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Original Article

Serum endotrophin levels in patients with heart failure with reduced and mid-range ejection fraction

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ABSTRACT

Background: Endotrophin, a type VI collagen cleavage product, has fibrosis, and insulin resistance effects. Type VI collagen also plays a role in cardiac fibrosis. In this study, we aimed to investigate the role of endotrophin in the pathogenesis of cardiac fibrosis by determining its levels in patients with heart failure with reduced and mid-range ejection fraction (EF). We also aimed to determine the possible association between endotrophin and treatment that prevents ventricular fibrosis.

Methods: Sixty patients with heart failure with reduced and mid-range EF and 27 volunteers with no cardiac failure were included in this study. In both groups, biochemical tests, EF, and endotrophin levels were measured. ELISA was performed for the determination of endotrophin levels.

Results: When compared with the control group, there was no significant difference for endotrophin levels in the patient group (p = .35). Participants in the study were divided into two groups according to their EFs, 40% and less, and 40–49%. They were classified according to their use of renin-angiotensin-aldosterone system (RAAS) blocking drugs. Endotrophin levels were significantly lower in patients with mid-range EFs between 40 and 49% (p = .03) using RAAS blockers.

Conclusion: This study is the first to evaluate the relationship between endotrophin and heart failure. Endotrophin levels were found to be low in patients with heart failure with mid-range EF who were using RAAS blockers. This suggests that RAAS blockers may influence endotrophin levels and thus could have a role in the prevention of remodelling.

1. Introduction

Fibrosis is a common pathologic consequence of extracellular matrix (ECM) dysregulation, which is caused by increased release of collagen family proteins [1]. Left ventricular remodelling develops in response to various pathophysiologic events. In adults, myocardial ECM consists primarily of type I and type III collagen. Type IV, V, and VI collagens are complementary to the collagen network [2]. Type I and type III collagen fibres contribute to cardiac fibrosis in patients with cardiac disease. Although a minor collagen type in the adult myocardium, Type VI collagen deposition was observed in patients with hypertension and diabetes [3]. Type VI collagen, along with type III collagen, is also implicated in hypertrophic cardiomyopathy-associated interstitial fibrosis and cardiac dysfunction [4].

In mice, type VI collagen stimulates cardiac myofibroblast differentiation and angiotensin 2-mediated cardiac fibroblasts produce type VI collagen, which is a constituent of post-infarction myocardial tissue [5]. Type VI collagen is a triple-helix molecule composed of A1, A2 and A3 polypeptide chains secreted from connective tissue, especially from adipose tissue [7]. Endotrophin is a newly identified adipokine that is a soluble proteolytic fragment of the A3 chain of type VI collagen [6]. Endotrophin also stimulates transforming growth factor-beta 1 (TGF-B1) production, adipose tissue fibrosis, and increases insulin resistance [7]. Type VI collagen is associated with many fibrotic diseases such as hepatic, renal, pulmonary and cardiac fibrosis [8].

In this study, we aimed to investigate the possible role of endotrophin, a profibrotic by-product of collagen VI, in the complex process of heart failure fibrosis, and to determine the relationship between endotrophin and renin-angiotensin-aldosterone system (RAAS) blockade.

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2. Materials and methods

2.1. Patients and clinical data collection

Sixty adult patients with heart failure with reduced and mid-range ejection fraction (EF) (mean age: 64.8 ± 13.5 years) who were admitted to our clinic were included in the study. The patients under follow-up at our clinic are those who have developed typical symptoms of heart failure and with structural failure in echocardiography. Patients with malignancy, chronic kidney disease, autoimmune disease, liver cirrhosis, serious infections, glitazone use, and pregnancy were excluded. The primary end-point of the study is to determine and match the levels of endotrophin of patients and control groups. Sample size calculation was made according to power analysis (based on previous articles about endotrophin).

Twenty-seven individuals (mean age: 61.2 ± 13.1 years) from our outpatient clinics without heart failure were included as the control group. The exclusion criteria for the control group were same as for the patient group. After obtaining informed consent from all participants, data including sex, age, body mass index (BMI), smoking status, medications, the aetiology of heart failure, New York Heart Association functional classification, echocardiography findings, and laboratory tests [glucose, haemoglobin, triglyceride, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, C-reactive protein (CRP), and creatinine] were recorded. BMI was calculated as weight (kg) / height squared (m^2).

Furthermore, the participants were divided into groups according to the use of RAAS blockers and EFs (EF < 40%, EF 40–49%). Blood samples were collected from all participants. Blood samples were left to stand for 30 min at room temperature, centrifuged for 10 min at 4000 rpm, and the obtained serum was stored at −80 °C until required for analysis.

2.2. Measurement of endotrophin

All stored blood samples were thawed only once and on the analysis day. Serum endotrophin levels were measured using an enzyme-linked immunosorbent assay (ELISA); human endotrophin ELISA kits (Sunred Biological Technology Catalogue No:201–12-9305) were used.

2.3. Performance characteristics of the endotrophin assay

The linear measurement range was: 1.5–300 ng/mL. The reported intra-assay and inter-assay coefficients of variability (CVs) were > 10% and < 12%.

2.4. Ethics committee approval

Our study was approved by University of Health Sciences, Okmeydanı Training and Research Hospital's ethics committee (Date/Number of decision: 07.02.2017 / 593).

2.5. Data analysis and statistics

Variables with normal distribution are expressed as mean ± standard deviation, and categorical variables are expressed as percentages (%). The Kolmogorov-Smirnov test indicated that endotrophin levels significantly deviated from normal distribution. The independent sample *t*-test was performed for comparisons of data with normal distribution between two independent groups. For non-normally distributed data (endotrophin only), the analysis was performed using the Mann-Whitney *U* test. The correlation of endotrophin with other variables was evaluated using non-parametric Spearman's correlation analysis.

Table 1
The laboratory and demographic data of the patient and control groups.

	Heart failure patients (n = 60)	Controls (n = 27)	p
Clinical assessment			
Age (years)	64.8 ± 13.5	61.2 ± 13.1	0.25
Female, n (%)	22 (36.6)	12 (44.4)	–
Male, n (%)	38 (63.4)	15 (65.6)	–
Smoking, n (%)	21 (33)	8 (29)	0.62
BMI (kg/m^2)	27.7 ± 3.21	27.4 ± 1.66	0.586
Ischemic heart disease, n (%)	40 (66.6)	0	< 0.0001
Hypertension, n (%)	33 (55)	13 (48)	0.55
Hyperlipidaemia, n (%)	6 (10)	2 (7.4)	0.69
Peripheral vascular disease, n (%)	4 (6.6)	0	0.17
Diabetes mellitus, n (%)	23 (38.3)	6 (22.2)	0.14
Systolic blood pressure (mm Hg)	117.7 ± 26.5	126.9 ± 13.1	0.03
Diastolic blood pressure (mm Hg)	72.1 ± 14.4	77.6 ± 6.93	0.01
NYHA stage	n (%)		
I	2 (3.3)		
II	12 (20)		
III	29 (48.3)		
IV	17 (28.3)		
The aetiology of heart failure	n (%)		
Ischemic heart disease	44 (73)		
Hypertensive cardiomyopathy	8 (13)		
Dilate cardiomyopathy	6 (10)		
Unknown	2 (4)		
Laboratory tests			
Glucose (mg/dL)	103.9 ± 18.09	94.8 ± 12.9	0.07
CRP (mg/L)	35 ± 41.9	11.8 ± 6.1	0.005
Total cholesterol (mg/dL)	143.9 ± 41.4	201.6 ± 34.2	< 0.0001
LDL cholesterol (mg/dL)	87 ± 32.8	123.2 ± 38.6	< 0.0001
HDL cholesterol (mg/dL)	38 ± 18.2	54 ± 13.8	< 0.0001
Triglyceride (mg/dL)	116.2 ± 61.6	173.1 ± 149.1	0.07
Haemoglobin (g/L)	12.06 ± 2.1	13.5 ± 1.47	< 0.0001
Ejection fraction (%)	32.4 ± 9.47	58.8 ± 2.74	< 0.0001
Creatinine (mg/dL)	0.96 ± 0.30,96 ± 0,3	0.86 ± 0.17	0.07
Treatment			
ACE inhibitor, n (%)	17 (28.3)	1 (1.6)	0.002
Ramipril 5 mg	9	0	
Perindopril 5 mg	2	1	
Ramipril 2.5 mg	6	0	
ANGII receptor antagonist, n (%)	9 (15)	11 (18.3)	0.008
Olmesartan 20 mg	1	0	
Candesartan 16 mg	3	2	
Losartan 80 mg	3	0	
Valsartan 320 mg	2	3	
Irbesartan 160 mg	0	1	
Telmisartan 80 mg	0	2	
Statin, n (%)	12 (20)	0	0.012
Diuretic, n (%)	51 (85)	0	< 0.0001
ASA, n (%)	19 (31.6)	0	0.001
Mineralocorticoid receptor antagonist, n (%)	16 (26.6)	0	< 0.0001
Spironolactone 25 mg	16	0	
Beta-blockers, n (%)	44 (73.3)	0	< 0.0001
Digital, n (%)	13 (21)	0	0.009

BMI: Body mass index, CRP: C-reactive protein

Table 2
Serum endotrophin levels of the patient-control group and subgroups.

Endotrophin levels	Heart failure patients (EF < 50%)	Controls	*p*
	n = 60	n = 27	
	75.2 ± 107.2	96.5 ± 111.7	0.35
	Heart failure patients with reduced EF (< 40%)	Heart failure patients with mid-range EF (40–49%)	*p*
	n = 48	*n* = 12	
	63.09 ± 32.8	143.8 ± 143.2	0.07

3. Results

The laboratory, history, treatment, and demographic data of the study groups are presented in Table 1. There was a statistically significant difference in CRP, LDL, HDL, total cholesterol, haemoglobin, and EF between the patients with heart failure and the control group. CRP levels were higher and LDL, HDL, total cholesterol, haemoglobin, ejection fractions were lower in the patient group. Nevertheless, there was no statistically significant difference between patients and controls in age, smoking ratio, BMI, triglyceride, creatinine, and glucose.

Serum endotrophin levels of the patient-control group and subgroups (reduced and mid-range EF) are presented in Table 2. There was no significance difference for endotrophin levels in the patient-control groups and reduced EF–mid-range EF groups.

The patients included in the study were grouped according to the European Society of Cardiology (ESC) classification with regard to EFs. Two groups were identified according to EF: reduced (< 40%) and mid-range (40–49%). These two groups were divided according to the use of RAAS blocking drugs and their serum endotrophin levels were measured (Table 3). A total of 8 patients used both angiotensinogen-converting enzyme (ACE) inhibitors and spironolactone at the same time. There was no statistically significant difference between the RAAS blocker users and non-users in patients with heart failure with reduced ejection fraction (EF < 40%) (p = .289). In the heart failure with mid-range EF group, the endotrophin levels of RAAS blocker users were significantly lower (p = .03). Additionally, endotrophin levels were found significantly lower (p = .03) among patients in the control group with hypertension using RAAS blockers.

The results of correlation analyses between endotrophin levels and other variables are summarized in Table 4. There was a positive correlation determined between the endotrophin levels of all participants and total cholesterol.

Table 4
Correlation analysis between endotrophin and the other variables (non-parametric Spearmen).

Correlation	*r*	*p*
Total cholesterol (mg/dL)	0.254	0.01
LDL cholesterol (mg/dL)	0.182	0.09
Triglyceride (mg/dL)	0.172	0.113
HDL cholesterol (mg/dL)	0.173	0.11
BMI (kg/)	0.125	0.249
Glucose (mg/dL)	0.05	0.64
EF (%)	0.17	0.11
Creatinine (mg/dL)	−0.02	0.85
LVEDD (mm)	−0.12	0.26
NYHA stage	−0.08	0.5
Age	−0.05	0.63

BMI: Body-mass index, EF: Ejection fraction, LVEDD: Left ventricle end-diastolic diameter.

4. Discussion

Left ventricular remodelling, which often starts after a myocardial infarction, may develop secondary to idiopathic cardiomyopathy or prolonged hypertension. All these adverse events bring about changes in the shape and structure of the left ventricle [9]. Although cardiac fibrosis is an important component of cardiac remodelling, it is an independent factor that leads to progression in heart failure, regardless of aetiology [10].

Cardiac fibrosis is the major determinant factor of myocardial stretch, left ventricular contractility, and cardiac arrhythmias [11,12]. Despite the reduced EF, myocyte necrosis and regional left ventricular dysfunction activate neurohumoral mechanisms to protect stroke volume [13]. In myocardial ischemic-damaged animal models, ACE inhibitors have been shown to inhibit fibroblast proliferation, collagen production, and left ventricular remodelling [14,15]. This success of ACE inhibitors is attributed to the reduction of angiotensin 2 [16] because angiotensin 2 has an important role in cardiac fibrosis. Aldosterone, which has adverse effects on post-infarction remodelling, is another important member of this system. Some of aldosterone's effects are vasoconstriction, inhibition of cardiac noradrenaline uptake, and stimulation of cardiac fibrosis [17–19]. Brialla and Weber showed that aldosterone triggered biventricular fibrosis in rats, and it was inhibited by aldosterone inhibitors [20].

In adults, the myocardial ECM predominantly consists of type I and type III collagen [22]. Type IV, V, and type VI are the remaining collagens of the collagen network [2]. In a recent study, it was determined that cardiac fibroblasts induced by angiotensin 2 produced type VI collagen, and the amount of type VI collagen increased during the development of remodelling after myocardial infarction [5]. In addition, in the same study, type VI collagen was thought to contribute to remodelling by preparing a suitable environment for myofibroblast differentiation [5]. In another study, it was shown that the absence of type VI collagen preserved long-term cardiac performance after myocardial infarction [22].

Endotrophin is a by-product produced by the contribution of matrix metalloproteinases in the posttranslational phase of the synthesis of the alpha 3 chain of type VI collagen [7]. Although it is mostly found in adipose tissue, it can be found and synthesized in many tissues. Endotrophin stimulates TGF-B1 secretion, adipose tissue fibrosis, angiogenesis, and inflammation [7]. Another important effect of endotrophin is increased fat tissue inflammation and fibrosis, leading to obesity-related systemic insulin resistance [7,10,24]. On the other hand, a recent study showed that procollagen VI (endotrophin) levels were found to be higher in women with angina pectoris [23]. Several studies have shown

Table 3
Serum endotrophin levels between groups according to RAAS blocker use.

	n	Endotrophin levels of RAS blocker users (ng/mL)	n	Endotrophin levels of non-users (ng/mL)	*p*
EF < 40%	27	58.8	21	68.5	0.289
EF 40–49%	7	55.4	5	278.9	0.03

EF: Ejection fraction, RAAS: Renin-angiotensin-aldosterone system

that TGF-B1 in myocardium plays an important role in collagen biosynthesis and myocardial fibrosis [21]. Endotrophin, a by-product of type VI collagen in the myocardium, has an effect on fibrosis, TGF-B1 secretion, insulin resistance may play a possible role in cardiac fibrosis. The correlation of type VI collagen and cardiac fibrosis has been determined in the literature [5].

In our study, there was no significant difference between patients with heart failure with reduced EF and the control group in terms of endotrophin levels. This may be because the cardiac fibrosis of the patients had developed but not continued when serum samples were taken from the patients or it may be because of the effects of the use of different drugs. It is a fact in the literature that cardiac fibrosis decreases in patients who receive RAAS blockers. In one study, ACE inhibitors were shown to reduce fibroblast proliferation, collagen production, and prevent left ventricular remodelling [4,5]. This success of ACE inhibitors is attributed to the reduction of angiotensin 2, which supports cardiac fibrosis [3]. Also, aldosterone has fibrotic effects [17–19]. The patients were divided into groups according to use of RAAS blockers to account of the effects of these drugs. In groups with EF 40–49%, endotrophin levels were significantly lower in RAAS-blocker users. This suggests that there may be a possible association between endotrophin release and RAAS. Previous studies have shown that angiotensin 2-stimulated cardiac fibroblasts produce type VI collagen [5]. It was also determined that in the pathophysiology of remodelling after myocardial infarction, type VI collagen was increased [5]. This suggests that low levels of endotrophin, a profibrotic by-product of type VI collagen, may be associated with the use of medication that reduces angiotensin 2 production in patients with heart failure with mid-range EF. No significant difference was found in the group with EF 40% and below. In this group with a very low EF, the number of myofibroblasts that may respond to RAAS blockade secondary to possible diffuse fibrosis may be low. Future molecular studies may direct the role of RAAS blockade in inhibiting fibrosis with the association of endotrophin.

In this study, a positive correlation was detected between endotrophin and total cholesterol. Karsdal et al. reported a positive correlation between BMI, triglyceride levels, and serum endotrophin levels, in contrast to our study [6]. This may be due to the use of drugs that affected the lipid profile of the study group. This difference may also be due to the effect of RAAS blockers on endotrophin level.

4.1. Limitations of the study

This study is limited by the small sample size. The other limitations of the current study are the non-parametric distribution of the endotrophin kit and the lack of adequate knowledge in the literature about endotrophin.

5. Conclusion

We investigated serum endotrophin levels in patients with heart failure. It is the first study in the literature on this subject.

Endotrophin levels were significantly lower in RAAS-blocker users of the mid-range heart failure group. Also, the levels were found to be significantly lower in the RAAS-blocker users of the control group. This study and future molecular studies will further clarify the role of endotrophin in heart failure and cardiac remodelling, and all of these with relation to RAAS. The effect of endotrophin on cardiomyopathy and fibrosis developing in patients with hypertension will be the subject of further research.

Declaration of interest

There are no conflicts of interest related to the manuscript.

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