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Serum VEGF-C levels as a candidate biomarker of hypervolemia in chronic kidney disease

Tuncay Sahutoglu, MD[a,*], Tamer Sakaci, MD[a], Nuri B. Hasbal, MD[a], Elbis Ahbap, MD[a], Ekrem Kara, MD[b], Mutlu C. Sumerkan, MD[c], Mustafa Sevinc, MD[a], Cuneyt Akgol, MD[a], Yener Koc, MD[a], Taner Basturk, MD[a], Abdulkadir Unsal, MD[a]

Abstract

Attaining and maintaining optimal "dry weight" is one of the principal goals during maintenance hemodialysis (MHD). Recent studies have shown a close relationship between Na^+ load and serum vascular endothelial growth factor-C (VEGF-C) levels; thus, we aimed to investigate the role of VEGF-C as a candidate biomarker of hypervolemia. Physical examination, basic laboratory tests, N-terminal pro b-type natriuretic peptide (NT-ProBNP), echocardiography, and bioimpedance spectroscopy data of 3 groups of study subjects (euvolemic MHD patients, healthy controls, and hypervolemic chronic kidney disease [CKD] patients) were analyzed. Research data for MHD patients were obtained both before the first and after the last hemodialysis (HD) sessions of the week. Data of 10 subjects from each study groups were included in the analysis. Serum VEGF-C levels were significantly higher in hypervolemic CKD versus in MHD patients both before the first and after the last HD sessions ($P = .004$ and $P = .000$, respectively). Healthy controls had serum VEGF-C levels similar to and higher than MHD patients before the first and after the last HD sessions of the week ($P = .327$ and $P = .021$, respectively). VEGF-C levels were correlated with bioimpedance spectroscopy results (r^2 0.659, $P = .000$) and edema (r^2 0.494, $P = 0.006$), but not with ejection fraction (EF) (r^2 −0.251, $P = .134$), blood pressures (systolic r^2 0.037, $P = 0.824$, diastolic r^2 −0.067, $P = .691$), and NT-ProBNP (r^2 −0.047, $P = .773$). These findings suggest that serum VEGF-C levels could be a potential new biomarker of hypervolemia. The lack of correlation between VEGF-C and EF may hold a promise to eliminate this common confounder. Further studies are needed to define the clinical utility of VEGF-C in volume management.

Abbreviations: CKD = chronic kidney disease, ECF = extracellular fluid, EF = ejection fraction, FHDW = first hemodialysis session of the week, LAD = left atrial diameter, LHDW = last hemodialysis session of the week, MHD = maintenance hemodialysis, PAP = pulmonary artery pressure, VCI = vena cava inferior index, VEGF-C = vascular endothelial growth factor-C.

Keywords: chronic kidney disease, hemodialysis, hypervolemia, VEGF-C

1. Introduction

The regulations of hydration status and gamut of electrolytes are some of the essential roles of the kidneys in physiology. Therefore, as the renal functions deteriorate, overhydration stands out as a common feature of chronic kidney disease (CKD) that healthcare professionals have been working on both to restore euvolemia and find out the optimal method for detection and monitoring of hypervolemia. In this regard, overhydration in

CKD may be arbitrarily categorized into 2 forms; overt hypervolemia with the presence of objective physical examination findings such as edema, lung rales and jugular venous distention, and obscure hypervolemia that is commonly regarded as the main cause of hypertension in dialysis population. As familiar to the nephrology community, despite straightforward detection and management of hypervolemic patients with undisputable clinical signs via traditional physical examination, concealed hypervolemia most often requires additional diagnostic tools such as ultrasonographic and bioimpedance spectroscopy assessments for a confident conclusion. Recent attention to the management of hidden hypervolemia in hemodialysis (HD), with the aid of bioimpedance spectroscopy and ultrasonographic surveillance, have been proven to yield improvement in cardiovascular outcomes and rates of mortality in randomized controlled trials.[1–4] However, bioimpedance spectroscopy and ultrasonographic evaluation of patients have not been adopted widely in daily practice, largely due to time limitation, necessity for experience and resource constraints. A reliable biomarker for volume expansion bears a potential to guide-treating physicians regarding fine tuning of volume management of patients with CKD as well as other conditions.

Recent studies have revealed that interstitial macrophages of the skin sense the accumulation of Na^+ within the interstitium and respond to accommodate the excesses of Na^+ and water, with vascular endothelial growth factor-C (VEGF-C) is their effective mediator.[5,6] We herein aimed to investigate whether VEGF-C has a potential role as a marker of volume status in patients with CKD.

2. Methods

2.1. Patients

There were 3 groups of subjects included in this study; 10 patients on chronic maintenance HD (MHD) for at least 3 months with euvolemia ascertained by physical examination, echocardiographic measures and bioimpedance spectroscopy, 10 CKD patients at predialysis stages with obvious hypervolemia evident by pretibial edema and lung rales, and 10 healthy subjects without any history of disease or medication use. Patients with a history of cancer, wound infection, peripheral arterial and/or venous disease, psychiatric disorders, infection with chronic hepatitis viruses or HIV, cirrhosis, infection, or hospital admission within the last 3 months were excluded.

Physical examination, bioimpedance spectroscopy, echocardiography, blood samplings for laboratory tests, and VEGF-C enzyme-linked immunosorbent assay (ELISA) were performed for healthy controls and hypervolemic patients in the morning at study admission and for MHD patients at both before the first HD and after the last HD sessions of the same week. Basic laboratory tests were run straightaway following blood samplings according to the usual laboratory processing, whereas serum samples for VEGF-C were stored at $-80°C$ until the test was performed.

2.2. Bioimpedance spectroscopy

Body Composition Monitor (Germany) (type 0BJA1394, Fresenius Medical Care AG & Co. KGaA, D-61343 Bad Homburg) was used for bioimpedance spectroscopy using 4 disposable electrodes each time and the results were expressed as positive or negative in milliliters.

2.3. VEGF-C test

VEGF-C levels were measured in the serum samples that were collected as described above and R&D Systems kit (Minneapolis, MN) (Catalog Number DVEC00) was used for the assay according to the user instructions.

2.4. Echocardiography

All echocardiography scans were performed by the same cardiologist who was blinded to the subject allocations.

2.5. Ethical approval and patients' consents

The study protocol was approved by the Ethics Committee of Sisli Hamidiye Etfal Education and Research Hospital. Written informed consent according to the Declaration of Helsinki was obtained from all participants.

2.6. Statistical analysis

Data were presented as mean ± standard deviation. Chi-square test was used for categorical variables and analysis of variance was used for parametric variables. Pearson's test was used for bivariate correlation analysis. Linear regression analysis was performed to plot the relationship between VEGF-C and bioimpedance results. P values less than .05 were considered statistically significant. IBM SPSS version 20 (Armonk, NY) was used for the statistical analysis.

3. Results

3.1. Baseline characteristics

Demographic, physical examination, echocardiography, bioimpedance, and laboratory parameters of all study subjects were given in Table 1. Hypervolemic patients were significantly older and had higher body mass index, greater volume load on bioimpedance, higher pulmonary artery pressure (PAP), larger left atrial diameter (LAD), lower left ventricular ejection fraction (EF), and lower vena cava inferior collapsibility index (VCI) than both MHD patients and healthy controls ($P < .05$).

All physical examination findings were similar in healthy and MHD patients, except for higher systolic and diastolic blood pressures in MHD patients recorded before the first HD sessions of the week (Table 1). In echocardiographic assessment, EF and VCI were not different between healthy controls and MHD patients, and LAD was significantly higher in MHD before the first HD sessions of the week (FHDW) and decreased to equal levels with healthy controls following the last HD sessions of the week (LHDW) (Table 1). Of note, bioimpedance results were at $+2560 ± 1726$ mL and dropped to $-1470 ± 781$ mL before FHDW and after LHDW in MHD patients, respectively.

N-terminal pro b-type natriuretic peptide (NT-ProBNP) levels were highly variable, high in both hypervolemic and MHD patients (before and after HD sessions), but there was not a significant difference between hypervolemic and MHD patients.

3.2. VEGF-C levels

VEGF-C levels were highly variable in all subjects (Table 1). Hypervolemic patients had significantly higher VEGF-C levels than MHD patients both before and after the HD sessions (Fig. 1). Healthy controls had similar levels of VEGF-C with MHD patients before the FHDW and significantly higher VEGF-C levels than MHD patients after LHDW. Although numerically higher in hypervolemic group, the difference with healthy controls was not at statistically significant level ($P = .222$).

3.3. Correlation analysis

In bivariate correlation analysis, VEGF-C and NT-ProBNP were significantly correlated with bioimpedance, presence of edema, lung rales, and LAD, but NT-ProBNP had additional significant correlations with blood pressure, history of hypertension, and EF (Table 2). VEGF-C and NT-ProBNP were not correlated.

In Fig. 2, the relationship between VEGF-C and bioimpedance was plotted using linear regression analysis via Enter method ($R^2 = 0.434$, $P = .000$).

3.4. ROC curve analysis

Figure 3 shows the receiver operating characteristic curve analysis of the relationship between VEGF-C levels and the presence of pretibial edema. When 5509.65 pg/mL was taken as a cutoff value for VEGF-C, the presence of pretibial edema could be predicted with 80% sensitivity and 90% specificity (area under the curve 0.850, $P = .001$, 95% confidence interval: 0.702–0.998).

4. Discussion

In this study, we had the opportunity to compare VEGF-C levels between euvolemic MHD patients, healthy controls, and hypervolemic patients, which revealed that clinically and echocardiographically confirmed euvolemic MHD patients had significantly lower VEGF-C levels (similar to healthy controls) than hypervolemic patients. Serum VEGF-C levels were lower in healthy controls than in hypervolemic CKD patients, which can be

Table 1

Demographic, anthropometric, physical examination, echocardiographic, and laboratory parameters of the 3 study groups with statistical comparisons.

	Before the first HD session of the week (n=10)	Healthy controls (n=10)	Hypervolemic patients (n=10)	After the last HD session of the week (n=10)	P
Demographic data					
Age, y	44.0±15.6	39.6±9.6	70.2±10.8	–	**.000**
Gender (F/M)	3/7	5/5	6/4	–	.43
AVF/catheter	8/2	–	–	–	NA
HD vintage, mo	23.2±16.9	–	–	–	NA
HT	7/10	0/10	9/10	–	**.000**
DM	3/10	0/10	7/10	–	**.000**
Smoking	5/10	4/10	1/10	–	.142
Physical examination					
BMI, kg/m^2	24.1±4.5	23.7±1.6	31.7±5.3	23.8±4.5	**.000**
Edema	0/10	0/10	10/10	0/10	**.000**
Rales	0/10	0/10	10/10	0/10	**.000**
JVD	0/10	0/10	4/10	0/10	**.010**
Systolic BP, mm Hg	142±20.9	104±10.7	138.8±24.7	111±20.2	**.000**
Diastolic BP, mm Hg	84±15.7	64±8.4	82.2±14.8	66.6±8.6	**.001**
Body composition monitor					
Bioimpedance, mL	+2560±1726	–	+6870±2490	−1470±781	**.000**
Echocardiography					
VCI, %	50.1±13.6	45.0±14.4	35.5±24.2	49.3±15.9	.467
EF, %	65.6±3.7	65.8±2.8	44.7±12.2	64.2±3.8	**.000**
LAD, cm	3.7±0.48	2.9±0.56	4.9±0.67	3.0±0.7	**.000**
PAP, mm Hg	33.5±3.5	–	60.5±20.8	20.7±2.9	**.001**
Laboratory tests					
Urea, mg/dL	145.8±29.9	27.7±8.7	153.2±80.84	–	**.000**
Creatitine, mg/dL	10.5±3.0	0.72±0.12	3.16±1.52	–	**.000**
Uric acid, mg/dL	6.8±1.4	4.2±0.8	7.1±2.1	–	**.001**
Na, mEq/L	132.9±4.0	138.3±2.3	137.6±5.8	–	**.020**
K, mEq/L	5.7±1.2	4.7±0.3	4.7±0.7	–	**.021**
Cl, mEq/L	93.3±4.7	98.7±1.7	98.6±7.4	–	**.040**
Albumin, g/dL	4.1±0.4	4.8±0.1	3.8±0.4	–	**.000**
Ca, mg/dL	8.0±1.2	9.5±0.2	8.7±0.5	–	**.001**
P, mg/dL	5.6±2.0	3.8±0.5	4.8±1.3	–	**.031**
iPTH	516.9±361.1	37.9±10.5	273.4±234.2	–	**.001**
CRP, mg/L	6.87±7.2	3.36±0.2	17.95±18.0	–	**.025**
Leukocytes	9705±2262	7791±3109	6559±1705	–	**.025**
Hemoglobin	11.5±1.0	13.8±0.8	9.6±1.5	–	**.000**
Platelets	239,200±56,815	251,800±39,521	206,400±71,299	–	.207
Ferritin, ng/mL	479.1±219.1	80.7±78.0	136.1±81.2	–	**.000**
NT-ProBNP, pg/mL	7558.7±10374	29.3±21.34	14215±12916	5254±7779	**.013**
VEGF-C, pg/mL	3787.8±1382	4945.8±1069	6262.9±2110	2895.6±1235	**.000**

AVF = arteriovenous fistula, BMI = body mass index, BP = blood pressure, CRP = C-reactive protein, DM = diabetes mellitus, EF = left ventricular ejection fraction, HD = hemodialysis, HT = hypertension, JVD = jugular venous distention, LAD = left atrial diameter, NT-ProBNP = N-terminal pro b-type natriuretic peptide, PAP = pulmonary artery pressure, VCI = vena cava index.
Bold and italic style was used to mark out statistically significant results.

explained by both high standard deviations and small number of patients. Significantly, healthy controls had significantly higher serum VEGF-C levels than MHD patients after the LHDW, which requires further clarification based on dynamic changes in VEGF-C levels during and in-between dialysis sessions.

The pathophysiology that underlies the increase in serum levels of VEGF-C in hypervolemia was asserted as the increase in Na$^+$ load leads to interstitial hypertonicity, which activates the tonicity responsive enhancer binding protein in the interstitial macrophages of the skin and downstream activation of transcription and secretion of VEGF-C.[6] An interesting observation made by Machnik et al[6] was that serum Na$^+$ concentrations were similar between low-salt- and high-salt-fed rats, whereas Na$^+$ concentrations in the skin carcasses were remarkably higher in the latter group, which suggests that Na$^+$ content of the skin increases independently from serum Na$^+$ concentrations with Na$^+$ overloading. This pathway is regarded
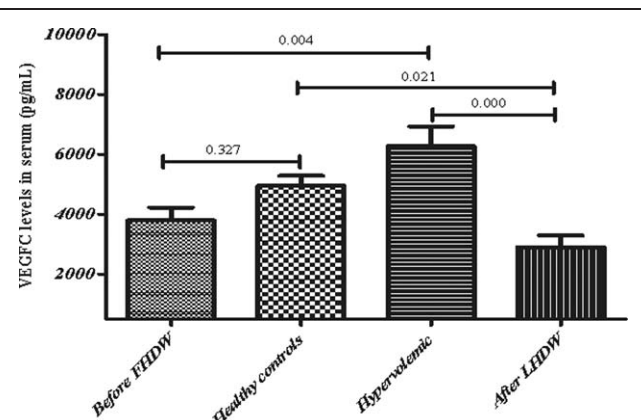

Figure 1. Vascular endothelial growth factor-C levels in serum across different groups of samples.

Table 2

Bivariate correlation analysis between VEGG-C, NT-ProBNP and conventional markers of volume status and echocardiographic parameters.

	VEGF-C		NT-ProBNP	
	r^2	P	r^2	P
Biompedance	0.659	.000	0.377	.040
Predialysis systolic BP	0.037	.824	0.624	.000
Predialysis diastolic BP	−0.067	.691	0.639	.000
History of HT	0.325	.080	0.408	.025
Edema	0.494	.006	0.456	.011
Rales	0.494	.006	0.456	.011
VCI	0.004	.983	−0.234	.214
EF	−0.251	.134	−0.518	.001
LAD	0.336	.042	0.443	.006
Na	0.078	.682	−0.040	.833
NT-ProBNP	−0.047	.773	−	−

BP = blood pressure, EF = left ventricular ejection fraction, HT = hypertension, LAD = left atrial diameter, NT-ProBNP = N-terminal pro b-type natriuretic peptide, VCI = vena cava inferior index.
Bold and italic style was used to mark out statistically significant results.

as a buffering mechanism that prevents the development of hypertension under high-salt diet, with supporting evidence from animal studies.[7,8]

Despite well-designed animal studies, there are a few human studies that describe the role of VEGF-C regarding volume regulation. High-salt diet was found to increase the levels of VEGF-C in both healthy subjects and patients with CKD.[9,10] In the study by Slagman et al,[10] high-salt diet resulted in increase in body weights of both healthy subjects and CKD patients, but rise of systolic and diastolic blood pressures concurred in only CKD patients. These findings, along with animal studies, suggest that Na$^+$ overload/hypervolemia could be evidenced by increase in VEGF-C levels before or even without the development of hypertension. Although a small number of subjects were studied, a statistically significant correlation was found between VEGF-C levels and bioimpedance, and the area under the curve of VEGF-C for predicting pretibial edema was found fairly high, which all point at a promising prospect for VEGF-C to be used as a biomarker of volume status.

The relationship between interstitial macrophages (hence VEGF-C) and Na$^+$ load may particularly be important from the perspective of a "cause and effect" of hypervolemia. The major determinant of extracellular fluid (ECF) volume is Na$^+$, and it is regulated through alterations in urinary Na$^+$ excretion; therefore, hypervolemic and hypovolemic states by other means reflect high and low Na$^+$ loads, respectively. Hence, the physiologic basis of the relationship between Na$^+$ and ECF volume could open a new research area; tracing a biomarker that has a direct correlation with Na$^+$ load for determining the ECF volume. In this regard, although NT-ProBNP is essentially a biomarker of left-ventricular stretch, rather than hypervolemia per se, we used it to compare whether VEGF-C would outperform as an associate of hypervolemia. Indeed, the correlation coefficient between VEGF-C and bioimpedance was higher than the correlation between NT-ProBNP and bioimpedance, and VEGF-C appeared to be exclusively associated with hypervolemia (edema, rales, but not EF), whereas NT-ProBNP was also associated with left-ventricular EF, systolic and diastolic blood pressures. These differences between VEGF-C and NT-ProBNP
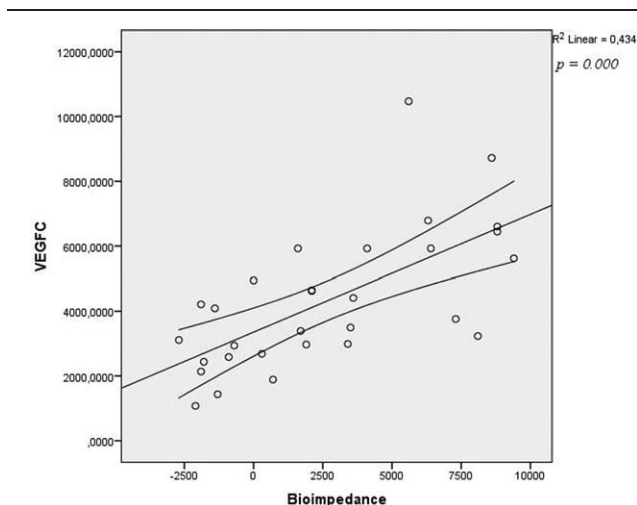

Figure 2. Linear regression analysis of vascular endothelial growth factor-C and bioimpedance (Enter method).
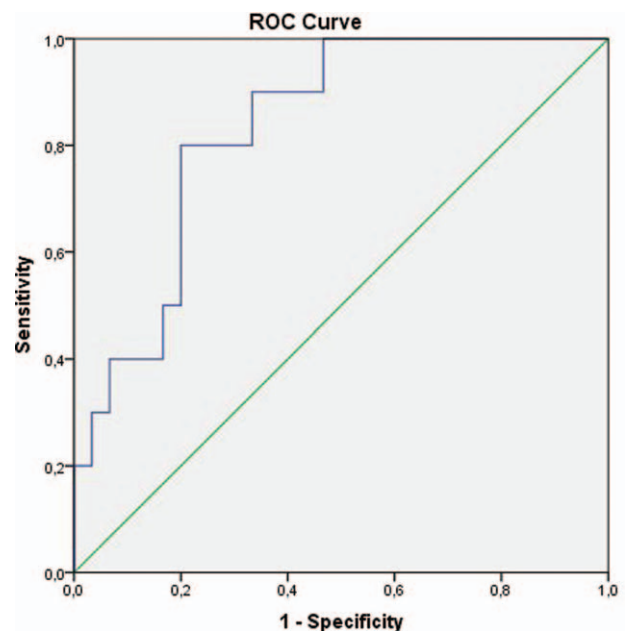

Figure 3. Receiver operating characteristic curve analysis of the relationship between vascular endothelial growth factor-C levels and pretibial edema (AUC: 0.850, P = .001, 95% confidence interval: 0.702–0.998). AUC = area under the curve.

might be important, because brain natriuretic peptides (BNPs) have been associated with hypervolemia and mortality in dialysis patients, but these peptides may not be specific biomarkers of hypervolemia.[11–14] Furthermore, the close association between CKD and left-ventricular dysfunction, hypertension, and coronary artery disease could render making a cutoff point for BNP to predict hypervolemia difficult. In this context, a specific biomarker of Na+ excess could be of great value for the distinction between hypervolemia and cardiac dysfunction.

5. Conclusion

In this study, we have shown that VEGF-C levels are high in hypervolemic and low in euvolemic (and hypovolemic) CKD patients. In addition, serum VEGF-C levels were significantly correlated with bioimpedance spectroscopy measurements. These results suggest that further studies are warranted to explore the relationship between serum VEGF-C levels and volume status and clinical outcomes in CKD patients.

Acknowledgments

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