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Research Article

Evaluation of the effects of various treatment modalities on angiogenesis in heart failure with reduced ejection fraction and heart failure mid-range ejection fraction patients without chronic kidney disease via angiostatin

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Abstract:

Introduction: It is called as heart failure with reduced ejection (HFrEF) while ejection fraction (EF) is lower than 40%. Patients with EF=40-50% is called as heart failure with mid-range ejection fraction (HFmrEF) which is considered as a subgroup of heart failure with preserved ejection fraction (HFpEF) rather than HFrEF. Angiostatin inhibits the proliferation of smooth muscles, endothelial cells, and mesenchymal stem cells. In this study, we aimed to investigate the clinical significance of angiostatin in HFrEF and HFmrEF patients without chronic kidney disease (CKD).

Body text: A total of 62 people consisting of patients with a diagnosis of HFrEF and HFmrEF without CKD (n = 25) and healthy (n = 37) subjects were included in this study. Blood samples were obtained and serum angiostatin, plasma N-terminal Pro-BNP analysis, and transthoracic echocardiography were performed.

Results and Discussion: The angiostatin level of HFrEF and HFmrEF group was significantly higher than the control group (94,32 (58,7-282,1); 47,14 (18,8-100,2); p<0.001; respectively). Average angiostatin level of HFrEF and HFmrEF patients using calcium chanel blocker (CCB) was significantly higher than the HF patients without CCB (200,4 (79,1 - 282,1); 83,5 (58,7 - 228,7; p = 0.021; respectively). Average angiostatin level of HFrEF and HFmrEF patients using spironolactone was significantly lower than the HF patients without spironolactone use (61,8 (58,7 - 64,1); 133,8 (62,3 - 282,1); p = 0.027; respectively).

Conclusion: Our study is the first study in this area. Angiostatin may be an important marker in HFrEF and HFmrEF patients. Use of spironolactone may induce angiogenesis and apoptosis and CCB may inhibit angiogenesis in HFrEF and HFmrEF patients. Further studies are required on this subject.

Keywords: Angiogenesis; heart failure; angiostatin, spironolactone

Introduction

Heart Failure is characterized as a clinical situation which consists of marked symptoms and signs caused by a cardiac abnormality, and it is called as heart failure with reduced ejection (HFrEF) while ejection fraction (EF) is lower than 40%. Patients with EF=40-50% is called as heart failure with mid-range ejection fraction (HFmrEF) which is considered as a subgroup of heart failure with preserved ejection fraction (HFpEF) rather than HFrEF. [1, 2]. Neurohumoral mechanisms have critical roles after the impairment of cardiac functions [3, 4].

For the angiostatin formation as a proteolytic molecule, plasminogen lysis metalloproteinase type II (MMP-2) and IX (MMP-9) [5]. Angiostatin inhibits the proliferation of smooth muscles, endothelial cells, and mesenchymal stem cells [5, 6]. It has been shown that, angiostatin level is increased in HFrEF patients with dilated cardiomyopathy [7]. Also, according to the several kinds of literature, significantly higher levels of angiostatin in chronic kidney disease (CKD) was demonstrated [8, 9].

The studies about angiostatin in HFrEF and HFmrEF patients are a limited number of patients and experimental animal studies [8, 9]. Also, we speculate that CKD may affect the levels of angiostatin in HF patients with CKD. However, studies reporting the association among circulating angiostatin levels and clinical information of patients with HFrEF and HFmrEF without CKD are very limited. From this point of view, we hypothesized that increased angiostatin formation may inhibit angiogenesis in HFrEF and HFmrEF patients without CKD and correlated them with other clinical features and treatments of these patients.

Body Text

A total of 62 people consisting of patients with a diagnosis of HFrEF and HFmrEF (n = 25), healthy (n = 37) in Gulhane Education and Research Hospital, Ankara, Turkey were included in the study. All of the patients underwent transthoracic echocardiography due to HF diagnosis in 2011, 2012 and 2013. The local ethics committee of Gulhane Education and Research Hospital has approved this study protocol. All procedures were according to the Helsinki Declaration of 1975 which revised in 2008 and all procedures were convenient to the ethical standards of the responsible committee on human experimentation (institutional and national). The patient group was older than 18 years old with HFrEF, HFmrEF, and EFs were under 40 and between 40-50 percent, respectively. The diagnosis of HFrEF and HFmrEF had been built according to the symptoms, signs of patients (European Society of Cardiology; 2016), echocardiographic findings and N-Terminal proBNP levels. The patients with infection, acute or chronic inflammatory disease, acute and chronic kidney disease, high sedimentation or c reactive peptide (CRP), have or suspected malignancy, chronic pulmonary obstructive disease, and cerebrovascular accident were excluded from the study.

Glomerular filtration rate (GFR) for the diagnosis of chronic kidney disease was determined by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [10]. Sera angiostatin levels were measured by using ELISA kits (Eastbiopharm, Hangzhou, China, catalog no: CK-E90461), Synergy HT plate reader (Bio-Tek Instruments Inc, Winooski, VT, USA). N-Terminal proBNP levels were detected in the plasma as quantitative by a magnetic immunochromatographic technique using MICT system. Clinical characteristics as natural history, medications and laboratory results of patients were compared by angiostatin.

We used the SPSS 16.0 software package for the evaluation of the results. For the descriptive statistics, discontinuous variables were indicated as numbers and percentages (%); continuous variables were indicated as a mean ± standard deviation. We used the Kolmogorov Smirnov test due to the evaluation of the normality of the data. Also, we used the chi-square test for categorical data. We used the Student's t-test for the continuous variables which distributed normally also for continuous variables which did not distribute normally we used Mann-Whitney U-test. A P value of less than 0.05 was considered significant.

Results and Discussion

The differences between patients with HFrEF, HFmrEF and the control group were analyzed and the demographics and clinical features of the study population together with the laboratory findings are presented in Table 1. In accordance with this, the average age of patients with HFrEF, HFmrEF patients and controls were similar. There were also no statistically significant differences among the groups in terms of gender, body mass index (BMI), hemoglobin, platelet, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, low-density lipoprotein (LDL), triglycerides (TG), total cholesterol, sedimentation, CRP (Table 1).

Pro BNP, serum angiostatin, white blood cell (WBC) and glucose levels were significantly higher in patients with HFrEF and HFmrEF than control group while EF was significantly lower in patients with HFrEF and HFmrEF than the control group (P<0.05 for all) (Table 1).

Table 1. Comparison of demographic features and clinical laboratory results of control group, HFrEF and HFmrEF group

	Control (n=37)	Patients (n=25)	P
Age, year	72,00 (49-88)	74,00 (55,0-88,0)	0. 595
Gender, F/M	17/20	14/11	0. 437
BMI, kg/m²	27,94 (16,4-39,2)	26,99 (19,5-40,0)	0. 260
Glucose (Fasting), mg/dL	98,00 (76,0-137,0)	126 (20-332)	<0.001
Urea, mg/dL	35,00 (5,15-92,00)	43,00 (4,00-70,00)	0.07
Creatinin, mg/dL	0,94 (0,61-1,35)	1,10 (0,64-1,3)	0.491
AST, U/L	22,00 (8,0-42,0)	23,00 (6,0-201,0)	0.236
ALT, U/L	16,00 (3,0-40,0)	19,00 (7,0-83,0)	0.206
Hemoglobin, g/dL	12,70 (10,20-16,23)	12,18 (11,00-16,63)	0.725
Platelet, 10^3/mm³)	278(91-430)	230 (77-481)	0. 173
White Blood Cell, /mm³	6000 (1700-17000)	7600 (4300-20600)	0. 003
Sedimentation, mm/h	15,00 (5,0-92,0)	19,00 (9,0-34,0)	0.724
CRP, mg/L	1,30 (,20-8,30)	1,60 (0,70-9,10)	0.277
NT-ProBNP,pg/mL	120,00 (35-410)	5705,00 (778-17857)	<0.001
EF, %	63,00 (50-67)	30,00 (20-45)	<0.001

LDL, mg/dL*	120,00 (53,0-183,0)	120,00 (56,0-213,0)	0.909
Triglyceride, mg/dL	134,00 (34,0-280,0)	120,00 (40,0-193,0)	0.089
HDL, mg/dL	45,00 (32,0-77,0)	40,00 (18,0-56,0)	0.008
T.Cholesterol, mg/dL*	201,00 (121,0-271,0)	184,00 (41,0-293,0)	0.060
Angiostatin, ng/mL	47,14 (18,8-100,2)	94,32 (58,7-282,1)	<0.001

*Variables, which are not normally distributed, were given as median (minimum, maximum)

ALT:Alanine aminotransferase, AST: Aspartate aminotransferase, BMI: Body Mass Index, CRP: C-reactive protein, EF: Ejection fraction, GFR: Glomerular filtration rate (estimated by CKD-EPI formula),HDL:High density lipoprotein, HFmrEF: Heart failure mid-range ejection fraction patient, HFrEF: Heart failure with reduced ejection fraction patient, LDL: Low density lipoprotein, NT-ProBNP: N Terminal Pro Brain Natriuretic Peptit, T. Cholesterol: Total Cholesterol

Also, there were no significant results of the correlation analyzes among angiostatin and pro-BNP, WBC, glucose, EF (Table2).

Table 2.The correlation of angiostatin levels with clinical features which are found to be significantly different in HFrEF and HFmrEF group

	Angiostatin	
	r	p
EF	0. 206	0. 324
NT-ProBNP	0. 210	0. 314
White Blood Cell	0. 224	0. 281
Glucose	0.057	0.787
HDL	0. 387	0. 056

EF: Ejection Fraction, HFmrEF: Heart failure mid-range ejection fraction patient, HFrEF: Heart failure with reduced ejection fraction patient, NT-ProBNP: N Terminal Pro Brain Natriuretic Peptit, HDL: High Density Lipoprotein.

The average angiostatin levels of the HFrEF and HFmrEF patients with diabetes mellitus (DM), coronary artery disease (CAD), hypertension (HT) and atrial fibrillation (AF) were not more than the HFrEF and HFmrEF patients without these diseases (Table 3). The average angiostatin levels of HFrEF and HFmrEF patients receiving angiotensin-converting enzyme (ACE) inhibitor, angiotensin receptor blocker (ARB), beta blocker, acetylsalicylic acid (ASA), enoxaparin, warfarin, statin, and insulin were not more than the HFrEF and HFmrEF patients who did not receive these medications significantly in HFrEF and HFmrEF patient group while average angiostatin level of HFrEF and HFmrEF patients calcium channel blocker (CCB) receiving was significantly higher than the HFrEF and HFmrEF patients without CCB using. Contrary average angiostatin level of HFrEF and HFmrEF patients spironolactone receiving was significantly higher than the HFrEF and HFmrEF patients without spironolactone receiving. The average angiostatin levels of all HFrEF and HFmrEF patients with or without disease were significantly more than the control group (Table 3).

Table 3. Comparison of angiostatin levels between HFrEF, HFmrEF group and control group

		Angiostatin			
	N	Mean ± SD		P¹	P²
Control	37	47,14 (18,8-100,2)			
DM (-)	12	82,9 (61-282,1)		0. 38	< 0,001
DM (+)	13	133,8(58,7-260,6)			
CAD (-)	18	112,8 (58,7-282,1)		0. 43	0,031
CAD (+)	7	84,2 (61-225,6)			
HT (-)	7	94,3 (58,7-228,7)		0.44	< 0,001
HT (+)	18	107,8 (62,3-282,1)			
AF (-)	19	133,8 (58,7-260,6)	83,8 (62,6-	0.53	0,029
AF (+)	6	282,1)			
ACE Inh or ARB(-)	14	84,2(61-234,1)		0.109	< 0,001
ACE Inh or ARB(+)	11	174,5(58,7-282,1)			
Spironalactone (-)	21	133,8 (62,3-282,1)		0.027	0,667
Spironalactone (+)	4	61,8 (58,7-64,1)			
Beta Blocker (-)	15	131,4 (61-260,6)		0.58	0,004
Beta Blocker (+)	10	83,3 (58,7-228,7)			

ASA (-)	15	131,4 (64,1-282,1)	0,747	0,006
ASA (+)	10	77,2 (58,7-260,6)		
Enoxaparin (-)	20	94,3 (58,7-282,1)	0,257	0,007
Enoxaparin (+)	5	226,3 (64,1-260,6)		
Warfarin (-)	20	112,8 (58,7-260,6)	0,768	0,029
Warfarin (+)	5	82,4 (62,6-282,1)		
Insulin (-)	19	94,3 (61-282,1)	0,811	0,589
Insulin (+)	6	129,3 (58,7-216,6)		
CCB (-)	17	83,5 (58,7-228,7)	0,021	< 0,001
CCB (+)	8	200,4 (79,1-282,1)		
Statin (-)	19	89,3 (61-282,1)	0,856	0,033
Statin (+)	6	118,6 (58,7-226,3)		

*Variables, which are not normally distributed, were given as median (minimum, maximum)

ACE Inh: Angiotensin Converting Enzyme Inhibitor, AF: Atrial Fibrillation, ARB: Angiotensin II Receptor Blocker, ASA: Acetylsalicylic acid, CAD: Coronary Artery Disease, CCB: Calcium channel blocker, DM: Diabetes mellitus, HT: Hypertension

P^1: Comparison of the accompanied diseases or medications used in HFrEF and HFmrEF group,

P^2: Comparison with control group

As far as we know, this is the first study evaluating and demonstrating the importance of serum angiostatin levels in HFrEF and HFmrEF patients without CKD clinically. Serum angiostatin levels were significantly higher in patients with HFrEF and HFmrEF than controls. Contrary, significantly lower levels of angiostatin were observed among HFrEF and HFmrEF patients with spironolactone therapy than patients without therapy.

In a clinical trial Yamahara et al. demostrated that angiostatin levels in patients with dilated cardiomyopathy were significantly higher than the control subjects [11]. Srikanth et al. created aortic stenosis on mice and demonstrated the average levels of angiostatin higher than the controls in mice with hypertrophic cardiomyopathy [12]. Our study was performed by humans with a larger amount of patients and control groups and angiostatin levels were significantly higher in the patient group.

Also, Tianfu et al. reported the significant higher level of urinary angiostatin in patients with lupus nephritis [8]. Basile et al. shpwed significant higher levels of average angiostatin in rats with acute and CKD [9]. Due to the fact that we excluded CKD patients with HFrEF and HFmrEF. With this configuration, we thought we would get better and objective results for angiostatin in HF patients without CKD.

Two very small studies demonstrated that CCB could improve exercise capacity and symptoms in HFpEF patients [13, 14]. Kim KH et al. demonstrated that a T-type CCB inhibits angiogenesis via suppression of hypoxia-inducible factor-1α [15]. Additionally, Munaron L. et al. reported targeting calcium channels may inhibit tumor vascularization and angiogenesis [16]. In our study, the level of angiostatin of HFrEF and HFmrEF patients receiving CCB is significantly higher than those who do not receive CCB medications. Contrary in the literature, benidipine induced coronary angiogenesis rather than inhibition of interstitial fibrosis [17]. Our patients were receiving dihydropyridine CCB medications. These findings support our results may indicate a negative effect of CCB medication on angiogenesis in HFpEF patients. Further studies and investigations are required in this regard.

Spironolactone use have prolonged the life in NYHA class III and IV HF patients in RALES study (2005), and was reported to shorten the duration of hospitalization. It was also demonstrated that spironolactone may be useful in cardiac remodeling and may have antifibrotic effects [18, 19]. In our study, the average level of angiostatin of HFrEF and HFmrEF patients using spironolactone is significantly lower than those who do not receive spironolactone, suggesting that spironolactone may have a significant effect on angiogenesis positively in patients with HFrEF and HFmrEF.

The current study has some limitations. Firstly, our study is limited to analysis of a single antiangiogenic molecule which makes it difficult to evaluate the unbalance of angiogenic/anti-angiogenic factors in patients with HFrEF and HFmrEF since other angiogenic factors have not been evaluated concurrently. However, we think that our study is meaningful and valuable for the reason that it has not been studied before in the literature and it is a clinical study. Finally, this study is based on a restricted number of patients and therefore cannot be ascertained whether these findings apply to other patients with HFrEF and HFpEF. Thus, larger clinical studies will be necessary for the confirmation of these findings.

Conclusion

In conclusion, CCB may have a negative effect on angiogenesis in HFrEF and HFmrEF patients via increasing on the contrary spironolactone may have positive effect on angiogenesis via decreasing angiostatin. Although no solid conclusion can be drawn from our study because of the small numbers of patients, it increases awareness about the clinical role of angiostatin in HFrEF and HFmrEF patients and the need for further studies.

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