

Research Article

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Evaluation of the effects of different treatment modalities on angiogenesis in heart failure patients with preserved ejection fraction via VEGF and sVEGFR-1

Korunmuş ejeksiyon fraksiyonlu kalp yetmezliği hastalarında anjiyogenez üzerine farklı tedavi modalitelerinin etkilerinin VEGF ve sVEGFR-1 aracılığı ile değerlendirilmesi

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Abstract

Objective: In this study, our aim was to investigate the clinical significance of VEGF, sVEGFR-1 in HFpEF patients.

Materials and methods: Seventy-two participants enrolled in this cross-sectional case-control study including HFpEF patients (n=41) and healthy (n=31) subjects. Blood samples were collected and serum VEGF, sVEGFR-1 analysis, and transthoracic echocardiography were performed.

Results and discussion: The average sVEGFR-1 level of HFpEF patient group was significantly higher than the control group (respectively 0.136 ng/L (0.04–0.34), 0.06 ng/L (0.01–0.25); $p < 0.001$). The average VEGF level of HFpEF patients using beta blocker was significantly higher than the HFpEF patients not using it (respectively 0.585 ± 0.194 ng/L; 0.349 ± 0.269 ng/L; $p = 0.025$). The average VEGF level of HFpEF patients using statins was significantly higher than the HFpEF patients

without a medication (respectively 0.607 ± 0.099 ng/L; 0.359 ± 0.273 ng/L; $p = 0.038$).

Conclusion: Our study is the first study demonstrating the relations among HFpEF, accompanying morbidities, VEGF and sVEGFR-1 levels. Statins and beta blockers may have positive effects on angiogenesis in HFpEF patients via increasing VEGF levels.

Keywords: Heart failure; VEGF; sVEGFR-1.

Öz

Amaç: Bu çalışmada, korunmuş ejeksiyon fraksiyonlu kalp yetmezliği (KEFKY) hastalarında VEGF, sVEGFR-1'in klinik önemini araştırmayı amaçladık.

Gereç ve yöntem: Çalışmaya KEFKY hastalar (n = 41) ve sağlıklı (n = 31) insanlardan oluşan toplam 72 kişi dahil edildi. Kan örnekleri alındı, serum VEGF ve sVEGFR-1 analizleri ve transtoraksik ekokardiyografi değerlendirmesi tüm katılımcılara yapıldı.

Tartışma: Hasta grubunun ortalama sVEGFR-1 düzeyi kontrol grubuna göre anlamlı derecede yüksekti (sırasıyla $0,136 \pm 0,060$; $0,074 \pm 0,050$; $<0,001$). Beta bloker kullanan hastaların ortalama VEGF düzeyi, Beta bloker kullanmayan hastalardan anlamlı derece daha yüksekti (sırasıyla $0,585 \pm 0,194$; $0,349 \pm 0,269$; $p = 0,025$). Statin kullanan KEFKY hastalarının ortalama VEGF düzeyi, statin kullanmayan KEFKY hastalarından anlamlı olarak daha yüksekti (sırasıyla $0,607 \pm 0,099$; $0,359 \pm 0,273$; $p = 0,038$).

Sonuç: Çalışmamız, eşlik eden morbiditeler, VEGF ve sVEGFR-1 düzeyleri ile KEFKY arasındaki ilişkileri gösteren ilk çalışmadır. Statinler ve beta blokerler artmış VEGF seviyeleri ile KEFKY hastalarında anjiyogenez üzerinde olumlu etkileri olabilir.

Anahtar kelimeler: Kalp yetmezliği; VEGF; sVEGFR-1.

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Introduction

Cardiac pump mechanism without an impairment and ejection fraction (EF) is over 50% is called heart failure with preserved ejection fraction (HFpEF) [1, 2].

Several neurohumoral mechanisms play role in the adaptation of the impairment of cardiac functions [3, 4]. As an important contributor of remodeling, formation of new vessels from performed vessels is a process that can be named so-called angiogenesis. Angiogenic molecules play critical roles in endothelial development, microvascular permeability and pathological angiogenesis [5]. Vascular endothelial growth factor (VEGF) is a well known angiogenic molecule. VEGFR-1 is a VEGF receptor which is known as transmembrane proteins. VEGFR-1 mediated signaling participates crucial action by enhancing the vascular permeability [6].

Soluble VEGFR-1 (sVEGFR-1) is produced from messenger RNA of VEGFR-1 and acts like decoying protein. It is probably a negative regulator of VEGF [7].

However, studies reporting the association among serum VEGF and sVEGFR-1 and clinical information of HFpEF patients are very limited and the role of VEGF and sVEGFR-1 in HFpEF patients is still an unclear situation.

From this point of view, we have hypothesized that increased sVEGFR-1 levels may cause the impaired angiogenesis in HFpEF patients and intended to evaluate HFpEF on VEGF (proangiogenic factor) and sVEGFR-1 (an angiogenesis inhibitor) in patients with HFpEF. In our opinion, this study will identify a physiopathological cause in HFpEF cases.

Methods

Subjects

Seventy-two participants consisting of HFpEF cases ($n=41$) and healthy subjects as the control group ($n=31$) were included in the study. It has been designed as a cross-sectional case-control study. Gulhane Faculty of Medicine Hospital regional ethics council approved this research with protocol number 1491-389-11/1539-267 on November 29th, 2011. The ethical norms of the council on human experimentation (institutional and national) was prepared by Helsinki Declaration in 1975, which was modified in 2008. All patients underwent transthoracic echocardiography for the diagnosis of HFpEF patients in 2011, 2012 and 2013. The symptoms, signs of patients

(European Society of Cardiology; 2012) and echocardiographic findings were recorded and used for diagnosis of the HFpEF. The patient group with HFpEF was composed of subjects older than 18 years old with ejection fraction over 50%. The exclusion criterias were the presence of infection, acute or chronic inflammatory disease and situation, high erythrocyte sedimentation rate or CRP, having or suspected malignancy, chronic obstructive pulmonary disease and cerebrovascular accident.

Sampling and laboratory measurements

We obtained fasting blood specimens via venipuncture in BD Vacutainer® serum tubes which include clot activator and polymer gel for sera isolation. We centrifuged samples for obtaining serum samples at 2000 *g* for 10 min. Just after, we analyzed the biochemical parameters. Two milliliter of serum samples were aliquoted and preserved at -80°C for further analyzes of VEGF and sVEGFR-1 up to working day.

Enzymatic and colorimetric methods with Olympus AU2700 (Beckman Coulter, USA) were used to perform measurements of total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), fasting blood glucose (FBG), urea and creatinine. We calculated low-density lipoprotein cholesterol (LDL-C) by Friedewald's formula [8]. Automated counter of blood cell (ABX Pentra 120, Horiba, Japan) has been used for complete blood count (CBC) analysis. We analyzed c-reactive protein (CRP) by immunoturbidimetric fixed rate technique by Olympus AU-2700 device (Beckman Coulter, USA).

We determined sera VEGF and sVEGFR-1 levels by commercial ELISA kits (Quantikine®, R&D Systems, Minneapolis, MN, USA) and Synergy HT plate reader (Bio-Tek Instruments Inc., Winooski, VT, USA) [9]. Intra-assay CV and interassay CV were $<6.5\%$, $<8.5\%$, respectively with sensitivity 9 pg/mL in order to evaluate VEGF, while intra-assay CV and inter-assay CV were $<3.8\%$, $<9.8\%$, respectively with sensitivity 13.3 pg/mL in order to evaluate sVEGFR-1.

Statistical analyzes

For statistical analysis, SPSS software (IBM SPSS, ver.24) was used. For the calculations, the statistical significance level (α) was taken as 5%. We noted *p*-value of 0.05 or less significant. We demonstrated categorical variables with numbers and percentages (%) for

the descriptive statistics; and also demonstrated the continuous (numerical) variables with median, mean, standard deviation, minimum, and maximum values. Non-parametric tests were used for the variables that were not normally distributed. Correlations were analyzed by the Spearman's nonparametric correlation test. In terms of parameters for comparing the means of the groups in order to evaluate categorical data χ^2 test was used and Mann-Whitney-U test was used in order to evaluate continuous variables that did not distribute normally. By multiple regression analysis method an estimation equation for the dependent variable "creatinine" is obtained with using independent variable "VEGF and sVEGFR-1". The predictive model obtained as a result of multiple regression analysis was statistically significant (Anova; $p < 0.05$). Assumptions of "residuals" were checked by Durbin-Watson test.

Results

The mean age of HFpEF patients and controls were similar (Table 1). The mean results were not different statistically

between groups in terms of VEGF, platelet, AST, erythrocyte sedimentation rate (ESR), CRP while mean serum sVEGFR-1, white blood cell (WBC), urea, creatinine, FBG, thyroid stimulating hormone (TSH), ejection fraction (EF) results were higher in HFpEF cases than controls significantly ($p < 0.005$). Hemoglobin, glomerular filtration rate (GFR), ALT, LDL-C, triglyceride, HDL-C, total cholesterol and ejection fraction (EF) levels were significantly lower in HFpEF group than control subjects ($p \leq 0.005$) (Table 1).

In correlation analysis, we determined sVEGFR-1 levels correlated positively and significantly ($r = 0.481$, $p < 0.001$) with serum creatinine levels in HFpEF cases. The correlation analyzes between serum sVEGFR-1 and GFR levels were significantly correlated negatively in HFpEF cases ($r = -0.559$, $p < 0.001$) (Table 2). The coefficients (B) that obtained by estimating creatinine variable with the help of the VEGF and sVEGFR-1 independent variables were demonstrated in Table 3. The increase in both arguments led to a significantly positive increase over creatinine. VEGF independent variable has no statistically significant effect alone (partial regression coefficients). On the contrary, the partial regression coefficient of sVEGFR-1 independent variable was statistically

Table 1: Comparison of demographic and laboratory features of patients with HFpEF and control group.

	HFpEF (n=41)	Control (n=31)	p-Value
Gender (M/F)	17/24	14/17	0.813 ^a
Age (years)	75.43 ± 8.04	74.54 ± 8.59	0.653
VEGF (ng/L)	0.33 (0.02–0.86)	0.34 (0.06–0.73)	0.941
sVEGFR-1 (ng/L)	0.136 (0.04–0.34)	0.06 (0.01–0.25)	<0.001
WBC ($10^3/\mu\text{L}$)	8.91 ± 3.82	6.5 ± 2.88	0.003
Hemoglobin (g/dL)	10.6 ± 1.69	13.14 ± 1.97	<0.001
Platelet ($10^3/\mu\text{L}$)	250.2 ± 116	278.2 ± 65.6	0.204
Urea	83 (22–389)	34 (23–87)	<0.001
Creatinine	1.9 (0.43–9.2)	0.87 (0.57–1.37)	<0.001
GFR (mL/min/1.73 m ²)	28 (5–88)	68 (55–139)	<0.001
AST (U/L)	21 (4–102)	22 (12–42)	0.926
ALT (U/L)	12 (3–41)	19 (9–40)	0.001
Glucose	102 (66–293)	95 (80–137)	0.198
LDL-C (mg/dL)	89.9 ± 26	109.4 ± 39.6	0.022
Triglyceride (mg/dL)	132 (51–184)	157 (34–330)	0.004
HDL-C (mg/dL)	40 (15–60)	44 (32–62)	0.004
T. Cholesterol (mg/dL)	153.6 ± 34.8	194.2 ± 39.7	<0.001
ESR (mm/hour)	19 (3–69)	21 (7–42)	0.222
hsCRP (mg/L)	2.1 (0.3–6)	1.2 (0.2–5)	0.13
EF	55.8 (45–65)	63 (55–67)	<0.001

Data are expressed as the mean ± SD. p-Values were calculated using independent-sample t and Chi square^a test.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; EF, ejection fraction; ESR, erythrocyte sedimentation rate; GFR, glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; hsCRP, high sensitive C-reactive protein; LDL-C, low-density lipoprotein cholesterol; sVEGFR-1, soluble vascular endothelial growth factor receptor-1; T. Cholesterol, total cholesterol; VEGF, vascular endothelial growth factor; WBC, white blood cells;

Bold values denotes significance at $p < 0.05$.

Table 2: Correlation between serum VEGF, sVEGFR-1 levels and laboratory features in HFpEF group (n=41)

	VEGF		sVEGFR-1	
	R (correlation coefficient)	p-Value	R (correlation coefficient)	p-Value
sVEGFR-1	–0.580	0.63	1	
WBC	0.022	0.85	0.215	0.07
Hemoglobin	0.110	0.35	–0.505	0.06
Urea	0.127	0.28	0.421	0.08
Creatinine	0.042	0.72	0.481	<0.001
GFR	0.037	0.76	–0.559	<0.001
ALT (U/L)	–0.066	0.58	–0.258	0.06
LDL-C (mg/dL)	–0.027	0.82	–0.205	0.08
Triglyceride (mg/dL)	0.208	0.07	–0.230	0.052
HDL-C (mg/dL)	–0.138	0.24	–0.056	0.64
T. Cholesterol (mg/dL)	–0.060	0.61	–0.231	0.07
EF	0.054	0.65	–0.334	0.06

Correlation analysis among variables was performed by using Spearman's correlation test. A p-value of <0.05 was considered significant. ALT, alanine aminotransferase; GFR, glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; sVEGFR-1, soluble vascular endothelial growth factor receptor-1; T. Cholesterol, total cholesterol; WBC, white blood cells; Bold values denotes significance at $p < 0.05$.

Table 3: Multiple regression analysis.

Model	Regression coefficients			t	p-Value
	Unstandardized coefficient		Standardized coefficient		
	B	Std. error	Beta		
(Constant)	–0.004	0.493			
VEGF	1.396	0.842	0.174		
sVEGFR1	13.289	2.920	0.477		
^a ANOVA (Regr.)	df=2			F=11.257	^b p<0.001
Model Summary	R=0.496	R ² =0.246	Adj. R ² =0.224		
Residuals Statistics	Predicted Value: 1.963		Durbin-Watson: 1.770		

Entered Method: ^aDependent Variable: creatinine; ^bPredictor: (Constant): sVEGFR-1, VEGF

Regr.: Regression, sVEGFR-1: Soluble vascular endothelial growth factor receptor 1, VEGF: Vascular endothelial growth factor.

significant. Residues were not correlated statistically because the Durbin-Watson statistics of residues were smaller than the predictive statistic. The increase of the sVEGFR-1 as an independent variable leads to a positive increase of creatinine.

We observed significantly higher sVEGFR-1 results in HFpEF cases who undergo dialysis than the HFpEF patients without dialysis (respectively 0.16 (0.09–0.33); 0.08 (0.008–0.24); $p=0.002$). Significantly higher VEGF results were also determined in HFpEF cases receiving beta-blocker and statin therapies versus without these therapies (respectively 0.58 (0.29–0.86); 0.32 (0.01–0.83); $p=0.02$; 0.56 (0.50–0.74); 0.32 (0.01–0.86); $p=0.03$). The sVEGFR-1 levels of HFpEF cases who have atrial fibrillation (AF), coronary artery disease (CAD), chronic kidney disease (CKD) were more the control subjects

significantly ($p=0.03$) while sVEGFR-1 levels of HFpEF patients undergoing dialysis were more than the HFpEF cases without undergoing dialysis significantly (respectively; 0.16 (0.09–0.33); 0.08 (0.008–0.24); $p=0.002$). Significantly higher levels of sVEGFR-1 results were also observed in HFpEF cases with acetylsalicylic acid, beta blocker, statin, enoxaparin, calcium channel blocker (CCB) and furosemide therapies versus control group ($p<0.05$) (Table 4).

Discussion

As far as is known, this is the primary trial demonstrating the importance of sera VEGF and sVEGFR-1 results together

Table 4: Comparison of VEGF, sVEGFR-1 levels and clinical information of patients with HFpEF.

Clinical information		VEGF			P1	P2	sVEGFR1			P1	P2
		Median	Minimum	Maximum			Median	Minimum	Maximum		
Control		0.34	0.05	0.72			0.05	0.008	0.24		
Dialysis	–	0.32	0.01	0.83	0.33	0.41	0.08	0.008	0.24	0.002	<0.001
Dialysis	+	0.34	0.02	0.86			0.16	0.09	0.33		
CKD	–	0.32	0.01	0.83	0.12	0.16	0.11	0.008	0.33	0.58	0.001
CKD	+	0.47	0.05	0.86			0.13	0.09	0.22		
CAD	–	0.31	0.02	0.83	0.14	0.15	0.10	0.008	0.33	0.68	0.001
CAD	+	0.51	0.01	0.86			0.13	0.04	0.22		
HT	–	0.34	0.02	0.86	0.89	0.92	0.07	0.0087	0.24	0.42	<0.001
HT	+	0.31	0.01	0.77			0.13	0.035	0.33		
AF	–	0.34	0.01	0.86	0.81	0.90	0.11	0.008	0.33	0.35	0.03
AF	+	0.22	0.02	0.70			0.12	0.04	0.13		
ARB	–	0.32	0.01	0.86	0.61	0.52	0.12	0.008	0.33	0.20	0.14
ARB	+	0.04	0.14	0.60			0.10	0.03	0.22		
Furosemide		0.32	0.01	0.86	0.96	0.78	0.12	0.008	0.33	0.27	0.05
Furosemide	+	0.40	0.08	0.60			0.12	0.04	0.27		
ASA		0.32	0.01	0.83	0.09	0.07	0.12	0.008	0.33	0.31	0.03
ASA	+	0.42	0.14	0.86			0.11	0.03	0.19		
Enoxaparin		0.34	0.01	0.86	0.98	0.92	0.11	0.008	0.33	0.50	0.002
Enoxaparin	+	0.32	0.08	0.77			0.13	0.04	0.27		
Beta Blocker	–	0.32	0.01	0.83	0.02	0.01	0.11	0.008	0.33	0.28	0.001
Beta Blocker	+	0.56	0.29	0.86			0.15	0.09	0.22		
CCB	–	0.32	0.01	0.86	0.46	0.27	0.11	0.008	0.33	0.47	0.039
CCB	+	0.38	0.08	0.64			0.12	0.04	0.18		
Statins	–	0.32	0.01	0.86	0.03	0.01	0.12	0.008	0.33	0.30	0.21
Statins	+	0.56	0.50	0.74			0.08	0.04	0.22		

P1, Comparison of the accompanied diseases or medications used in HFpEF group, P2, Comparison with control group

AF, Atrial fibrillation; ARB, Angiotensin II receptor blocker; ASA, Acetylsalicylic acid; CAD, Coronary artery disease; CCB, Calcium channel blocker; CKD, Chronic kidney disease; HT, Hypertension.

in HFpEF cases. Serum sVEGFR-1 results were significantly more HFpEF cases than control subjects.

As previously demonstrated by numerous studies, VEGF and its receptors promote angiogenesis, while sVEGFR-1 decreases angiogenesis by inhibiting VEGF [10]. In 2010, Kaza et al. have demonstrated an increase in sVEGFR-1 in rat models with hypertrophied myocardium [11]. From this point of view, it brings to mind that increasing levels of sVEGFR-1 levels may be important in terms of angiogenesis and pathophysiology in HFpEF patients. Additionally, DiMarco et al. have found the level of sVEGFR-1 higher than the control group due to inflammation likely to the previous studies. Also, a high level of sVEGFR-1 was demonstrated as an independent risk factor for CKD [12]. Significantly higher levels of sVEGFR-1 were observed among HFpEF patients undergoing dialysis than the HFpEF patients without dialysis in our study. Also in correlation analysis, sVEGFR-1 levels were significantly positively correlated with creatinine levels in HFpEF patients. The correlation analyzes between serum

sVEGFR-1 and GFR results were negatively correlated among HFpEF cases significantly. Multiple regression analysis models was found to be statistically significant as mentioned above. The increase of the sVEGFR-1 as an independent variable leads to a positive increase of creatinine. We think that the increase of this sVEGFR-1 is due to low clearance. All these findings bring to mind that sVEGFR-1 levels in HFpEF patients were affected by renal functions and the chronic inflammatory situation of CKD. Additionally, sVEGFR-1 may be one of the mechanism of antiangiogenesis in HFpEF cases with CKD.

However, the VEGF levels in our patient group did not differ from the control group despite the change in the sVEGFR-1 levels. According to some of the literature, VEGF levels of HF cases were more than the control subjects. Friehs et al. demonstrated that receiving VEGF causes retardation the beginning of HF in rabbits with a pressure overload model [13]. Gustafsson et al. showed that exercise training induces angiogenesis in HF cases most probably by VEGF gene expression [14]. Contrast to these kinds

of literature, we have found the VEGF levels were not significantly higher than the control groups. The contribution of VEGF released from platelets and leukocytes in serum is the major difference between plasma and serum. Many points associated with analyzing circulating VEGF are described in a review by Jelkmann and demonstrated that VEGF should be analyzed in plasma [15]. Also, the use of anticoagulant and antiaggregant when collecting the plasma can affect the results [16]. In addition, not only the anticoagulants and antiaggregants but also the analyzing center, centrifuge, and method for analyzing have been found to be independent factors for the analysis of circulating plasma levels of VEGF [17]. With that in mind, it is because of that difficult to compare different studies reporting the levels of VEGF in the blood since there are a lot of factors that can influence the analysis.

In the comparison of VEGF, sVEGFR-1 levels and medication status of patients with HFpEF, we demonstrated the mean level of sera VEGF in patients using beta-blockers was significantly more in the HF cases and the control subjects. In the literature as similar to our results Rudolf A. et al. have demonstrated that carvedilol raises plasma VEGF in HF cases [18]. It is previously well known that VEGF also increases in hypoxic situations [19]. It is not fully understood why VEGF results raised by a potentially anti-ischaemic medication. Thirteen of patients were with CAD in our patient group for explaining the contribution of ischemic situation. Previous studies also demonstrated the induction of angiogenesis by the reduction of heart rate [20]. We want to speculate that the anti-ischemic effect of beta-blockers in HF cases should be because of impairment of angiogenesis with the VEGF production as the angiogenic mediator.

Erbs et al. have demonstrated the increased sera VEGF results in the HF cases with reduced ejection and additionally demonstrated the effect of statins on angiogenesis positively except the effect on the lipids [21]. Cantoni et al. also have shown rosuvastatin increased capillary formation with VEGF by evaluating the effect of rosuvastatin in human mesenchymal stem cells [22]. Our study was the first study evaluating the effect of statins on VEGF in HFpEF. None the patients using statin have also accompanied beta-blocker using. From this point of view, statins may induce angiogenesis via increasing the VEGF results in HFpEF.

This study has some limitations. Firstly, it is limited analyzing only one angiogenic molecule, VEGF and only one anti-angiogenic molecule sVEGFR-1, which makes it difficult to evaluate the disequilibrium of angiogenic/anti-angiogenic factors in HFpEF cases since other angiogenic factors have not been evaluated concurrently. However,

we think that the study is meaningful and valuable due to the fact that it has not been studied before in the literature and it is a clinical study. Finally, this study is based on a limited number of patients and thus, cannot ascertain whether these findings apply to other patients with HFpEF. Therefore, clinical studies with more participants should be suitable for approval of these findings.

In conclusion, statins and beta-blockers may have a positive effect on angiogenesis in HFpEF patients via increasing VEGF levels. Although no solid conclusion can be drawn from our study due to the small numbers of patients, it increases awareness about the physiopathological role of sera VEGF in HFpEF patients and the need of further studies.

Declaration of conflict of interest: None

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