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Peripheral Arterial Occlusive Disease – PAOD

Expression of Vascular Cell Adhesion Molecule-1 in Peripheral Artery Disease is Enriched in Patients with Advanced Kidney Disease

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Background: Serving as an inflammatory biomarker in patients under regular hemodialysis (HD), the arterial tissue expression of vascular cell adhesion molecule 1 (VCAM-1) in patients with different renal function has rarely been investigated and remains unclear.

Methods: Fifty-one consecutive patients with peripheral arterial disease (PAD) who underwent percutaneous transluminal angioplasty were recruited and divided into a normal renal function group, chronic kidney disease (CKD) group, and HD group. Background disease, clinical and angiographic severity, and serum level of VCAM-1 in the three groups were analyzed. The tissue expression of VCAM-1 was quantitatively demonstrated by immunohistochemical (IHC) staining and protein extraction from cell membranes in another amputated cohort.

Results: In PAD patients, the serum level of VCAM-1 was significantly elevated in the HD group compared with the other two groups (1990.2 ± 607.1 ng/ml vs. 1547.9 ± 511.2 ng/ml vs. 1161.0 ± 435.8 ng/ml, $p < 0.001$). Serum VCAM-1 was a prognostic factor of major adverse cardiac or limb events (odds ratio: 1.002, 95% confidence interval: 1.001-1.003, $p = 0.003$). The expression of VCAM-1 was higher in the PAD amputated arterial tissue of CKD and HD patients as demonstrated by quantitative analysis of IHC staining and quantitative membrane protein extraction.

Conclusions: VCAM-1 is a cardiovascular prognostic biomarker. Both serum level and the tissue expression of VCAM-1 were significantly higher in PAD patients with advanced kidney disease.

Key Words: Chronic kidney disease • Hemodialysis

INTRODUCTION

Cardiovascular disease is an important cause of morbidity and mortality in patients with chronic kidney disease (CKD).[1] Patients with CKD have a higher frequency of peripheral arterial occlusive disease, and especially the more severe types of Rutherford categories 5 and 6.[2,3] CKD is also a significant predictor of long-term outcomes in patients with peripheral arterial disease (PAD).[2]

The prevalence of CKD among PAD patients is high. In a multicenter registry of PAD patients enrolled in France, 44.3% of the cohort had moderate to severe renal dysfunction [estimated Glomerular filtration rate (eGFR) < 60 mL/min per 1.73 m^2]. In this cohort, CKD was also an independent predictor of 1-year mortality.[4]

PAD patients with CKD often present with more severe disease. These patients are difficult to manage by percutaneous transluminal angioplasty due to notable

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vessel calcification, multiple lesion sites, and high percentage of total occlusion. The progression of atherosclerosis in CKD patients can be related to non-traditional risk factors, including inflammation, oxidative stress, and endothelial dysfunction.[5,6]

Vascular cell adhesion molecule 1 (VCAM-1) is a protein associated with vascular inflammation. VCAM-1 protein mediates the adhesion of circulating white blood cells associated with inflammation, including lymphocytes, monocytes, eosinophils, and basophils to vascular endothelium. It also plays a role in leukocyte-endothelial cell signal transduction and the development of atherosclerosis.[7] Although circulating levels of VCAM-1 have been proposed to be associated with vascular atherosclerosis and considered to be predictors of all-cause death, cardiovascular death, and incidence of cardiovascular events in hemodialysis (HD) patients, evidence of higher tissue expressions of VCAM-1 in the atherosclerotic vascular tissue of CKD and HD patients has seldom been mentioned or proven.[8-10] Moreover, the HD procedure itself does not alter the serum level of adhesion molecules including VCAM-1.[11]

Regarding the relationship between VCAM-1 and PAD, patients with PAD are more likely to have significantly elevated levels of circulating soluble adhesion molecules.[12] Since the VCAM-1 level is higher in both advanced kidney disease and PAD patients, we hypothesized that the serum and tissue levels of VCAM-1 would be enriched in CKD and HD patients with PAD, and thus we aimed to evaluate the interaction between VCAM-1 expression and PAD disease severity in patients with different stages of renal disease.

MATERIALS AND METHODS

Patient source

From June 2011 to June 2012, we enrolled consecutive PAD patients who underwent percutaneous transluminal angioplasty (PTA) of the lower extremities at a single hospital (National Cheng-Kung University Hospital) in Taiwan. Details of the study protocol were explained to all participants and written informed consent was obtained before the study. The study was conducted according to the principles expressed in the Declaration of Helsinki and was approved by the Medical Ethics Committee of National Cheng-Kung University Hospital. All patients were documented to have PAD by angiography. The patients were divided into three groups based on renal function "normal renal function group" (normal group) was defined as an eGFR $\geq$ 60 mL/min per 1.73 m^2; "chronic kidney disease group" (CKD group) was defined as an eGFR between 15 mL/min per 1.73 m^2 and 60 mL/min per 1.73 m^2; and "end-stage renal disease under hemodialysis group" (HD group) was defined as an eGFR $\leq$ 15 mL/min per 1.73 m^2 and under HD. The patients' baseline characteristics were obtained from chart review, including past disease history and current medications. Three years later, the charts of the enrolled patients were reviewed again.

Clinical outcomes

The clinical outcomes of interest were major adverse cardiovascular events (MACEs) and major adverse limb events (MALEs) three years after the index PTA procedures. MACE was defined as stroke, myocardial infarction, and death. MALE was defined as acute or chronic limb ischemia needing re-intervention, and major amputation due to a vascular event above the forefoot.[13] The clinical outcomes were obtained from chart review and were confirmed by two physicians independently.

Severity of PAD

The clinical disease severity of PAD was determined using Rutherford classification.[14] Stage 0 was defined as asymptomatic. Stage 1 was defined as mild claudication. Stage 2 was defined as moderate claudication. Stage 3 was defined as severe claudication. Stage 4 was defined as ischemic resting pain. Stage 5 and stage 6 was defined as ischemic ulceration not exceeding ulcers of the digits of the foot, and severe ischemic ulcers or frank gangrene, respectively. The angiographic severity of PAD was presented as Bollinger score.[15]

Blood sampling

Blood samples were taken from the patients before the PTA procedure. Ten milliliter of blood was drawn at the arterial puncture site into a vacutainer without anticoagulants or other additives. Serum was separated by centrifugation at 2000 x *g* for 15 minutes at 4 °C, and then stored at -80 °C. All frozen samples were subsequently defrosted, and serum levels of VCAM-1 were

measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Human sVCAM-1/CD106 Quantikine ELISA Kit, R&D Systems Europe Ltd, UK).

Tissue sampling and immunochemical staining

Specimens from four groups of patients were collected. Group 1 (two cases) as normal controls, obtained from patients with no PAD, diabetes mellitus (DM) or CKD. Group 2 (two cases) from patients with PAD but with normal renal function. Group 3 (one case) from a patient with PAD and CKD. Group 4 (two cases) from patients with PAD and end-stage renal disease (ESRD) under HD. All patients underwent below the knee amputation. The investigated vessels (posterior tibial artery or peroneal artery) were dissected out. Endothelial cells and the smooth muscle cells on the main artery, and the vasa vasorum on the outermost layer of this artery were evaluated. Other smaller vessels and capillaries within adjacent adipose tissue were not included during the microscopic examination. Each artery from the patients in the four groups were preserved for cell lysate, and hematoxylin and eosin and immunohistochemical stained slides were reviewed at the same time. Immunohistochemistry of VCAM-1 was performed on the blocks of the collected specimens using rabbit anti-VCAM-1 (1:100 dilution, Proteintech).

Quantitative analysis of the VCAM-1 immunohistochemistry stain

The TissueGnostics GmbH (Vienna, Austria) platform provides tools to quantify protein expression levels in immunohistochemically (HistoQuest) stained tissue slides. The software is based on single cell detection by identifying nuclear structures.[15,16]

An initial contextual user analysis phase included visual assessment of the virtual slides from a pathological point of view. Whether or not the tissue was stained was first defined by an experienced pathologist. After the HistoQuest analysis of the slides, the degree of staining was quantified.

VCAM-1 extraction from the tissue

Amputated tissues were used. Since VCAM-1 is mainly a membrane protein, a Mem-PER^{TM} Plus Membrane Protein Extraction Kit (Thermo Scientific, USA) was used for membrane protein extraction. About 40 mg of soft tissue was placed in a 5 mL microcentrifuge tube for protein extraction. Then the membrane-associated protein of interest, VCAM-1, was further quantitatively analyzed using a Human VCAM-1 ELISA Kit (ScienCell, USA).

Statistical analysis

Categorical variables were expressed as number (%), and continuous variables were presented as mean ± standard deviation. Correlation coefficients between covariates of interest were calculated. Univariate logistic regression analysis was performed to investigate the independence of risk factors associated with MACEs and MALEs. Regarding the baseline characteristics, p values were tested using the chi-square test for categorical variables and ANOVA for continuous variables. Comparisons of serum VCAM-1 levels and tissue VCAM-1 expressions were conducted using nonparametric statistics with the Mann-Whitney U test and Kruskal-Wallis H test. Post-hoc analysis of the serum VCAM-1 level was performed using the Dunn test. All statistical analyses were performed using SPSS v.22.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Baseline characteristics, clinical laboratory data, and medications

Fifty-one patients were enrolled, including 18 in the normal renal function group, 10 in the CKD group, and 23 in the HD group (Table 1). The mean ages of the three groups were 68.7 ± 10.8, 72.9 ± 7.1, and 68.5 ± 9.2 years, respectively. All three groups were male predominant. Regarding the clinical risk factors, in the HD group and CKD group, more patients had hypertension and DM compared with the normal renal function group (hypertension: 91%, 91%, 61%, $p = 0.037$; DM: 91%, 90%, 33%, $p < 0.001$). The other clinical risk factors, including dyslipidemia, stroke, and coronary artery disease (CAD) were similar in these three groups.

As for the lab data, anemia was more frequent in the patients with CKD and ESRD under HD. The hemoglobin (Hb) levels in the three groups (HD, CKD, and normal renal function group) were 10.2 ± 0.9, 11.3 ± 1.8, and 12.7 ± 2.1 g/dl, respectively ($p < 0.001$). The mean

eGFR values of the three groups were 10 ± 4, 36 ± 17, and 70 ± 10 mL/min per 1.73 m^2, respectively, and the mean serum creatinine levels of the three groups were 5.8 ± 2.2, 2.4 ± 2.0, and 0.98 ± 0.2 mg/dl, respectively. There were no significant differences in white blood cell count, platelet count, liver enzymes, and lipid profiles of the three groups. Since medications such as antiplatelet agents and statin have been reported to protect the endothelium by hindering VCAM-1 expression, the medication history of the three groups was analyzed.[17-19] The use of antiplatelet medications including aspirin, clopidogrel, and cilostazol was similar in these three groups. There was also no significant difference in statin usage between the three groups.

PAD severity and PTA procedure success rate

The clinical severity of PAD showed a more serious trend in the patients with poor renal function (Table 1). The mean Rutherford scores in the HD, CKD, and normal renal function groups were 4.6 ± 0.9, 4.2 ± 0.8, and 3.8 ± 1.1, respectively (p = 0.068). However, the severity of angiography, presented as Bollinger score, was similar in

Table 1. Baseline characteristics of the enrolled PAD patients

	HD (n = 23)	CKD (n = 10)	Normal renal function (n = 18)	p value
Age (yrs)	68.5 ± 9.2	72.9 ± 7.1	68.7 ± 10.8	0.465
Male	12 (52)	6 (60)	13 (72)	0.426
Risk factors				
Hypertension	21 (91)	9 (90)	11 (61)	0.037*
DM	21 (91)	9 (90)	6 (33)	< 0.001*
Dyslipidemia	7 (30)	7 (70)	6 (33)	0.080
Stroke	5 (21)	5 (50)	5 (28)	0.257
CAD	17 (74)	7 (70)	10 (55)	0.451
Clinical severity				
Rutherford classification	4.6 ± 0.9	4.2 ± 0.8	3.8 ± 1.1	0.068
Angiography severity				
Bollinger score	46.2 ± 16.5	55.1 ± 21.5	40.5 ± 23.0	0.3116
Lab data				
WBC (*10^3)	9.0 ± 3.8	6.5 ± 1.5	8.2 ± 2.1	0.090
Hb	10.2 ± 0.9	11.3 ± 1.8	12.7 ± 2.1	< 0.001*
Platelet (*10^3)	243 ± 110	216 ± 67	236 ± 53	0.719
eGFR	10 ± 4	36 ± 17	70 ± 10	< 0.001*
Creatinine	5.8 ± 2.2	2.4 ± 2.0	0.98 ± 0.2	< 0.001*
GPT	37 ± 43	19 ± 8	25 ± 14	0.329
GOT	51 ± 60	22 ± 10	312 ± 11	0.404
Total cholesterol	168 ± 30	204 ± 31	191 ± 33	0.060
TG	165 ± 87	187 ± 149	135 ± 70	0.519
LDL	105 ± 33	111 ± 34	127 ± 13	0.355
Medication				
Aspirin	13 (57)	7 (70)	14 (77.8)	0.347
Clopidogrel	10 (44)	6 (60)	9 (50)	0.680
Cilostazol	19 (83)	8 (80)	9 (50)	0.058
Statin	4 (17)	4 (40)	6 (33)	0.321
PTA procedure success	22 (95.7)	9 (90.0)	18 (94.4)	0.408
MACE/MALE	17 (73.9)	5 (50.0)	3 (16.7)	0.001*

Data are presented as mean ± standard deviation or number (percentage). The p value was tested using Chi-square test for categorical variables and ANOVA test for continuous variables. * Indicates significant p values in different groups.
CAD, coronary artery disease; CKD, chronic kidney disease; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; Hb, hemoglobin; HD, hemodialysis; LDL, low-density lipoprotein; MACE, major adverse cardiac events; MALE, major adverse limb events; PAD, peripheral arterial disease; PTA, percutaneous transluminal angioplasty; TG, triglyceride; WBC, white blood cell count.

these three groups. The overall PTA procedure success rate was 96.1%. The success rate of the index PTA procedure was similar in the three groups (HD vs. CKD vs. normal renal function: 95.7% vs. 90.0% vs. 94.4%, p = 0.408). Each group had one patient with PTA procedure failure, all of whom underwent surgical bypass surgery thereafter.

Serum VCAM-1 concentration

There were significant differences in serum VCAM-1 concentration among the three groups (1990.2 ± 607.1 ng/ml in the HD group, 1547.9 ± 511.2 ng/ml in the CKD group, and 1161.0 ± 435.8 ng/ml in the PAD normal renal function group, p < 0.001). The HD group had a higher concentration than the other two groups (Figure 1).

Analysis of clinical outcomes and risk variables

MACEs and MALEs were analyzed three years after the index procedure. In the HD group, 17 patients (73.9%) had a MACE or MALE. Five patients (50.0%) in the CKD group had a MACE or MALE. Three patients (16.7%) in the normal renal function group had a MACE or MALE. Overall, 25 patients (49.0%) in this cohort had a MACE or MALE.

The serum level of VCAM-1 in the patients with or without MACEs/MALEs was also analyzed. In the patients with a MACE or MALE, the serum level of VCAM-1 was 1914 ± 567.1 ng/ml, which was significantly higher than that in the patients without a MACE or MALE (1363 ± 611.3 ng/ml, p = 0.001; Figure 2).

Risk variables which may have been associated with MACEs and MALEs, including age, CKD/HD, DM, hypertension, stroke, CAD, dyslipidemia, Rutherford ≥ 4, Bollinger score, and serum VCAM-1 concentration were investigated using logistic regression analysis. The results showed that the presence of CKD/HD [odds ratio (OR) = 9.998, 95% confidence interval (CI) = 2.380-42.005, p = 0.002], DM (OR = 6.286, 95% CI = 1.502-26.308, p = 0.012), and elevated serum VCAM-1 (OR = 1.002, 95% CI = 1.001-1.003, p = 0.003) were significantly associated with MACEs and MALEs (Table 2).

Immunohistochemical staining of PAD tissues

Cross sections of posterior tibial arteries or peroneal arteries were obtained from amputated lower extremities, including PAD patients with normal renal function, CKD, and HD.

Immunohistochemical staining of VCAM-1 was detected in the cytoplasm of endothelial cells and smooth muscle cells in the HD amputated arterial tissues (Figure 3A). VCAM-1 staining was also detected in the endothelial cells but not the smooth muscle cells in CKD amputated arterial tissues (Figure 3B). Only scattered endothelial cells showed low to moderate intensity in the control group with normal renal function (Figure 3C).

The staining results were further analyzed and quantified by HistoQuest. Examples of quantitative immu-

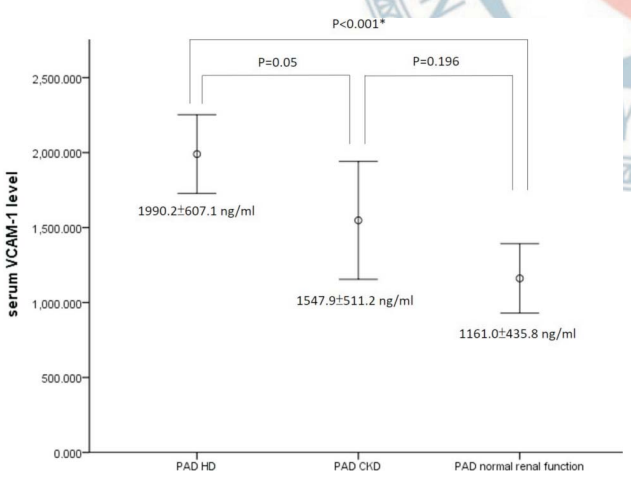

Figure 1. *Serum VCAM-1 level in PAD patients of different renal function. Comparison of serum VCAM-1 level of PAD patients with different renal function. Serum level of VCAM-1 was elevated in HD group in comparison with the normal renal function group (p < 0.001), and with the CKD group (p = 0.02). HD, hemodialysis; MACE, major cardiac events; MALE, major adverse limb events; VCAM-1, vascular cell adhesion molecule 1.*

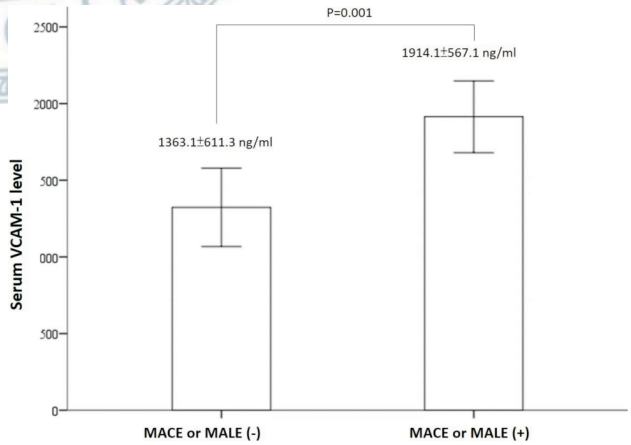

Figure 2. *Serum VCAM-1 level in PAD patients with different clinical outcome. Comparison of serum VCAM-1 level of PAD patients with clinical outcome. Serum level of VCAM-1 was elevated in patients with MACE or MALE in comparison with other patients without MACE or MALE (p = 0.001). Abbreviations are in Figure 1.*

nohistochemical stain analysis of three patients with different renal function are shown in Figure 4A-C. The stained ratio in the CKD and HD groups was 9.26 ± 4.47% and was significantly higher than that in the normal renal function group (2.61 ± 0.40%, p = 0.028, Figure 4D). According to these data, the VCAM-1 distribution was significantly higher in the PAD patients with advanced kidney disease, including CKD and HD.

Table 2. Univariate logistic regression analysis of risk variables with associated end point (MACE or MALE)

	Crude OR (95% CI)	p value
Age	1.030 (0.970-1.094)	0.333
CKD/HD	9.998 (2.380-42.005)	0.002*
DM	6.286 (1.502-26.308)	0.012*
Hypertension	1.575 (0.386-6.423)	0.527
Stroke	1.277 (0.382-4.270)	0.691
CAD	1.607 (0.495-5.217)	0.430
Dyslipidemia	1.067 (0.346-3.284)	0.910
Rutherford ≥ 4	2.045 (0.670-6.244)	0.209
Bollinger score	0.987 (0.954-1.020)	0.435
VCAM-1	1.002 (1.001-1.003)	0.003*

Data are presented as odds ratio (95% confidence interval). The p value was tested using logistic regression. * Indicates significant p values in different groups.
CAD, coronary artery disease; CI, confidence interval; CKD, chronic kidney disease; DM, diabetes mellitus; HD, hemodialysis; MACE, major cardiac events; MALE, major adverse limb events; OR, odds ratio; VCAM-1, vascular cell adhesion molecule 1.

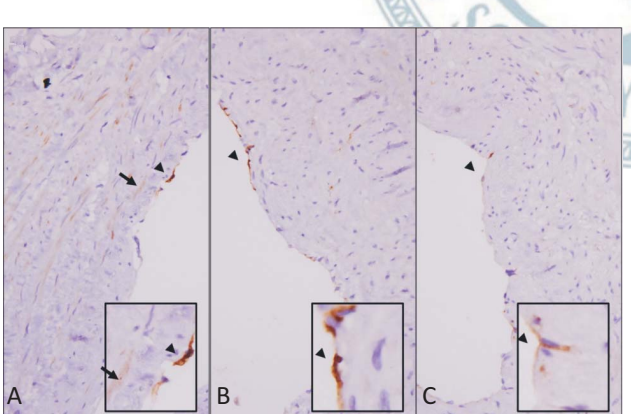

Figure 3. *VCAM-1 immunohistochemical stain in human arterial tissue. Cross section of artery (posterior tibial artery or peroneal artery) in (A) PAD with HD, (B) PAD with CKD, and (C) normal control from patient with normal renal function. Immunohistochemical stain of VCAM-1 was positive (brown in color) on the cytoplasm of endothelial cells (arrowhead) and smooth muscle cells (arrow) in (A), positive on the endothelial cells but not the smooth muscle cells in (B), and only focally weak positive in (C). Abbreviations are in Figure 1.*

Quantification of tissue VCAM-1 by ELISA in the PAD tissues

To demonstrate the consistency of serum level and tissue expression of VCAM-1, quantification of cell membrane protein by ELISA was performed. VCAM-1 tissue expression was quantified using a membrane protein extraction kit. The arterial tissue expression of VCAM-1 was significantly higher in the CKD and HD groups (5648.4 ± 1077.7 pg/ml) compared with the normal renal function group (945.0 ± 349.7 pg/ml, p < 0.001, Figure 5). These results consistently demonstrated that the serum level of VCAM-1 and tissue expression of VCAM-1 were significantly higher in the PAD patients with advanced kidney disease.

DISCUSSION

The main findings of this study were: (1) the serum level of VCAM-1 was elevated in PAD patients with advanced kidney disease; (2) serum VCAM-1 level contributed to cardiovascular events including MACEs and MALEs; and (3) the tissue expression of VCAM-1 was higher in vessel tissues of CKD and HD patients.

VCAM-1 is a cell surface adhesion molecule involved

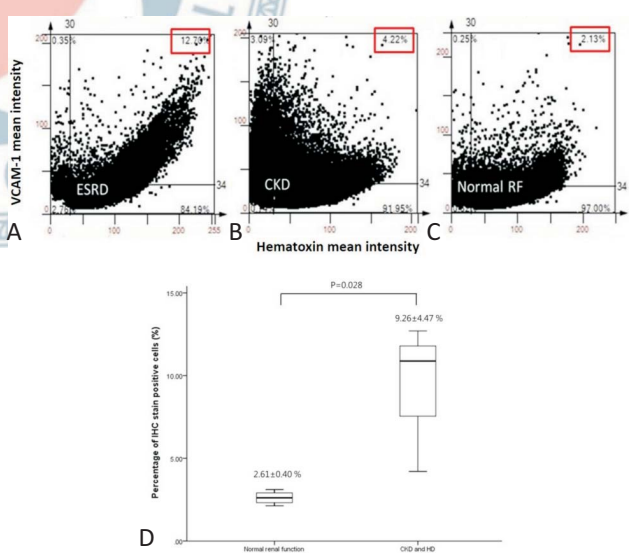

Figure 4. *Quantitative analysis of immunohistochemical stain in human arterial tissues. Examples of quantitative immunohistochemical stain analysis using HistoQuest of three patients with different renal function were displayed in (A)-(C). Enrichment of VCAM-1 expression in CKD and HD patients in comparison with normal renal function patients (p = 0.028) was illustrated in (D). Abbreviations are in Figure 1.*

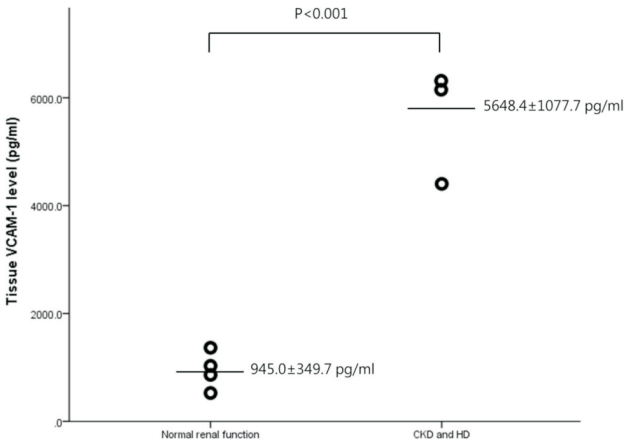

Figure 5. *Cell membrane expression of VCAM-1. Quantitative cell membrane VCAM-1 expression analysis was performed by membrane protein extraction kit and ELISA. Expression of VCAM-1 was enriched in the PAD amputated arterial tissues of CKD and HD and patients in comparison with that of normal renal function. Abbreviations are in Figure 1; ELISA, enzyme-linked immunosorbent assay.*

in the recruitment of leukocytes to endothelial cells on arterial walls during the pathogenesis of atherosclerosis. A high serum level of VCAM-1 has been associated with arteriovenous fistula stenosis, atherosclerosis, and long-term mortality in HD patients.[10,20,21] In the present study, we found that the serum level of VCAM-1 was elevated in both the HD patients and also in the patients with MACEs or MALEs. The clinical impact of elevated serum VCAM-1 was thus clearly confirmed in our study.

Although significant elevation of serum VCAM-1 in HD patients has been reported, the arterial tissue expression of VCAM-1 has seldom been reported. In this study, we demonstrated that the tissue expression of VCAM-1 in amputated lower extremities was significantly higher in the PAD arterial tissues of HD patients. We also found that VCAM-1 was abundantly expressed not only in arterial endothelial cells, but also in arterial smooth muscle cells in the PAD patients undergoing HD. VCAM-1 is generally considered to be present in vascular endothelial cells with the role of trapping macrophages in the atherosclerotic process. Only a few animal studies have mentioned the expression of VCAM-1 in vascular smooth muscle cells. Bro et al. reported that the expression of VCAM-1 was higher within aortic vascular smooth muscle cells in a mouse model of chronic renal failure.[22] In our study, we found that the VCAM-1 expression in arterial smooth muscle cells was more significant in the arterial smooth muscle cell layer of the patients with advanced kidney disease compared to those with normal renal function. In our opinion, the VCAM-1 expression within endothelial cells and vascular smooth muscle cells in the CKD and HD groups may have had a combined effect in aggravating the progression of atherosclerosis in these patients. Further studies are necessary to clarify the role of VCAM-1 in vascular endothelial cells and smooth muscle cells individually.

In terms of the relationship between tissue expression and serum level of VCAM-1, previous studies have shown that inflammatory cytokines such as TNF-α and IL-1β can stimulate TIMP-3-regulated VCAM-1 ectodomain (soluble VCAM-1) shedding from the cell surface to serum[23,24] In our study, the tissue expression and serum level of VCAM-1 were similar in the patients with different renal function.

Treatment against VCAM-1 to ameliorate atherosclerosis has been developed. Human antibodies against human VCAM-1 were used in a previous study to validate the hypothesis that blocking VCAM-1 ameliorates atherosclerosis in apolipoprotein E-deficient mice. The anti-VCAM-1 antibodies attenuated atherosclerosis by improving plaque inflammation and stability, and also inhibited the adhesion of inflammatory cells to the endothelium.[25] Recently, vitamin D administration has also been reported to be effective in reducing serum levels of VCAM-1.[26] However, it is doubtful whether reducing serum levels of VCAM-1 will translate to a similar reduction in the tissue expression of VCAM-1. Meanwhile, the long-term cardiovascular outcomes after reducing VCAM-1 still need to be investigated. Nevertheless, lowering VCAM-1 still shed light on attenuating atherosclerosis in patients with advanced kidney disease.

Study limitation

The present study was mainly limited by the number of patients. The baseline characteristics also showed that more patients in the CKD and HD groups had diabetes mellitus and hypertension, which may have interfered with the results. Moreover, due to ethical issues and reluctance of the patients, it was difficult to obtain amputated lower extremities to show more significant results. Finally, since this was a PAD cohort study, it was difficult to obtain normal individual's serum to check

the VCAM-1 level under normal circumstances.

CONCLUSION

In summary, our study demonstrated that the serum and tissue expressions of VCAM-1 were consistently higher in patients with advanced kidney disease. The serum VCAM-1 level also contributed to MACEs and MALEs in the HD patients with PAD. VCAM-1 can serve as a promising marker of atherosclerosis and a predictor of cardiovascular outcomes.

CONFLICT OF INTEREST

All the authors declare no conflicts of interest.

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