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Relationship between fortilin levels and coronary ischemia in heart failure

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ABSTRACT

Objectives: Fortilin is a multifunctional protein that protects cells against apoptosis. We aimed to investigate the levels of fortilin in patients with heart failure.

Methods: Patients with ejection fraction (EF) below 40% were divided into two groups according to coronary angiography results: those with ischemic heart failure (Group 1) and those with non-ischemic heart failure (Group 2). Patients with normal anatomy and EF over 50% were included in the control group (Group 3).

Results: A total of 119 patients were prospectively included in the study. A total of 81 patients (41 patients with ischemic heart failure and 40 patients with non-ischemic heart failure) were included in the heart failure group. 38 patients with EF >50 and normal coronary anatomy were included in the control group. There was no significant difference in serum fortilin levels between the study groups (Group 1: 5.5±2.6 ng/mL, Group 2: 6.1±3.8 ng/mL, and Group 3: 5.6±3.6 ng/mL; P=0.693). Fortilin did not show a correlation with any other variables.

Conclusion: In our study, there was no significant difference in fortilin levels between the groups, and no relationship was found between coronary ischemia and fortilin levels in heart failure.

Keywords: Heart failure, fortilin, ejection fraction, pro-BNP

Cellular changes, apoptosis, and various factors also play a role in the pathogenesis of heart failure. In the early stages of heart failure, cardiac physiology attempts to adapt through several compensatory mechanisms to maintain cardiac output and meet systemic demands. With increased wall tension, the myocardium tries to compensate for the loading findings that increase wall tension and deterioration through cardiac remodeling. This paradoxical need for increased cardiac output eventually leads to myocardial cell death and apoptosis to meet myocardial demand. As apoptosis continues, the reduction in cardiac output with increased demand leads to an ongoing cycle of increased neurohumoral stimulation and maladaptive hemodynamic and myocardial responses [1]. Only recently has slowing or reversing remodeling become a goal of treatment for capillary insufficiency. Left ventricular diastolic and end-systolic volume and EF data; it support the beneficial effects of therapeutic agents such as angiotensin-converting enzyme inhibitors and beta-adrenergic blocking agents on the remodeling process. These agents also provide benefits in terms of morbidity and mortality [2].

Pathological remodeling may occur following

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pressure overload, volume overload, or cardiac injury and may be localized or widespread depending on etiology. In each of these processes, remodeling can change from a compensatory process to a maladaptive one. Other components include the interstitium, fibroblasts, collagen, and the coronary vasculature. Remodeling and myocyte hypertrophy are associated with a series of cellular changes that underlie structural remodeling, including myocyte loss due to apoptosis or necrosis, fibroblast proliferation, and fibrosis [3].

Many forms of myocardial remodeling are associated with increased expression of fetal heart-specific genes. Additionally, post-translational modifications can affect protein function and quantity, altering many aspects of cellular homeostasis. Fortilin, also called histamine-releasing factor, is a 172 amino acid polypeptide that was originally reported to be abundantly expressed in tumor cells. Fortilin is considered to be present in the cytosol, nucleus, mitochondria, and blood and plays a crucial role in normal physiological function. It has been reported to protect cells against apoptosis and promote cell proliferation. First, fortilin binds to a tumor suppressor protein (p53). Binding both destabilizes the protein and prevents it from transcriptionally activating pro-apoptotic molecules such as B-cell lymphoma gene 2-related proteins [4-7].

Fortilin directly binds to and negatively regulates tumor suppressor protein 53 and endoplasmic reticulum stress processing protein (IRE1α), both of which promote cardiomyocyte loss and subsequent capillary rarefaction. Second, fortilin cooperates with B-cell lymphoma gene 2 family proteins to inhibit apoptosis. It binds to the anti-apoptotic myeloid cell leukemia 1, a B-cell lymphoma gene 2 family protein. Fortilin also binds another anti-apoptotic B-cell lymphoma 2 protein and protects against apoptosis. Third, fortilin binds calcium, clears it intracellularly, sequesters it, and protects cells against calcium-dependent apoptosis. Fortilin exhibits cytokine-like functions, including histamine release, induction of cytokines and chemoattractants, enhancement of B cell proliferation, and immunoglobulin production during late-phase allergic inflammation [8-10]. Fortilin expression increases in human atherosclerotic lesions. Previous studies demonstrated that fortilin-deficient mice developed decreased atherosclerotic lesions associated with increased macrophage apoptosis, and decreased macrophage infiltration [11]. Fortilin may contribute to the progression of atherosclerosis. Blood fortilin levels were suggested to be a promising biomarker for apoptosis [12]. Heart failure is associated with apoptosis. Preventing apoptosis may be effective in the treatment and progression of heart failure. Perhaps, due to low fortilin levels, heart failure may progress faster in some patient groups and the prognosis may be worse. For this purpose, in this study, we aimed to examine fortilin levels in chronic heart failure patients.

METHODS

Patients presenting to our institution's outpatient clinic were prospectively enrolled. Patients with ischemic and non-ischemic heart failure who had undergone coronary angiography were included. Healthy individuals with no significant lesions on coronary angiography were included as the control group. Detailed cardiovascular examination and transthoracic echocardiography were performed for each patient during the outpatient evaluation. Demographic data were recorded. Blood pressure was measured in all patients, followed by fasting morning blood samples. Patients with ejection fraction (EF) below 40% were divided into two groups according to coronary angiography results: those with ischemic heart failure (Group 1) and those with non-ischemic heart failure (Group 2). Patients with left ventricular ejection fraction less than 40% on echocardiography and coronary artery stenosis less than 50% on coronary angiography were included in Group 2. Patients with normal anatomy and EF over 50% were included in the control group (Group 3). Three groups were formed with individuals aged between 18 and 75 years, ensuring similar gender and age characteristics. The GE Vivid S5 echocardiography machine was used for transthoracic echocardiographic evaluation of the patients. The Modified Simpson method was used to obtain quantitative results in evaluating left ventricular dimensions, mass, and contractile functions. EF values were calculated using this method.

Exclusion criteria were as follows: patients with LVEF between 40-50%, rheumatic diseases, acute inflammation, liver and kidney failure (patients with glomerular filtration rate <15 mL/h), patients with thyroid hormone disorders, the first 40 days after acute

myocardial infarction, the first 90 days after bypass and percutaneous coronary intervention, left ventricular hypertrophy, moderate-to-severe aortic and mitral stenosis, history of prosthetic valve surgery, chronic obstructive pulmonary disease, asthma, lung parenchymal diseases, and patients with a history of stroke.

Fortilin Analysis Method and Levels

Samples were centrifuged at 2500 rpm for 15 minutes. The obtained serum samples were aliquoted into microcentrifuge tubes and stored at -80°C until the day of analysis. On the day of the study, samples were brought to room temperature. Serum fortilin levels were measured using a commercial kit and the ELISA (Enzyme-Linked Immunosorbent Assay) method. Mass measurements were made using the ELISA principle.

Statistical Analysis

Statistical analyses were performed using IBM SPSS version 20 (IBM Corp., Armonk, NY, USA). Numerical variables were presented as mean ± standard deviation or median (min-max), and categorical variables were presented as number and percentage (n, %). Whether the variables followed a normal distribution was evaluated using the Kolmogorov-Smirnov test. For three-group comparisons, parametric variables with normal distribution were evaluated using ANOVA, while non-parametric variables with normal distribution were evaluated using the Kruskal-Wallis test. If there was a difference between the three groups in the ANOVA test, the Tukey test was used for pairwise comparisons between groups. In the Kruskal-Wallis test, if there was a difference between the three groups in non-parametric variables, Dunn's test was used, and categorical variables were compared using the Chi-square test. The Bonferroni test was used for pairwise comparisons in categorical tests. Variables with differences between groups were included in correlation analysis to investigate the correlations of pro-BNP and fortilin. A P-value <0.05 was considered statistically significant.

RESULTS

A total of 119 patients were prospectively included in the study. A total of 81 patients (41 patients with ischemic heart failure and 40 patients with non-ischemic heart failure) were included in the heart failure group. Thirty-eight patients with EF >50 and normal coronary anatomy were included in the control group. There was no difference in gender, body mass index, diabetes, smoking status, and family history rates between the groups (Table 1). However, patients in the control group were younger (Group 1: 65.3±4.6, Group 2: 63.5±10.8, and Group 3: 55.3±10.1; P<0.001). There was a difference in hypertension between the ischemic heart failure and control groups (Group 1: 31 [75.6%], Group 2: 24 [60%], and Group 3: 18 [47.4%]; P=0.035). The rates of atrial fibrillation were similar between both heart failure groups but significantly lower in the control group (Group 1: 10 [24.4%], Group 2: 13 [32.5%], and Group 3: 1 [%2.6]; P=0.003). In terms of medical treatment, the use of beta-blockers, mineralocorticoid receptor antagonists or angiotensin receptor blockers and diuretics was higher in group 1 than in the other groups. Additionally, statin use was higher in group 1 because the patient had coronary artery disease. In groups 1 and 2, EF and Tricuspid Annular Plane Systolic Excursion (TAPSE) were lower, while left ventricular diameters and Left Ventricular Mass Index (LVMI) were higher compared to the control group (Table 2).

There were significant differences in urea, creatinine, pro-BNP, and CRP levels between both heart failure groups and the control group (Table 3). There was no significant difference in serum fortilin levels between the study groups (Group 1: 5.5±2.6, Group 2: 6.1±3.8, and Group 3: 5.6±3.6; P=0.693). Fortilin did not show a correlation with any other variables. A moderate positive correlation was observed between Pro-BNP and GFR, creatinine, low-density lipoprotein cholesterol (LDL-C), left ventricular systolic diameter (LVSD), and left ventricular diastolic diameter (LVDD) (Table 4).

DISCUSSION

In our study, Fortilin was found to be similar in heart failure and normal individuals. When compared between patient groups, although urea, glomerular filtration rate, and creatinine values were slightly higher in the ischemic heart failure groups, this did not affect fortilin levels. Although mild increases in urea and cre-

Table 1. Clinical characteristics of study groups

Variables	Ischemic HF Group (n=41)	Non-ischemic HF Group (n=40)	Control Group (n=38)	P value
Age (years)	65.3±4.6	63.5±10.8	55.3±10.1	**<0.001**
Male, n (%)	32 (78)	25 (62.5)	21 (55.3)	0.090
Body Mass Index (kg/m²)	27.6±3.6	27.7±3.9	28.5±3.6	0.481
Diabetes, n (%)	23 (56.1)	18 (45)	13 (34.2)	0.148
Hypertension, n (%)	31 (75.6)	24 (60)	18 (47.4)	**0.035**
Smoking, n (%)	22 (53.7)	15 (37.5)	13 (34.2)	0.168
Family History, n (%)	11 (26.8)	7 (17.5)	3 (7.9)	0.090
HF Duration (years)	5 (2-20)	4.2 (1-10)	-	0.203
Functional capacity, NYHA	1.8±0.8	2.0±0.9	-	0.921
AF, n (%)	10 (24.4)	13 (32.5)	1 (2.6)	**0.003**
Medications				
Beta-blocker, n (%)	40 (97.6)	30 (75)	23 (60.5)	**<0.001**
Digoxin, n (%)	5 (12.2)	7 (17.5)	-	0.502
ACEI and ARB, n (%)	31 (75.6)	23 (57.5)	19 (50)	0.054
Diuretic, n (%)	30 (73.2)	23 (57.5)	3 (7.9)	**<0.001**
MRA, n (%)	24 (58.5)	22 (55)	1 (2.6)	**<0.001**
Anticoagulant, n (%)	11 (26.8)	13 (32.5)	2 (5.3)	0.009
Antiplatelet, n (%)	33 (80.5)	30 (75)	30 (78.9)	0.521
Sacubitril-valsartan, n (%)	4 (9.8)	9 (22.5)	-	0.118
SGLT2 inhibitors, n (%)	3 (7.3)	6 (15)	2 (5.3)	0.290
Statin, n (%)	33 (80.5)	20 (51.3)	27 (71.1)	**0.018**

ACEI=Angiotensin-Converting Enzyme Inhibitor, ARB=Angiotensin Receptor Blocker, AF=Atrial fibrillation, HF=Heart Failure, MRA=Mineralocorticoid Receptor Antagonist, SGLT2=Sodium-Glucose Cotransporter 2 Inhibitor

Table 2. Comparison of echocardiographic and angiographic variables among groups

Variables	Ischemic HF group (n=41)	Non-ischemic HF group (n=40)	Control group (n=38)	P value
EF (%)	34 (24-40)	31.5 (15-40)	59.9 (50-70)	**<0.001**
Left ventricular diastolic diameter (cm)	58±6.2	58±7.7	46.4±3.7	**<0.001**
Left ventricular systolic diameter (cm)	47.2±8	47±7.6	31.4±4.3	**<0.001**
TAPSE (mm)	18.6±2.9	19.3±4.1	25±4	**<0.001**
LVMI (g/m^2)	204±76	131±29	112±39	**<0.001**
Systolic pulmonary artery pressure (mmHg)	35±12	31±13	--	0.317

EF=Ejection Fraction, HF=Heart Failure, TAPSE=Tricuspid Annular Plane Systolic Excursion, LVMI= Left Ventricular Mass Index

Table 3. Comparison of biochemical and hematological variables among groups

Variables	Ischemic HF Group (n=41)	Non-Ischemic HF Group (n=40)	Control Group (n=38)	P value
Fasting blood sugar (mg/dl)	102 (66-392)	105 (67-210)	103 (75-181)	**0.046**[b,c]
Urea (mg/dl)	42±12.5	37.5±12.5	31.2±10.4	**<0.001**[b]
GFR	72±21	75±24	93.9±22.6	**<0.001**[b,c]
Creatinine (mg/dl)	1.04±0.3	0.9±0.3	0.85±0.18	**0.009**[b]
Total Cholesterol (mg/dl)	159±39	164±49	176±40	0.200
HDL Cholesterol (mg/dl)	44±11	44±13	48±11	0.209
LDL Cholesterol (mg/dl)	80±31	93±37	102±36	**0.019**[b]
Triglycerides (mg/dl)	152±15	136±71	128±53	0.268
CRP (mg/dl)	5.5 (2.6-10.5)	10.2 (0-59.5)	3.5 (0.3-20)	**0.015**[c]
Uric Acid (mg/dl)	5.9±1.8	6.3±2.5	5.07±1.4	**0.018**[c]
Hemoglobin (mg/dl)	13.1±1.7	13.4±3.4	13.3±1.9	0.857
Hematocrit (%)	40±4.5	39±4.2	40±5.5	0.374
Platelet Count (n)	242±52	221±49	233±51	0.205
Pro-BNP (ng/mL)	892 (20-8750)	2752 (10-35000)	104 (10-852)	**<0.001**[b,c]
Fortilin (ng/mL)	5.5±2.6	6.1±3.8	5.6±3.6	0.693
HbA1c (mmol/mol)	7±1.6	6.3±1.6	5.6±0.7	**<0.001**[b,c]

BNP=Brain Natriuretic Peptide, CRP=C-Reactive Protein, HF=Heart Failure, LDL-C=Low-Density Lipoprotein Cholesterol, GFR= Glomerular Filtration Rate, HDL-C= High-Density Lipoprotein Cholesterol

[a]difference between ischemic heart failure and non-ischemic heart failure groups (P<0.05)

[b]difference between ischemic heart failure and control group (P<0.05)

[c]difference between non-ischemic heart failure and control group (P<0.05)

atinine are expected in heart failure, we thought that this difference was not of clinical importance because we excluded patients with a glomerular filtration rate below 15 in our study. Additionally, HbA1c levels were slightly higher in the ischemic vascular insufficiency group. We thought that this was because diabetes is an important CAD risk factor and we evaluated that blood sugar control was not better in the ischemic vascular insufficiency group. Interestingly, Pro-BNP values were higher in the non-ischemic vascular insufficiency group than in the ischemic vascular insufficiency group. As it is known, heart failure worsens in the presence of atrial fibrillation. We attributed this increase in the non-ischemic heart failure group to the higher atrial fibrillation rates in this group.

There are no studies on blood levels of fortilin in the literature on heart failure. When we started the study, we assumed that it might affect slowing down cardiomyocyte loss, as far as we understood from animal experiments [11]. Interestingly, fortilin did not correlate with any study parameters. However, in clinical studies, fortilin is expected to reduce cardiomyocyte loss due to its anti-apoptotic properties. We did not see this effect. The fact that fortilin is incompatible with other cardiological parameters makes us think that more detailed studies should be conducted on fortilin. That's why we did a study, but we couldn't find a relationship. If fortilin correlated with pro-BNP, it would make our study meaningful. However, there was no correlation between them. Fortilin also did not correlate with EF and heart diameters. As is known, chronic heart failure is the main cause of programmed death. Factors that trigger programmed death are important. Although fortilin is expected to slow programmed death, no data is showing this in clinical trials. Interestingly, triggering properties of fortilin have been de-

Table 4. Correlation of Pro-BNP and fortilin with other variables

Variable	Pro-BNP		Fortilin	
	r	P value	r	P value
Age	0.088	0.586	0.006	0.970
Fasting blood sugar	-0.062	0.702	-0.118	0.462
Urea	0.226	0.156	-0.125	0.438
GFR	0.468	**0.002**	0.028	0.864
Creatinine	0.355	**0.023**	-0.111	0.490
LDL-C	0.524	**<0.001**	0.247	0.120
C-reactive protein	0.237	0.136	-0.174	0.276
Uric Acid	0.148	0.357	0.200	0.209
HgA1C	-0.030	0.852	-0.154	0.335
TAPSE	-0.364	0.019	-0.038	0.812
LVMI	0.047	0.770	0.029	0.859
LVSD	0.383	**0.013**	0.072	0.653
LVDD	0.368	**0.018**	0.008	0.959
EF	-0.199	0.299	-0.123	0.443
Fortilin	-0.072	0.654	-	-

EF=Ejection Fraction, LDL-C=Low-Density Lipoprotein Cholesterol, GFR=Glomerular Filtration Rate, LVSD=Left Ventricular Systolic Diameter, LVDD=Left Ventricular Diastolic Diameter, LVMI=Left Ventricular Mass Index, TAPSE=Tricuspid Annular Plane Systolic Excursion, HgA1C=Glycated hemoglobin A1C

tected in hypertension and atherosclerosis [13, 14]. This is a confusing situation.

The groups were determined as ischemic vascular insufficiency, non-ischemic vascular insufficiency, and control group. Although care was taken to ensure that the individuals included in the groups were balanced in terms of demographic characteristics, the vascular insufficiency groups were more balanced in terms of demographic characteristics, while the age was younger and the rates of atrial fibrillation and hypertension were lower in the control group. Including gender-balanced numbers of individuals in both heart failure groups proved challenging. One of the difficulties encountered in patient selection was that connective tissue diseases were accepted as exclusion criteria in individuals in the non-ischemic vascular insufficiency group.

Limitations

Although the patient group in our study was small, the lack of a relationship does not reduce the value of our study. For this reason, it is thought that more detailed histopathological studies are needed rather than clinical studies to evaluate the relationship of fortilin with vascular insufficiency.

CONCLUSION

There was no significant difference in fortilin levels between the groups, and no relationship was found between coronary ischemia and fortilin levels in heart failure. Further studies with larger sample sizes are needed to investigate the role of fortilin in heart failure pathophysiology and its potential as a therapeutic target.

Ethics Committee Approval

This study was approved by Tekirdağ Namık Kemal University, Non-invasive Clinical Research Ethics Committee (Decision No: 2921.277.11.21, Date: 30.11.2021

Authors' Contribution

Study Conception: ŞA; Study Design: ŞA; Supervision: AD, CA; Funding: N/A; Materials: SG; Data Collection and/or Processing: SG; Statistical Analysis and/or Data Interpretation: AD, CA; Literature Review: AD, SG; Manuscript Preparation: AD, CA and Critical Review: ŞA, CA, AD.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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