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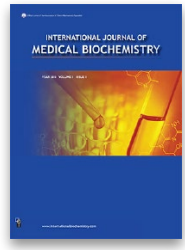

Research Article

Angiostatin levels in systolic heart failure patients with chronic kidney disease

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Abstract

Objectives: 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 angiogenesis and the proliferation of mesenchymal stem cells and there are limited studies about angiostatin in HF patients in the literature. Many studies have focused on the effect of angiostatin on endothelial cell apoptosis. By this study, we aimed to evaluate the angiostatin levels in systolic HF patients with chronic kidney disease (CKD).

Methods: A total of 69 people consisting of patients with a diagnosis of systolic HF with CKD (n=29) and healthy (n=40) subjects were included the current study. After obtaining blood samples, we evaluated serum angiostatin, plasma N-terminal Pro-BNP, creatinine, and transthoracic echocardiography was performed.

Results: The angiostatin level of patient group was significantly higher than the control group (163 (48-336); 58.14 (18.1-167); p=0.02; respectively). Average angiostatin level of HF patients receiving beta blocker therapy was significantly higher than the HF patients without beta-blocker (105.3 (50.7-220.7); 70.4 (35-224); p=0.02; respectively).

Conclusion: About this topic, our study is first. Angiostatin may be an important marker in systolic HF patients with CKD. Use of beta-blocker may inhibit angiogenesis and induce apoptosis in HF patients with CKD. Further studies are required on this subject.

Keywords: Angiogenesis, angiostatin, chronic kidney disease, heart failure

CKD is characterized by alterations in kidney function, which manifest in various ways depending upon the underlying causes and the severity of the disease [1-3] When ejection fraction (EF) is lower than 40% heart failure (HF) with reduced ejection (HFrEF) occurs, and when EF is in the range of 40-50% heart failure with mid-range ejection fraction (HFmrEF) occurs. These conditions considered as systolic HF [4, 5].

Angiostatin, a naturally occurring protein by fragmentation of plasminogen, is a potent endogenous inhibitor of mesenchymal stem cells, endothelial cells and angiogenesis [6]. Although the mechanism of action has not been completely lightened, many studies have focused on the effect of angiostatin on endothelial cell apoptosis in the literature [7-9].

Binding of angiostatin to plasma membrane-localized ATP synthetase suppresses the ATP metabolism in the endothelial cells thus downregulates the endothelial cell proliferation [10].

According to the literature, local synthesis of angiostatin following acute kidney disease suggest a possible role for angiostatin in this activity [11].

However, studies reporting the relationship between plasma angiostatin and characteristics and laboratory findings of patients with systolic HF patients with CKD are limited. Conse-

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quently, we assumed that the increase of angiostatin in the plasma should contribute to the impairment of angiogenesis in HF patients with CKD and investigated the relationship with the laboratory findings and medications of the patients.

Materials and Methods

Study sample and study protocol: This comparative cross-sectional study was conducted on 69 people totally consisting of HF patients with CKD (n=29) and normal people without disease (n=40) who applied nephrology or internal medicine outpatients of Gulhane Training and Research Hospital, Ankara, Turkey between the times 2011 and 2013. All of the procedures that we followed were in accordance with the ethical standards of the committee on human trials (institutional and national) was prepared by Helsinki Declaration in 1975, and it was changed in 2008. This study was approved by Gulhane Education and Research Hospital local ethics council with protocol number 1491-181-12/1539-491 on February 28th, 2012. The EFs were under 40 and between 40-50 percent, respectively for the patients with HFrEF, HFmrEF. All the patients and the cotrol goup participants were older than 18 years old. 17 of the participants were male, 23 of the participants were female in the control group, and 17 of the patients were male, and 12 of the patients were female in the patient group. The diagnosis of systolic HF was built by the symptoms, physical examinations of patients (European Society of Cardiology; 2016), echocardiographic results and N-Terminal proBNP levels. We excluded the patients from the study with the rheumatic disease or clinical signs of infection, atrial fibrillation, chronic obstructive pulmonary disease, high c reactive protein (>5 mg/L) and/or known malignancy. Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used for determining. Glomerular filtration rate (GFR) CKD [12]. There were 15 patients with dibates mellitus in patient group and all of them were on insulin treatment. In the patient group 25 patients were receiving angiotensin converting enzyme inhibitor (ACE Inh) or angiotensin II receptor blocker (ARB), 14 patients were receiving beta blocker and acetylsalicylic acid (ASA), 12 patients were receiving calcium channel blocker (CCB).

Blood samples as serums were collected from all of the participants and serum angiostatin levels of the HF patients with CKD and control group have been compared. The associations with angiostatin and clinical findings like demographic features, laboratory findings, comorbidities, and especially medications have been evaluated in the HF patient with CKD group.

After overnight fasting, baseline blood samples of each participant have been drawn from antecubital veins and gathered in BD Vacutainer® venous blood collection tubes containing clot activator and gel for serum isolation. We separated serum samples by centrifugation at 2000 g for 10 min. After that, we performed the analysis of the biochemical parameters without freezing while 2-3 mL of serum samples were aliquoted and instantly frozen at −80°C for further analyses of angiostatin until examination of the samples.

We evaluated high sensitive C-Reactive Protein (hs-CRP) in serum by immunoturbidimetric fixed rate method by Olympus AU-5800 autoanalyzer (Beckman Coulter, USA). Enzymatic and colorimetric methods with Olympus AU2700 (Beckman Coulter, USA) were used to measure alanine aminotransferase (ALT), aspartate aminotransferase (AST), fasting blood glucose (FBG), urea and creatinine. Automated counter of blood cell (ABX Pentra 120, Horiba, Japan) has been used for complete blood count (CBC) analysis. N-terminal pro-brain natriuretic peptide (NT-proBNP) levels were detected by the magnetic immunochromatographic technique using MICT system.

Angiostatin (Cat.No: CK-E90461) levels were analyzed by using quantitative ELISA kits (Hangzhou Eastbiopharm Co., Ltd, China). Measurements were applied using ELISA plate reader Bio-Tek Synergy HT (Biotek Instruments Inc., Winooski, VT, USA). Intra-assay CV and inter-assay CV were <10% and <12%, respectively while the minimum detectable dose of angiostatin was less than 20 ng/mL.

Statistical analysis

Clinical characteristics, history of medication and laboratory results of patients, were compared by angiostatin.

We used the Statistical Package for Social Science v 16.0 software package (SPSS, Chicago, IL, USA) for evaluating the statistical analyses. Discontinuous variables were shown as numbers and percentages (%) for the descriptive statistics, and mean±standard deviation or median (25th–75th interquartile range) was used for the evaluation of continuous variables. We evaluated the normality of the data by the Kolmogorov Smirnov test.

Comparisons of two groups were assessed for noncontinuous variables with the Mann-Whitney-U test. Spearman test was used for the nonparametric correlations. The P-values of less than 0.05 were considered to be statistically significant.

Results

The differences between HF patients with CKD and the control group were presented in Table 1. There was no statistically significant difference between groups in terms of age, gender, glucose, ALT, AST, hemoglobin, platelet, hs-CRP, white blood cell, erythrocyte sedimentation rate (ESR), while angiostatin, urea, creatinine and NT-ProBNP were significantly higher in patients with CKD than controls, (P<0.001 for all). Also, the glomerular filtration rate (GFR), ejection fraction (EF) were significantly lower in HF patients with CKD than controls (P≤0.05).

In correlation analysis, serum angiostatin levels were not significantly correlated with GFR, urea, creatinine, EF, and NT-ProBNP.

Also, we observed significantly average a higher level of angiostatin in HF patients with CKD with beta-blocker than the

Table 1. Comparison of demographic and laboratory features of HF patients with ChKD and control group

	Control (n=40)	Patients (n=29)	P
Age, year	71.53 (48-86)	71.72 (49-87.0)	0.695
Gender, F/M	23/17	17/12	0.434
Glucose (Fasting), mg/dL	99.07 (80-137)	115.72 (56-332)	0.09
Urea, mg/dL	36.75 (5-45)	119.75 (43-244)	<0.001
Creatinin, mg/dL	0.89 (0.59-1.24)	3.69 (1.69-11)	<0.001
GFR,mL/min/1.73 m^2	80.21 (67-110)	23.02 (10-45)	<0.001
AST, U/L	23.00 (12.0-70.0)	25.00 (10-64)	0.216
ALT, U/L	21.00 (6.0-83.0)	16.00 (2-56)	0.266
Hemoglobin, g/dL	13.28 (9.3-16.22)	11-4 (7.5-15.6)	0.655
Platelet, $10^3/mm^3$)	271(91-394)	222 (87-507)	0.275
White Blood Cell, $/mm^3$	6121 (3700-10400)	7575 (3900-14500)	0.061
Sedimentation, mm/h	15.00 (5.0-92.0)	26.00 (12-41)	0.452
CRP, mg/L	1.5 (0.2-5.1)	1.8 (0.9-7.1)	0.356
NT-ProBNP,pg/mL	122.00 (31-230)	13542 (664-35000)	<0.001
EF, %	60.57 (55-65)	37.00 (15-45)	<0.001
Angiostatin, ng/mL	58.14 (18.1-167)	163 (48-336)	<0.001

*Variables, which are not normally distributed, were given as median (minimum, maximum)
ALT:Alanine aminotransferase, AST: Aspartate aminotransferase, CRP: C-reactive protein, EF: Ejection fraction, GFR: Glomerular filtration rate (estimated by ChKD-EPI formula), NT-ProBNP: N Terminal Pro Brain Natriuretic Peptit.

Table 2. Comparison of angiostatin levels and clinical information of patients with HF patients with ChKD

		Angiostatin		
	n	Mean±SD	P^1	P^2
Control	40	58.14 (18.1-167)		
DM (-)	14	88.9 (60-283.1)	0.24	<0.001
DM (+)	15	121.8 (58.7-260.6)		
CAD (-)	19	102.8 (60.7-212.1)	0.21	0.044
CAD (+)	10	88.2 (66-134.6)		
HT (-)	13	92.3 (45.7-200.7)	0.34	<0.001
HT (+)	16	107.8 (62.3-282.1)		
ACE Inh or ARB (-)	4	82.2 (66-211.1)	0.112	< 0.001
ACE Inh or ARB (+)	25	134.5 (50.7-201.1)		
Beta Blocker (-)	15	70.4 (35-224)	0.02	0.004
Beta Blocker (+)	14	105.3 (50.7-220.7)		
ASA (-)	15	113 (66.1-200.1)	0.447	0.005
ASA (+)	14	87.2 (59.7-210.6)		
CCB (-)	17	82.5 (58.7-228.7)	0.521	<0.001
CCB (+)	12	80.4 (79.1-282.1)		

*Variables, which are not normally distributed, were given as median (minimum, maximum)
ACE Inh: Angiotensin Converting Enzyme Inhibitor, 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 patient group, P^2: Comparison of the accompanied diseases or medications of the patients with control group.

HF patients without beta-blocker. We did not observe any significant difference among angiostatin and comorbidities in the patinet group. We observed significant higher levels of angiostatin of patients with comorbidities than the control group (Table 2).

Discussion

To the best of our knowledge, this cross-sectional trial evaluating and demonstrating the importance of serum angiostatin levels in HF patients with CKD is the first. The primary novel findings of this study indicate that serum levels of angiostatin are markedly increased in HF patients with CKD compared to healthy subjects. Also, significantly higher angiostatin levels were determined in HF patients with CKD patients receiving beta-blocker therapy versus without therapy in the patient group.

Angiostatin has been the focus of compact study for the treatment of cancer where it has been shown to be an effective antiangiogenic agent, and there are some data concerning the involvement of angiostatin in angiogenesis and inhibiting the mesenchymal stem cell [13, 14].

On the other hand, studies are demonstrating the alterations of angiostatin levels in the setting of kidney disease. Basile et al. demonstrated that local synthesis of angiostatin following acute kidney disease in rat models suggests a possible role for angiostatin in this activity and furthermore, angiostatin expression does not occur in healthy rat kidney [11]. Besides, Tianfu et al. showed the elevation of angiostatin in the urine of patients with lupus nephritis significantly [15].

Additionally, Yamahara et al. showed the increase of angiostatin in dilated cardiomyopathy patients significantly higher than the healthy people [16]. Srikanth et al. created hypertrophic cardiomyopathy on mice experimentally and demonstrated the increase of angiostatin [17]. Accordingly, thus leading to tubular hypoxia/ischemia have to be considered for angiostatin, due to anti-angiogenic effects. However, there is a lack of studies demonstrating changes in systemic concentrations of angiogenic factors in patients with HF patients with CKD. Considering the role of angiostatin in depressing neovascularization in CKD patients and by demonstrating increased serum angiostatin levels in CKD patients in the present study, we think that it could be an important physiopathological molecule in HF patients with CKD. Additionally we observed significantly higher levels of angiostatin in HF patients with CKD and comorbidities than the control group. But we did not observe specific high level of angiostatin in a specific patient group in HF patient group. Because of that reason we do not think the comorbidities in patient group do not affect the angiostatin levels in HF patients with CKD.

Another important finding of medication-angiostatin association in our HF patients with CKD was the relationship with the use of beta-blockers. We observed significantly higher angiostatin levels among HF patients with beta-blocker therapy than those without. There are some studies about the effect of beta-blockers on angiogenesis [18, 19]. These are the effects of propranolol in infantile hemangioma and the anti-angiogenic effect of beta-blockers in breast cancer patients. According to our findings, we hypothesize that the use of beta-blockers may reduce angiogenesis via increasing angiostatin in HF patients with CKD. This may be one of the antiangiogenic and apoptotic mechanisms of beta-blockers in this patient population. Contrary, in the patient group other medications except beta-blocker treatment are not significantly diffferent than the other medications. We hipothise that ather medications do not affect the angiostatin levels in HF patients with CKD.

Firstly, the limitation of the current study is limited to the analysis of only one anti-angiogenic agent, angiostatin, which makes it hard to reveal the angiogenic/anti-angiogenic factors in HF patients with CKD. Furthermore, we think that the present study is significant and worthy of the reason that it has not been demonstrated before in the literature. Finally, the number of participants in this study is limited and thus cannot ascertain whether these findings apply to other patients with HF patients with CKD. Accordingly, further clinical trials with more number of participants should be necessary for confirmation of the current results.

Conclusion

Consequently, circulating angiostatin levels are higher in HF patients with CKD. Beta-blocker may harm angiogenesis in HF patients with CKD by increasing angiostatin levels by the effect on angiogenesis and apoptosis. Finally, the current study provides hope that targeting neovascularization might be a novel approach to retard the progression of HF patients with CKD.

Conflict of interest: None declared.

Ethics Committee Approval: This study was approved by Gulhane Education and Research Hospital local ethics council with protocol number 1491-181-12/1539-491 on February 28th, 2012.

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Authorship contributions: Concept – I.E.; Design – T.O.; Supervision – K.S.; Fundings – R.A.; Materials – R.A.; Data collection &/or processing – I.E.; Analysis and/or interpretation – F.Y., E.S.; Literature search – B.Y.; Writing – I.E.; Critical review – F.N.A.

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