/L in the control and patient groups, respectively (p = 0.012). The results were given within a 95% confidence interval (CI). A positive correlation was determined between M30 and ADMA in the patient group ($p < 0.001$, r= 0.627) (Figure 1). No statistically significant correlation was determined between M30, creatinine (p = 0.327, r = - 0.18), eGFR (p = 0.851, r = - 0.03), NT-pro-BNP (p= 0.404, r= 0.157), troponin I (p = 0.638, r = 0.241), WBC (p = 0.805, r = 0.04), and ejection fraction (EF) (p= 0.861, r= 0.032). No statistically significant correlation was determined between ADMA, creatinine (p = 0.710, r = -0.07), WBC (p = 0.690, r = 0.07), EF (p = 0.144, r= 0.322), eGFR (p = 0.780, r = -0.053), NT- pro-BNP (p = 0.775, r = -0.054), and troponin I (p = 0.341, r = 0.064) in patients. The clinical features of the patients are given in Table 2.

Discussion

Recent evidence suggests that apoptosis is involved at multiple points in HF, although the molecular biology and biochemistry of the apoptotic death machinery are far from being completely resolved in HF [5]. In this study, M30 concentrations were found to be higher in patients than in the healthy control group. There have been few studies conducted on the concentrations of M30 in cardiovascular disease [18-19]. It has been revealed that cardiac lipofuscin-laden lysosomes obtained from patients with ischemic, congestive, and hypertrophic cardiomyopathy contain M30 [19]. Adlbrecht et al. [18] reported elevated levels of M30 in patients with acute myocardial infarction. Recent evidence has suggested that caspases, which are a group of cysteine proteases, play a crucial role in the apoptosis of myocyte and the formation of M30 and thereby have a crucial role in HF [20,21]. In previous research of sheep fitted with variable aortic constriction devices, it was indicated that activated cardiomyocyte caspase enzymes play an important role during the transition to heart failure [20]. Merkle et al. [21] demonstrated the upregulation of myocardial caspase-1 in a murine heart failure model. In a study by Narula J et al. [22], increased caspase 3 activation was reported in patients with heart failure. The reason for increased M30 concentrations can be assumed to be associated with increased caspases activation. M30 can therefore be considered a biomarker in the monitoring of myocardial damage associated with apoptosis in patients with heart failure.

In the present study, no associations were found between the concentrations of NT-pro-BNP, EF, troponin I, and M30. Natri-

Table 1. Laboratory parameters of patients and controls

Characteristics	Patients (n: 30)	Control (n:30)	P value
AST (IU/L)	25.68 ± 9.79	23.97 ± 5.94	0.556
ALT (IU/L)	22 ± 14.26	22.97 ± 14.02	0.360
HDL-C (mg/dL)	34.72 ± 10.16	44.89 ± 9.34	< 0.001
LDL-C (mg/dL)	96.66 ± 32.21	86.20 ± 15.15	0.116
TCHOL (mg/dL)	157.10 ± 49.50	140.55 ± 20.82	0.100
TG (mg/dL)	108.60 ± 49.17	99.55 ± 40.58	0.445
eGFR (ml/min/1,73 m2)	73.41 ± 30.49	99 ± 9.57	0.014
Creatinine (mg/dL)	1.08 ± 0.85	0.93 ± 0.17	0.457
PT (sec)	11.78 ± 0.69	11.3 ± 0.32	0.392
aPTT (sec)	29 ± 2.81	31 ± 1.25	0.283
INR	1.02 ± 0.5	1.03 ± 0.2	0.427
Na (mEq/L)	134.50 ± 4.50	136 ± 2	0.642
K (mEq/L)	3.79 ± 0.65	4.07 ± 0.30	0.846
WBC (10^3 mcL)	7.70 ± 1.65	8.10 ± 1.5	0.153

aPTT: activated partial thromboplastin time, ALT: Alanin aminotransferase, AST: Aspartat aminotransferase, HDL-C: High density lipoprotein cholesterol, INR: International normalized ratio, K: Potassium, LDL-C: Low density lipoprotein cholesterol, Na: Sodium, PT: prothrombin time, TCHOL: total cholesterol, TG: Triglyceride TSH: Thyroid stimulant hormone, WBC: White blood cell. Results were given as mean ± SD and with 95% confidence intervals.

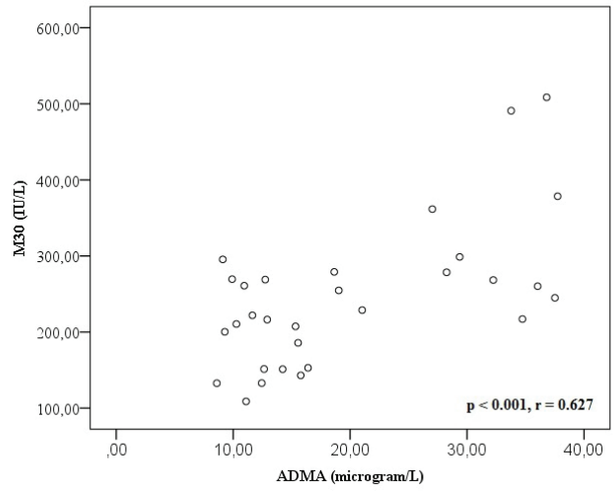

Figure 1. A scatterplot matrix with Pearson correlation to demonstrate the association between M30 and ADMA

Table 2. Clinical features of heart failure patients (n=30)

Clinical features	Values
NYHA functional class III/IV	24/6
Diabetes mellitus (yes/no)	11/19
Hypertension (yes/no)	22/8
Hyperlipidemia (yes/no)	6/24
Obesity (yes/no)	11/19
COPD (yes/no)	7/23
CEID (yes/no)	9/21
Chronic kidney disease (yes/no)	6/24
Ischemic/non-ischemic etiology	12/18
SPAP (mm/Hg)	39 ± 16
EF (%)	27 ± 3
Concomitant right ventricular systolic dysfunction (yes/no)	10/20

EF: Ejection fraction, CEID: Cardiac implantable electrical devices, COPD: Chronic obstructive pulmonary disease, SPAP: Systolic pulmonary artery pressure, TR: Tricuspid regurgitation, NYHA: New York Heart Association

uretic peptides including BNP and NT-pro-BNP, an amino-terminal propeptide equivalent to BNP, are reliable biomarkers for the diagnosis, prognosis determination, and treatment of heart failure [23]. From the results of the current study, it was concluded that M30 may not be used for the diagnosis and prognosis determination of HF as there was no correlation between M30 and NT-pro-BNP and EF. However, caution must be applied in the evaluation of this conclusion because of the low sample size. Further studies are required with larger sample sizes to evaluate the diagnostic performance of M30 in patients with HF.

Although an association has been reported in literature between ADMA concentrations and heart failure [14], the role of ADMA has not been completely defined. The ADMA concentrations were found to be higher in the patients than in the control group in the present study. Previous studies have reported elevated ADMA concentrations in patients with heart failure [24-27]. The findings of the current study are in accordance with these previous studies. A positive correlation between ADMA and M30 concentrations was determined in the current study. It is a well-known fact that there is an association between ADMA and apoptosis. Different molecular mechanisms such as the p38MAPK-dependent signaling pathway, accumulation of cytochrome c, and activation of endoplasmic reticulum stress have been described in ADMA-related apoptotic processes in different conditions [22,28,29]. The current study results suggest that increased concentrations of ADMA trigger the caspases activation in HF. Small sample size was major limitation of our study.

In conclusion, M30 can be used as an apoptotic serum marker in patients with heart failure. In addition, the activated apoptotic cascade caused by increased ADMA concentrations can contribute to the molecular mechanism of the formation and progression of HF. Therefore, further studies are warranted with respect to the potential therapeutic utility of the regulating of ADMA concentrations and caspase inhibition with potential DDAH activity and caspase inhibitors and its improved delivery system to the heart in patients with HF.

Competing interests

The authors declare that they have no competing interests.

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