Decreased serum level and increased urinary excretion of vascular endothelial growth factor-C in patients with chronic kidney disease

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Abstract

Background: Interstitial tonicity increases vascular endothelial growth factor-C (VEGF-C), a lymphangiogenic factor in salt-induced hypertension. Therefore, it can be assumed that changes of serum VEGF-C level may be associated with increasing blood pressure. However, there is no report about the changes of serum VEGF-C levels in patients with chronic kidney disease (CKD). The aims of this study were to investigate the changes of serum and urine VEGF-C levels in patients with CKD stage 3–4 and to evaluate the relationship between blood pressure and serum VEGF-C levels in the patients with CKD stage 5 and hemodialysis.

Methods: Glomerular filtration rate (GFR) was assessed by the Modification of Diet in Renal Disease equation. Blood pressure and VEGF-C levels (serum and urine) were measured by enzyme-linked immunosorbent assay (ELISA) in nine patients with stage 3–4 CKD, 41 hemodialysis patients, and eight healthy individuals.

Results: The median serum level of VEGF-C in patients with stage 3–4 CKD and stage 5 hemodialysis significantly decreased in comparison with healthy individuals. Urinary VEGF-C excretion increased in patients with stage 3–4 CKD compared with healthy control patients. For 41 hemodialysis patients, the serum level of VEGF-C in patients with stage 1 or stage 2 hypertension with hemodialysis did not significantly increase when compared with prehypertension hemodialysis patients.

Conclusion: We demonstrated that circulating levels of VEGF-C were decreased in patients with CKD, and the decrease of VEGF-C in patients with stage 3–4 CKD coincided with an increase in the urinary excretion of VEGF-C.

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Introduction

Vascular endothelial growth factor (VEGF) is a key molecule for angiogenesis and lymphangiogenesis [1]. The VEGF families are composed of six different proteins: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor. The VEGF binds three highly related receptor tyrosine kinases (RTKs), VEGFR-1, VEGFR-2, and VEGFR-3. One of the members of the VEGF family,
VEGF-C is known as a strong lymphangiogenic growth factor [2]. VEGF-C has high affinity for both VEGFR-2 and VEGFR-3 [2]. The VEGF-C through VEGFR-2 or VEGFR-3 has a critical role in mitogenesis, migration, and survival of lymphatic endothelial cells [3].

Recently, it was suggested that the mononuclear phagocyte system is involved in the control of interstitial volume and blood pressure (BP) homeostasis. High salt diet feeding activates the system is involved in the control of interstitial volume and blood pressure and 17 with stage 2 hypertension. Patients with CKD stage 3 or 4 were defined by the Modification of Diet in Renal Disease (MDRD) estimated glomerular filtration rate (eGFR) between 30–59 mL/min/1.73 m² and 15–29 mL/min/1.73 m², respectively. The dialysis patients had maintained hemodialysis three times a week and no medical events in the 6 months prior to inclusion in this study. The number of anuric HD patients was 38. In all of these patients, a detailed history and laboratory examination data were extracted by electronic medical records. Eight healthy patients were included as control patients. Edema was defined by depressing the skin with a finger by applying pressure to the pretilial area. Cerebrovascular disease included cardiovascular events such as myocardial infarction and/or revascularization procedures, stroke, new-onset or worsening congestive heart failure, and peripheral arterial disease.

**BP measurement**

BP was measured oscillometrically in the supine position in a quiet environment with an automated device (Dinamap, Critikon Co., Tampa, FL, USA) in healthy control patients and those with CKD stages 3 and 4. The mean of three consecutive measurements, taken 5 minutes apart after a rest of at least 20 minutes, was taken for analysis. In the HD patients, BP was assessed noninvasively (Phoenix Hemodialysis System, Gambro, Lund, Sweden) prior to and after hemodialysis in the supine position. We also measured BP every hour during hemodialysis. Mean value of at least four measurements was used for analysis. The patients were classified as having prehypertension (BP 120–139/80–89 mmHg), stage 1 hypertension (BP 140–159/90–99 mmHg), and stage 2 hypertension (BP ≥ 160/100 mmHg) [16].

**Antihypertensive medication**

Antihypertensive medication was evaluated by electrical medical records. Medication was quantified using the Anatomical Therapeutic Chemical (ATC) classification system. According to the World Health Organization recommendation, the defined daily dose was used as a measuring unit [17].

**Blood VEGF-C measurement**

After 2 hours of hemodialysis, blood samples for measurement of VEGF–C were drawn with the patient in a supine position. The serum level of VEGF–C was measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s guidelines (eBioscience, San Diego, CA, USA).

**Measurement of urinary VEGF–C**

VEGF–C urine levels were measured simultaneously with blood levels. The urine level of VEGF–C was measured by ELISA according to the manufacturers guidelines (eBioscience). Urine VEGF–C (pg) per urine creatinine (mg) was calculated by the urine level of VEGF–C (pg/mL) divided by urine creatinine (mg/dL).
Fractional excretion of VEGF–C

Fractional excretion (FE) of VEGF–C is the percentage of the VEGF–C filtered by the kidney that is excreted in the urine. This formula is represented mathematically as:

\[
\text{FE}_{\text{VEGF–C}} (\%) = 100 \times \frac{\text{Urine VEGF–C} \times \text{Serum creatinine}}{\text{Serum VEGF–C} \times \text{Urine creatinine}}
\]

Biochemical parameters

All measures were taken at the same clinical visit; prior to a midweek session of HD or at clinic review for patients with CKD stage 3–4. Serum and urine creatinine and serum cholesterol were measured using a Hitachi 7180 autoanalyzer (Hitachi High-Technologies Corporation, Tokyo, Japan). Serum samples from patients with hemodialysis were taken prehemodialysis.

Statistical analyses

Data are presented as median (25–75 percentiles) or as mean ± standard deviation (SD). Normality tests and other analyses were performed using the SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). The stage 3–4 CKD subgroups, HD patients, and the healthy control patients were analyzed using the Student t-test. The Chi-square test (or Fisher’s exact test) was used for comparing categorical variables. Nonparametric Mann-Whitney U tests were used for non-normally distributed variables (VEGF–C levels, urine VEGF–C/urine creatinine ratio, and FE of VEGF–C). The degree of clinical correlation was estimated by variable Pearson method. Statistical significance was accepted at a two-sided P value of < 0.05.

Results

Patient demographics

The clinical characteristics of the study participants are described in Table 1. The mean age of control patients was significantly lower than those of patients with CKD stage 3–4 and HD patients (31.3 ± 6.7 years in healthy control patients, 62.2 ± 14.4 years in patients with CKD stage 3–4, CKD, and 61.3 ± 13.3 years in HD patients; P < 0.01). The BPs and serum creatinine levels were significantly higher in patients with CKD stage 3–4 and HD patients compared with normal control patients (BP 141.8 ± 14.4/78.8 ± 14.5 mmHg in patients with CKD stage 3–4, 152.8 ± 12.3/83.1 ± 10.1 mmHg in HD patients, and 122.5 ± 8.1/76.2 ± 6.4 mmHg in healthy control patients, P < 0.01; mean serum creatinine level, 3.98 ± 2.06 mg/dL in patients with CKD stage 3–4, 7.37 ± 2.80 mg/dL in HD patients, and 0.82 ± 0.20 mg/dL in healthy control patients, P < 0.001). The mean eGFR was 61.3 ± 13.3 mL/min/1.73m2 in patients with CKD stage 3–4, 5.9 ± 1.9 mL/min/1.73m2 in HD patients, and 107.1 ± 22.4 mL/min/1.73m2 in healthy control patients (P < 0.01). There were no statistically significant differences in male-to-female ratio (2/7 in patients with CKD stage 3–4, 19/22 in HD patients, and 6/2 in healthy control patients) and percentage of smokers (3/9 in patients with CKD stage 3–4, 21/41 in HD patients, and 6/2 in healthy control

| Parameters | Controls (n = 8) | CKD stage 3–4 (n = 9) | CKD stage 5 with HD (n = 41) |
|------------|-----------------|----------------------|-----------------------------|
| Age (y)    | 31.3 ± 6.7      | 62.2 ± 14.4a         | 61.3 ± 13.3a                |
| Sex (male/female) | 6/2     | 2/7                 | 19/22                      |
| Smokers (n, %) | 3 (37.5) | 3 (33.3)            | 21 (51.2)                  |
| SBP (mmHg)  | 122.5 ± 8.1     | 141.8 ± 14.4         | 152.8 ± 12.3a              |
| DBP (mmHg)  | 76.2 ± 6.4      | 78.8 ± 14.5          | 83.1 ± 10.1b               |
| Serum Cr (mg/dL) | 0.8 ± 0.2 | 3.9 ± 2.1a          | 7.4 ± 2.8a                |
| eGFR (mL/min/1.73m2) | 107.1 ± 22.4 | 161 ± 8.2a      | 5.9 ± 1.9a                 |
| Total cholesterol (mg/dL) | 187.5 ± 37.8 | 181.4 ± 64.1 | 198.3 ± 56.9              |
| Diabetes mellitus (n, %) | 0 (0)    | 2 (22.2)            | 11 (26.8)                 |
| Cerebrovascular disease (n, %) | 0 (0)    | 1 (11.1)            | 8 (19.5)                  |
| Cancer (n, %) | 0 (0)     | 0 (0)               | 8 (19.5)                  |
| Diuretic (n, %) | 0 (0)    | 4 (44.4)            | 7 (17.0)                  |
| Edema (n, %) | 0 (0)      | 2 (22.2)            | 4 (9.7)                   |
| No. of patients with antihypertensive drug (n, %) | 0 (0) | 9 (100)*          | 38 (92.6)*                |

* P < 0.01 versus control subjects.

Table 2. The number of concomitant total antihypertensive drugs (The number of antihypertensive drugs/person)

| Antihypertensive drugs | CKD stage 3–4 (n = 9) | CKD stage 5 with HD (n = 41) |
|------------------------|-----------------------|-----------------------------|
| Calcium antagonist     | 9 (1.00)              | 37 (0.97)                   |
| β-blocker              | 1 (0.11)              | 4 (0.11)                    |
| α-blocker              | 0 (0)                 |                             |
| α- and β-blocker       | 4 (0.44)              | 14 (0.37)                   |
| ACE inhibitor          | 0 (0)                 | 22 (0.58)                   |
| ARB                    | 8 (0.89)              | 12 (0.32)                   |
| Vasodilator            | 0 (0)                 | 7 (0.18)                    |
| Diuretic               | 4 (0.44)              |                             |

ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker.
Urinary excretion of VEGF-C was increased in patients with CKD stage 3 compared with healthy control patients; patients with CKD stage 3 had 156.4 ± 44.1 ng of urine VEGF-C per mg of urine creatinine to healthy control patients, 3.53 ± 1.60 ng of urine VEGF-C per mg of urine creatinine in patients with CKD stage 3–4 and 0.00–0.03% in healthy control patients; P < 0.01 (Fig. 1B). These data suggested that urinary excretion of VEGF-C was increased in patients with CKD stage 3–4.

Decreased serum VEGF-C levels in patients with CKD stage 3–4

As shown in Table 3, median serum VEGF-C levels decreased in patients with CKD stage 3–4 compared with healthy control patients [114.6 (49.0–353.1) vs. 2341.1 (1679.1–7952.1) pg/mL; P < 0.05]. Median serum VEGF-C levels of the HD patients were also decreased in comparison with healthy control patients [146.4 (98.6–245.8) vs. 2341.1 (1679.1–7952.1) pg/mL; P < 0.05].

Increased urinary excretion of VEGF-C in patients with CKD stage 3–4

To evaluate whether urinary VEGF-C excretion was increased, we measured urine VEGF-C per urine creatinine and FE of VEGF-C in patients with CKD stage 3–4. The urine VEGF-C (pg) per urine creatinine (mg) were significantly increased in patients with CKD stage 3–4 compared with healthy control patients [40.1 (27.7–44.1) ng of urine VEGF-C per mg of urine creatinine to healthy control patients, 156.4 (82.0–283.0) ng of urine VEGF-C per mg of urine creatinine in patients with CKD stage 3–4; P < 0.01] (Fig. 1A). The FE of VEGF-C was also increased in patients with CKD stage 3–4 compared with healthy control patients [3.53 (1.60–14.11)% in patients with CKD stage 3–4 and 0.01 (0.00–0.03)% in healthy control patients; P < 0.01] (Fig. 1B). These data suggested that urinary excretion of VEGF-C was increased in patients with CKD stage 3–4.

Figure 1. Urinary excretions of VEGF-C in patients with CKD stage 3–4 (CKD stages 3 and 4) and healthy control subjects (Control). (A) Urine levels of VEGF-C per urine creatinine in patients with CKD stage 3–4 and healthy control subjects. (B) Fractional excretion of VEGF-C in patients with CKD stage 3–4 and healthy control subjects. *P < 0.05 versus control. CKD, chronic kidney disease; VEGF-C, vascular endothelial growth factor.

No relationship between serum VEGF-C and degree of hypertension

The concentrations of serum VEGF-C in patients with stage 1 hypertension or stage 2 hypertension tended to be higher than those in healthy control patients, but the differences did not reach statistical significance (536.5 ± 982.2 pg/mL to stage 1 hypertension; 386.8 ± 991.4 pg/mL to stage 2 hypertension, and 116.2 ± 30.6 pg/mL prehypertension; P > 0.05). Thus, our data showed that no significant differences in the serum concentrations of VEGF-C were detected between prehypertension, stage 1 hypertension, and stage 2 hypertension group in HD patients. We also evaluated the correlation between serum levels of VEGF-C and the number of antihypertensive drugs in HD patients. Our data showed that calcium antagonists were more frequently used with concomitant drugs in patients with stage 1 and stage 2 hypertension than in healthy control patients. However, other antihypertensive concomitant drugs were similar in all groups except calcium antagonists. The serum levels of VEGF-C were not correlated with the number of antihypertensive drugs used in patients with CKD stage 5. CKD stage 5. We demonstrated that serum VEGF-C levels decreased in the patients with CKD stage 3–4 and stage 5 and HD compared to healthy controls, and urinary excretion of VEGF-C was increased in patients with CKD stage 3–4.

Discussion

In this study, our results also demonstrated that the serum levels of VEGF-C in patients with CKD stage 5 were not correlated with the number of hypertensive drugs used. A diet high in salt has been implicated in hypertension. However, individual variations exist in the sensitivity of the BP to salt and various mechanisms for salt-sensitive hypertension [18]. The kidney is an organ that regulates total body sodium for homeostasis of extracellular fluid volume [19]. The extracellular volume consists of two distinct body fluid compartments, including the interstitial and the intravascular spaces. Because
extracellular body fluids are in equilibrium, excess interstitial sodium is readily moved into the bloodstream for external renal sodium excretion. It has been reported that lymphatic vessels drain the interstitial fluid [20]. Therefore, one of the critical functions of lymphatic system is regulation of the interstitial fluid in sodium excessive state.

Machnik et al [5] demonstrated that leukocyte in the skin interstitial space is involved in the development of hypertension. Mononuclear phagocyte cells such as macrophages participated in the pathogenesis of hypertension. A diet high in salt leads to interstitial hypertonic Na+ accumulation and activation of TonEBP in mononuclear phagocyte cells in the skin [4,5]. These authors suggested that TonEBP increases VEGF-C production and increases lymphangiogenesis [6,7]. Therefore, VEGF-C can be associated with sodium excessive state and activation of high TonEBP in high salt diet-induced hypertension.

Because VEGF-C is involved in salt-induced hypertension and hypertension in patients with CKD is linked to volume expansion, we assumed that serum levels of VEGF-C increase in the patients with hypertension and CKD. However, our data demonstrated that serum levels of VEGF-C in patients with CKD stage 3–4 and HD patients had significantly lower levels of VEGF-C than control patients. Therefore, the extra renal fluid homeostasis mechanism via the VEGF-C system in patients with CKD and hypertension can be evaluated in the future.

There is a tendency that the mean level of serum VEGF-C increased in stage 1 and stage 2 hypertension groups compared with prehypertension in HD patients. However, the differences did not reach statistical significance. Because there are various factors that can be involved in blood pressure regulation in patients with end-stage kidney disease, we also evaluated the relationship between the number of antihypertensive drugs used and stage of hypertension. Our results showed that there was no relationship between the number of antihypertensive drugs used and stage of hypertension. There may be many factors that can affect serum levels of VEGF-C in normal patients or those with CKD. However, to our knowledge, there are few reports about the regulating factors and metabolism of serum VEGF-C. Therefore, further studies are needed in this field in the future.

Lely et al [14] demonstrated that serum levels of VEGF-C increased in patients with preeclampsia. However, our results demonstrated that blood levels of VEGF-C decreased in patients with CKD stage 3–4 with hypertension. Also, our data regarding serum VEGF-C level in patients with CKD were different from those of patients with preeclampsia. We also found that urinary excretion of VEGF-C was increased in patients with CKD stage 3–4 compared with healthy control patients. Thus, all of these data suggested that decreased serum levels of VEGF-C may be associated with increased excretion of VEGF-C in patients with CKD stage 3–4. To our knowledge, these are the first data in the English literature about increased urinary excretion of VEGF-C in patients with CKD. However, further investigation is needed regarding the exact mechanism of VEGF-C kidney excretion.

There was evidence that increased serum levels of VEGF-C are associated with cancer and cancer metastasis [21–25]. Thus, we evaluated the prevalence of cancer in each group. Our demographic data showed that cancer prevalence in patients with CKD stage 3–4 and HD patients was not different compared with that in healthy control patients (Table 1). Although healthy control patients in this study were younger than other patients, the prevalence of diabetes mellitus and cerebrovascular disease was not different between groups. The patients with CKD stage 3–4 tended to take more diuretics compared with those in the healthy control group. However, statistical significance was not reached (P = 0.08).

Recently, Slagman et al [15] demonstrated that VEGF-C levels in patients with CKD and high salt intake are higher than in patients with CKD and low salt intake. By comparison, the serum levels of VEGF-C were low in our study of patients with CKD stage 3 and 4. The difference in serum VEGF-C levels between the study by Slagman et al and this study may be due to the difference in kidney function: creatinine clearance in the study by Slagman et al was 82 ± 6 mL/min and eGFR in this study was 16.1 mL/min/1.73 m2.

The clinical significance of this study was to develop the role of VEGF-C in patients with hypertension and CKD. Our data demonstrate that serum VEGF-C levels were decreased and urinary VEGF-C excretion was increased in patients with CKD stage 3 and 4. Our results also showed that serum VEGF-C levels were decreased in HD patients. We suggest that decreased serum VEGF-C levels may be associated with increased urinary excretion of VEGF-C. Furthermore, we suggest that the decreased serum levels of VEGF-C may be associated with hypertension in patients with CKD. However, we could not explain about the exact mechanism of decreased serum VEGF-C level in HD patients.

The current study has several limitations. Patients with CKD stages 3 and 4 and the normal control population are a relatively small group. There were fundamental differences of the three studied populations in age and the number of patients using antihypertensive drugs. Thus, these findings should be considered carefully in interpreting the results.

Our results demonstrate that circulating levels of VEGF-C decreased in patients with CKD and the decreased circulating VEGF-C levels may be associated with increased excretion of VEGF-C in patients with CKD stage 3–4. Additional studies are needed to evaluate the clinical relevance of the decreased lymphangiogenic factor, VEGF-C, in patients with CKD.

Conflict of interest

No conflict of interest.

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References

[1] Eilken HM, Adams RH: Dynamics of endothelial cell behavior in sprouting angiogenesis. *Curr Opin Cell Biol* 22:617–625, 2010
[2] Ferrara N: Vascular endothelial growth factor: Basic science and clinical progress. *Endocr Rev* 25:581–611, 2004
Strumberg D: Sorafenib for the treatment of renal cancer. J Clin Invest 123:407, 2012

Izzedine H, Ederhy S, Goldwasser F, Soria JC, Milano G, Cohen A, Lely AT, Salahuddin S, Holwerda KM, Karumanchi SA, Rana S: Slagman MC, Kwakernaak AJ, Yazdani S, Laverman GD, van den Born J, Titze J, Navis G: Vascular endothelial growth factor C levels are modulated by dietary salt intake in proteinuric chronic kidney disease patients and in healthy subjects. Nephrol Dial Transplant 27:978–982, 2012

Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo Jr JL, Jones DW, Materson BJ, Oparil S, Wright Jr JT, Roccella EJ: National Heart, Lung, and Blood Institute Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; National High Blood Pressure Education Program Coordinating Committee. The seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC-7 report. JAMA 289:2560–2572, 2003

Official version of the German Anatomical Therapeutic Chemical (ATC) Classification with defined daily doses (DDD) 2009: Deutsches Institut für Medizinische Dokumentation und Information, Cologne, 2009.

Guyton AC, Coleman TG, Cowley Jr. AW, Liard JF, Norman Jr. RA, Manning Jr RD: Systems analysis of arterial pressure regulation and hypertension. Ann Biomed Eng 1:254–281, 1972

Lifton RP, Charagi AV, Geller DS: Molecular mechanisms of human hypertension. Cell 104:545–556, 2001

Go WY, Liu X, Roti MA, Liu F, Ho SN: NFAT5/tonebp mutant mice define osmotic stress as a critical feature of the lymphoid microenvironment. Proc Natl Acad Sci U S A 101:10673–10678, 2004

Kimura H, Kato H, Tanaka N, Inose T, Faried A, Sohda M, Nakajima M, Fukai Y, Miyazaki T, Masuda N, Manda R, Fukuchi M, Ojima H, Tsuchida K, Kawanoh H: Preoperative serum vascular endothelial growth factor-C (VEGF-C) levels predict recurrence in patients with esophageal cancer. Anticancer Res 28:165–169, 2008

Tamura M, Ohta Y: Serum vascular endothelial growth factor-C level in patients with primary nonsmall cell lung carcinoma: A possible diagnostic tool for lymph node metastasis. Cancer 98:1217–1222, 2003

Vihinen PP, Hilli J, Vuoristo MS, Syrjanen KJ, Kahari VM, Pyrhonen SO: Serum VEGF-C is associated with metastatic site in patients with malignant melanoma. Acta Oncol 46:678–684, 2007

Wang TB, Deng MH, Qiu WS, Dong WG: Association of serum vascular endothelial growth factor-C and lymphatic vessel density with lymph node metastasis and prognosis of patients with gastric cancer. World J Gastroenterol 13:1794–1797, 2007

Yu XM, Lo CY, Lam AK, Leung P, Luk JM: Serum vascular endothelial growth factor C correlates with lymph node metastases and high-risk tumor profiles in papillary thyroid carcinoma. Ann Surg 247:483–489, 2008