



## Research Article

# Angiostatin levels in systolic heart failure patients with chronic kidney disease

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### Abstract

**Objectives:** Heart failure (HF) with reduced ejection fraction (HFrEF) is defined as an ejection fraction (EF) of less than 40%. An EF of 40% to 50% is known as HF with mid-range EF, which is considered a subgroup of HF with preserved ejection fraction, rather than HFrEF. Angiostatin inhibits angiogenesis and the proliferation of mesenchymal stem cells. Though many studies in the literature have focused on the effect of angiostatin on endothelial cell apoptosis, studies about angiostatin levels in HF patients are limited. The aim of this study was to evaluate the angiostatin level in systolic HF patients with chronic kidney disease (CKD).

**Methods:** A group of 69 individuals, consisting of patients with a diagnosis of systolic HF with CKD (n=29) and healthy (n=40) subjects, was included in the current study. Serum angiostatin, plasma N-terminal pro-brain type natriuretic peptide, and creatinine levels were assessed, and transthoracic echocardiography was performed.

**Results:** The angiostatin level of the patient group was significantly higher than that of the control group (median: 163 ng/mL [25<sup>th</sup>-75<sup>th</sup> interquartile range: 48-336 ng/mL] and median: 58.14 ng/mL [25<sup>th</sup>-75<sup>th</sup> interquartile range: 18.1-167 ng/mL], respectively; p=0.02). The angiostatin level of HF patients receiving beta blocker therapy was significantly higher than that of the HF patients who were not taking a beta blocker (105.3 ng/mL [50.7-220.7 ng/mL] and 70.4 ng/mL [35-224 ng/mL], respectively; p=0.02).

**Conclusion:** To the best of our knowledge, this study is the first to examine this subject. Angiostatin may be an important marker in systolic HF patients with CKD. The use of a beta blocker may inhibit angiogenesis and induce apoptosis in HF patients with CKD. Further studies are required.

**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]. Heart failure (HF) with reduced ejection (HFrEF) occurs when the ejection fraction (EF) is less than 40%. When the EF is in the range of 40% to 50%, it is classified as HF with mid-range EF (HFmrEF). These conditions are considered systolic HF [4, 5].

Angiostatin is a naturally occurring protein, formed through the process of the fragmentation of plasminogen. It is a

potent endogenous inhibitor of mesenchymal stem cells, endothelial cells, and angiogenesis [6]. Many studies have focused on the effect of angiostatin on endothelial cell apoptosis, though the mechanisms of action are still not yet completely understood [7, 8].

The binding of angiostatin to plasma membrane-localized adenosine triphosphate (ATP) synthase suppresses ATP metabolism in the endothelial cells, and thus downregulates endothelial cell proliferation [9].

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According to the literature, local synthesis of angiotensin following acute kidney disease suggests a possible role for angiotensin in this activity [10].

However, studies examining the relationship between plasma angiotensin and the characteristics and laboratory findings of systolic HF patients with CKD are limited. The hypothesis of this study was that an increase in angiotensin in the plasma might contribute to the impairment of angiogenesis in HF patients with CKD, and the relationship with the laboratory findings and medications of the patients were investigated.

## Materials and Methods

### Study sample and study protocol

This comparative, cross-sectional study was conducted with a total of 69 individuals: HF patients with CKD (n=29) and participants without disease (n=40) who presented at the nephrology or internal medicine outpatient facilities of Gulhane Training and Research Hospital, Ankara, Turkey, between 2011 and 2013. All of the procedures applied were performed in accordance with the ethical standards of the committees on human trials (institutional and national) and the Declaration of Helsinki. This study was approved by Gulhane Education and Research Hospital local ethics council with protocol number 1491-181-12/1539-491 on February 28, 2012. The EF was less than 40% and between 40% and 50%, respectively, for the patients with HF<sub>r</sub>EF and HF<sub>m</sub>rEF. All of the patients and the control group participants were older than 18 years of age. In all, 17 of the participants in the control group were male and 23 were female. In the patient group, 17 were male, and 12 were female. The diagnosis of systolic HF was based on the symptoms, a physical examination (European Society of Cardiology, 2016), echocardiographic results, and N-terminal pro-brain natriuretic peptide (NT-proBNP) level. Patients with rheumatic disease or clinical signs of infection, atrial fibrillation, chronic obstructive pulmonary disease, high C-reactive protein (CRP) value (>5 mg/L) and/or known malignancy were excluded from the study. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to determine the glomerular filtration rate (GFR) [11]. There were 15 patients with diabetes mellitus in the patient group and all were on insulin treatment.

The serum angiotensin level of the HF patients with CKD and the control group were compared. The angiotensin level and the clinical findings of demographic features, laboratory results, comorbidities, and medications in the HF patient were compared with the CKD group to assess possible relationships. After an overnight fast, baseline blood samples were drawn from an antecubital vein in each participant into a BD Vacutainer venous blood collection tube containing clot activator and gel for serum isolation (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Centrifugation at 2000 g for 10 minutes was performed to separate the serum. A portion was used for analysis of the biochemical parameters without freezing,

while 2 to 3 mL of the serum sample was aliquoted and immediately frozen at  $-80^{\circ}\text{C}$  for further analysis of angiotensin.

High sensitivity C-reactive protein (hs-CRP) in the serum was evaluated using the immunoturbidimetric fixed rate method and an AU5800 autoanalyzer (Beckman Coulter, Inc., Brea, CA, USA). Enzymatic and colorimetric methods were used to measure alanine aminotransferase (ALT), aspartate aminotransferase (AST), fasting blood glucose, urea, and creatinine with the same autoanalyzer. An automated blood cell counter (ABX Pentra 120, Horiba Ltd., Kyoto, Japan) was used for complete blood count analysis.

The angiotensin level was analyzed using quantitative enzyme-linked immunosorbent assay (ELISA) kits (Cat.No: CK-E90461; Hangzhou Eastbiopharm Co., Ltd, Hangzhou, China). Measurements were made using the Bio-Tek Synergy HT ELISA plate reader (Biotek Instruments Inc., Winooski, VT, USA). The intra-assay coefficient of variation (CV) and inter-assay CV results were <10% and <12%, respectively, while the minimum detectable dose of angiotensin was less than 20 ng/mL.

### Statistical analysis

The clinical characteristics, history of medications, and the laboratory results of the participants were compared according to the angiotensin level.

SPSS for Windows, Version 16.0 (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analyses. Discontinuous variables were shown as numbers and percentages for the descriptive statistics, and mean $\pm$ SD or median (25<sup>th</sup>–75<sup>th</sup> percentile, interquartile range) was used for the evaluation of continuous variables. Normality of the data was evaluated with the Kolmogorov-Smirnov test.

The Mann-Whitney U test was applied to assess differences between independent groups and the Spearman test was used for nonparametric correlations. P values of less than 0.05 were considered statistically significant.

## Results

The differences between HF patients with CKD and the control group are presented in Table 1. There was no statistically significant difference between groups in terms of age, gender, or levels of glucose, ALT, AST, hemoglobin, platelet count, hsCRP, white blood cell count, or erythrocyte sedimentation rate. Levels of angiotensin, urea, creatinine, and NT-ProBNP were significantly higher in patients with CKD than in the controls ( $p<0.001$  for all). Also, the glomerular filtration rate (GFR) and EF were significantly lower in HF patients with CKD than in the controls ( $p\leq 0.05$ ).

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

A significantly higher level of angiotensin was observed in HF patients with CKD who used a beta blocker than in the HF patients who did not use a beta blocker. No significant difference

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

	Control (n=40)	Patients (n=29)	P
Age, years	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 <sup>2</sup>	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 <sup>3</sup> /mm <sup>3</sup>	271(91-394)	222 (87-507)	0.275
White blood cell, /mm <sup>3</sup>	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

\*Non-normally distributed variables are presented as median (minimum-maximum); ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CKD: Chronic kidney disease; CRP: C-reactive protein; EF: Ejection fraction; GFR: Glomerular filtration rate; HF: Heart failure; NT-ProBNP: N-terminal pro-brain natriuretic peptide.

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

	n	Angiostatin		
		Mean±SD	P <sup>1</sup>	P <sup>2</sup>
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 that were not normally distributed are presented as median (minimum-maximum); ACEI: Angiotensin-converting enzyme inhibitor; ARB: Angiotensin II receptor blocker; ASA: Acetylsalicylic acid; CAD: Coronary artery disease; CCB: Calcium channel blocker; CKD: Chronic kidney disease; DM: Diabetes mellitus; HF: Heart disease; HT: Hypertension; P<sup>1</sup>: Comparison of the accompanying diseases or medications used in patient group; P<sup>2</sup>: Comparison of the accompanying diseases or medications of the patients with control group.

was seen in an analysis of angiostatin and comorbidities in the patient group; however, a significantly higher level of angiostatin was measured in the patients with comorbidities when compared with 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 indicated that serum levels of angiostatin were markedly increased in HF patients with CKD compared with healthy subjects. Also, significantly higher angiostatin levels were determined in HF patients with CKD patients receiving beta blocker therapy versus those in the patient group who were not using a beta blocker.

Angiostatin has been a focus of study for the treatment of cancer and it has been shown to be an effective antiangiogenic agent. Some data have been reported concerning the involvement of angiostatin in angiogenesis and inhibition of the mesenchymal stem cell [12, 13].

There are also studies demonstrating a change in angiostatin level in the presence of kidney disease. Basile et al. [10] demonstrated that local synthesis of angiostatin following acute kidney disease in rat models suggested a possible role for angiostatin in this activity and furthermore, angiostatin expression did not occur in a healthy rat kidney. In addition, Tianfu et al. [14] demonstrated a significant elevation of angiostatin in the urine of patients with lupus nephritis.

Yamahara et al. [15] reported that the angiostatin level in dilated cardiomyopathy patients was significantly higher than that of the healthy individuals. In a mouse experiment, Srikanth et al. [16] found increased angiostatin in hypertrophic cardiomyopathy. A progression to tubular hypoxia/ischemia has to be considered due to the anti-angiogenic effects of angiostatin.

There is a lack of studies demonstrating changes in systemic concentrations of angiogenic factors in HF patients with CKD. Angiostatin could be an important physiopathological molecule in HF patients with CKD, considering its role in depressing neovascularization in CKD patients and given the results of increased serum angiostatin levels in CKD patients in the present study. Additionally we observed significantly higher levels of angiostatin in HF patients with CKD and comorbidities than in the control group. However, we did not observe a significantly high level of angiostatin in a specific HF patient group. Therefore, we do not think the comorbidities in the patient group affected the angiostatin level in HF patients with CKD.

Another important finding was the medication-angiostatin association observed in our HF patients with CKD. A significantly higher angiostatin level was seen among HF patients using beta blocker therapy than in those who did not. There is some research in the literature about the effect of beta blockers on angiogenesis related to the effects of propranolol in infantile hemangioma and the anti-angiogenic effect of beta blockers in breast cancer patients [17, 18]. 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. There was no significant difference in the patient group when comparing the use of medications other than a beta blocker. We hypothesize that other medications do not affect the angiostatin level in HF patients with CKD.

The current study is an analysis of only 1 anti-angiogenic agent, angiostatin, which makes it hard to reveal the angiogenic/anti-angiogenic factors in HF patients with CKD. In addition, the number of participants in this study is limited, and thus cannot ascertain whether these findings apply to other HF patients with CKD. Nonetheless, we think that the present study is significant in that these findings have not been demonstrated before in the literature. Further clinical trials with a larger number of participants are necessary for confirmation of the current results.

## Conclusion

Circulating angiostatin levels were found to be higher in HF patients with CKD. Beta blocker use may harm angiogenesis in HF patients with CKD by increasing angiostatin levels through the effect on angiogenesis and apoptosis. Our results suggest 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 28<sup>th</sup>, 2012.

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