Original Paper

Soluble Urokinase Plasminogen Activator Receptor is Associated with Coronary Artery Calcification and Cardiovascular Disease in Patients Undergoing Hemodialysis

Wenjuan Wu^a Yanhai Cui^b Juan Hu^a Ruyi Liao^a Sijia Li^a Liyi Mo^a Lixia Xu^a
Yuanhan Chen^a Zhiwen Lian^a Xiulan Tian^a Zhuo Li^a Ruizhao Li^a Li Zhang^a
Huaban Liang^a Jianchao Ma^a Ting Lin^a Zhonglin Feng^a Wei Dong^a
Guibao Ke^a Pingjiang Ge^c Zhiming Ye^a Wenjiang Wang^a Bing Zhang^a
Wei Shi^a Xinling Liang^a Shuangxin Liu^a

^aDepartment of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangdong Geriatrics Institute, Guangzhou, ^bDepartment of Radiology, Guangdong General Hospital, Guangdong Academy of Medical Science, Guangzhou, ^cDepartment of Otolaryngology, Guangdong General Hospital, Guangdong Academy of Medical Science, Guangzhou, China

Key Words

Hemodialysis • SuPAR • Vascular calcification • Cardiovascular diseases

Abstract

Background/Aims: Cardiovascular disease (CVD) is an important cause of morbidity and mortality in hemodialysis patients. Vascular calcification is thought to play an important role in causing CVD. Soluble urokinase plasminogen activator receptor (suPAR) is a biomarker strongly predictive of cardiovascular outcomes in the pathogenesis of diabetic patients with renal disease treated with hemodialysis. We investigated the relationship between suPAR and coronary artery calcification (CAC) in patients undergoing maintenance hemodialysis.

Methods: A total of 99 adult hemodialysis patients were enrolled in this study. Plasma samples were analyzed for suPAR with an enzyme-linked immunosorbent assay and the CAC score was determined with multidetector computed tomography. The occurrence of cardiovascular events and all-cause mortality during follow-up were recorded from January 1, 2010 to June 1, 2016. **Results:** In 99 patients treated with maintenance hemodialysis, 91 (91.9%) had varying degrees of CAC, and suPAR correlated positively with the CAC score in a Spearman analysis. In a mean follow-up period of 33 months, 36 patients (36.4%) experienced at least

W. Wu, Y. Cui and J. Hu contributed equally to this work.

Xinling Liang
and Shuangxin Liu

Department of Nephrology, Guangdong General Hospital,
Zhongshan Er Lu 106, Guangzhou City, Guangdong (China)
E-Mail xinlingliang_ggh@139.com; 13543456446@139.com

one cardiovascular event. When the quartiles of suPAR concentrations were used as the cutoff points for a subgroup analysis, the incidence of CVD and all-cause mortality was much higher in the higher quartiles of suPAR. In a univariate Cox regression analysis, high suPAR was a risk factor for CVD and all-cause mortality. **Conclusion:** suPAR is associated with the CAC score and is a risk factor for new-onset CVD in patients undergoing hemodialysis.

Introduction

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Chronic kidney disease (CKD) is a growing public health problem, affecting over 10% of the world's population [1]. At least 2 million patients are receiving dialysis for end-stage renal disease (ESRD) throughout the world [2]. Despite advances in dialysis technology, the morbidity and mortality of dialysis patients has not improved much and is unacceptably high. Cardiovascular disease (CVD)-related morbidity and mortality in ESRD patients undergoing hemodialysis is 10–30-fold higher than in the general population. Vascular calcification is an important cause of morbidity and mortality in hemodialysis patients.

Soluble urokinase plasminogen activator receptor (suPAR), a new inflammatory biomarker, is produced during the inflammatory response. When the urokinase plasminogen activator receptor (uPAR) is cleaved from the cell membranes of endothelial cells and macrophages, it becomes a soluble factor [3]. Previous studies have shown that suPAR is associated with the coronary artery calcification (CAC) score (CACS) independently of the systematic coronary risk, and provides prognostic information on the CVD risk in the general population [4–7]. suPAR is also a biomarker strongly predictive of cardiovascular outcome in the pathogenesis of diabetic renal disease patients treated with hemodialysis [8–9]. However, it is unclear whether suPAR levels are associated with CAC in patients undergoing hemodialysis. Therefore, we investigated the relationship between suPAR, CAC, and CVD in patients treated with maintenance hemodialysis.

Materials and Methods

Study design

This was a prospective study carried out between January 2010 and June 2016 at the Nephrology Department of the Guangdong General Hospital (Guangdong, China). All experiments were approved by the Ethical Committee of Guangdong General Hospital (GDREC 2010201H). The study subjects were informed of the purpose and nature of the study and gave their written informed consent to their participation.

Ninety-nine adult patients (18–75 years), consisting of 53 males and 46 females, who had been treated with ongoing hemodialysis for at least 6 months at Guangdong General Hospital, were enrolled in the study and invited to undergo an multidetector computed tomography scan. Patients with an active infection (high-sensitivity C-reactive protein [hs-CRP] > 12 mg/L) were excluded. Among the remaining study subjects, 63 had glomerulonephritis, 14 had diabetic nephropathy, 12 had hypertensive nephropathy, 4 had polycystic kidney, 2 had renal artery stenosis, 2 had obstructive nephropathy, 1 had malignant renal tumor, and 1 had an unknown etiology. The median age was 52.0 ± 14.3 years and the median period of dialysis was 72.5 ± 58.6 months. All the patients underwent routine hemodialysis with a 4 h dialysis session three times a week, with standard high-flux dialysis membranes. The dialysate contained the following concentrations: Na 135–140 mmol/L, K 2.0–3.0 mmol/L, Ca 1.25–1.5 mmol/L, and bicarbonate 25.0 mmol/L. The patients were evaluated for dialysis adequacy every 3 months and spKt/V was > 1.2 in all patients.

Biochemical analyses

Blood was drawn before dialysis at the time of the regular dialysis treatment. Blood levels of suPAR were measured in frozen EDTA-plasma samples stored at -80°C . The samples were analyzed with the human uPAR Quantikine® ELISA Kit (R&D Systems, Minneapolis, MN), according to the instructions of the manufacturer.

Serum calcium, phosphorus, albumin (Alb), hemoglobin (Hb), alkaline phosphatase (ALP), hs-CRP, and intact parathyroid hormone (iPTH) were measured with standard procedures at the Department of Laboratory at Guangdong General Hospital.

CAC

CACS was determined with a noncontrast cardiac computed tomography scan performed during an inspiratory breath hold, by an experienced cardiologist. To assess CAC, the Radiology Department used a GE 64-slice CT scanner (GE LightSpeed VCT, Massachusetts) with the following technical settings: gantry rotation time 500 ms, 2.5 mm collimation, 120 kV tube voltage, 200 mA tube current, and prospectively electrocardiography-triggered scan acquisition gating at 50% of the R-R interval.

The CACS was calculated by adding the scores from all the foci in the coronary arteries, and is given in Agatston units (U) [10]. To make it more manageable in the clinical setting, CACs was divided into five categories: 0, 1–9, 10–99, 100–399, > 400 U, corresponding to none, very low, low, moderate, and severe coronary calcification [11]. A cut-off value of 100 U, which is generally accepted as corresponding to significant coronary calcification, was chosen for the final analysis [12].

Recording CVD events and mortality

Patients were followed-up prospectively at our dialysis center until June 1, 2016. If the patient was referred for peritoneal dialysis or kidney transplantation, they were deemed to be lost to follow-up, starting from the date of the last actual visit. The cardiovascular end-points were recorded based on clinical and biochemical data. The primary end-points were overall mortality and the time to the first cardiovascular event. The latter was a composite of death from cardiac causes, nonlethal myocardial infarction (MI), myocardial ischemia, coronary intervention, and ischemic stroke (ICD-10) [13], whichever occurred first. Only one event per subject was included in the analysis.

Statistical analyses

Data are expressed as means (standard deviations) for normally distributed variables or medians with the 25th and 75th percentiles for nonnormally distributed variables. The normally distributed continuous variables were compared with Student's *t* test, whereas the nonnormally distributed continuous variables were analyzed with the nonparametric Mann–Whitney *U* test. Categorical variables are presented as frequencies and the corresponding percentages, and a χ^2 test or Fisher's exact test was used to compare them, as appropriate. Variables with a nonnormal distribution were log-transformed using the natural logarithm. The correlations between the levels of suPAR and other variables were calculated with Spearman's rank correlation coefficients. The cumulative incidence of end-point events was calculated with the Kaplan–Meier method. The time to mortality and the time to the first cardiovascular event were analyzed with the Cox proportional hazards model. The results are expressed as ratios of the expected geometric means between groups, with their 95% confidence intervals. Two-sided *P* < 0.05 was considered statistically significant. All statistical analyses were performed with SPSS 23.0.

Results

Patient characteristics

The study sample comprised 99 subjects, of whom 53 were men and 46 women. The patients were divided two groups according to their cardiovascular events (Table 1). Compared with the no-CVD group, the CVD group had higher CACS, higher hs-CRP levels, and had experienced longer periods of dialysis. Plasma suPAR levels were significantly higher in the CVD group than in the no-CVD group (*P* = 0.043). There were no significant differences between the CVD group and no-CVD group in the parameters age, sex, serum calcium, phosphorus, iPTH, Hb and Alb.

Table 1. Comparison of patient characteristics in the CVD and no-CVD groups. Abbreviations: iPTH, intact parathyroid hormone; hs-CRP, high-sensitivity C-reactive protein; CAC, coronary artery calcification; CVD, cardiovascular disease; suPAR, soluble urokinase plasminogen activator receptor. Data are means $\pm$ SD, medians (25–75 percentiles), or numbers (%) P values are reported for differences between the CVD group and no-CVD group

Characteristics	No CVD (n=63)	CVD (n=36)	P
Age (year)	50.0 $\pm$ 14.3	55.4 $\pm$ 14.0	0.074
Male (n, %)	34 (54%)	19 (52.8%)	0.91
Time on hemodialysis (month)	60 (34,72)	74 (48,117)	0.009
Calcium (mmol/L)	2.3 $\pm$ 0.2	2.3 $\pm$ 0.4	0.584
Phosphorous (mmol/L)	2.0 $\pm$ 0.7	1.8 $\pm$ 0.9	0.380
iPTH (pg/mL)	314 (178,1003)	413 (119,1586)	0.670
Hemoglobin (g/L)	105 $\pm$ 20.0	106.8 $\pm$ 21.4	0.678
Albumin (g/L)	33.9 $\pm$ 4.1	32.8 $\pm$ 5.1	0.211
Alkaline phosphatase (U/L)	153.8 $\pm$ 236.4	199.5 $\pm$ 218	0.344
hs-CRP (mg/L)	2.9 (1.9, 4.2)	3.8 (2.7, 4.9)	0.021
CACS (score)	71.5 (17.8, 190.4)	910 (308, 1403)	<0.001
suPAR (pg/mL)	3449 (864, 6533)	5803 (2948, 7666)	0.043
Hypertension (n, %)	51 (81%)	31 (86.1%)	0.518
Diabetes mellitus (n, %)	8 (12.7%)	8 (22.2%)	0.220

Comparison of suPAR in diabetic and nondiabetic patients treated with maintenance hemodialysis

Among the patients treated with maintenance hemodialysis, 16 had diabetes and 83 were not diabetic. Although suPAR was higher in the diabetic group than in the nondiabetic group, the difference was not significant (Fig. 1).

Relationship between CACS and suPAR

When the relationship between CACS and suPAR was examined with a Spearman rank analysis, the two variables were found to be positively associated ($r = 0.315$, $P = 0.001$) (Fig. 2). Furthermore, when CACS was divided into two groups, $CACS < 100$ and $CACS \geq 100$, the suPAR plasma levels were significantly higher in the $CACS \geq 100$ group (4808 [2350, 7267] pg/mL) than in the $CACS < 100$ group (2742 [644, 6572] pg/mL; $P = 0.048$).

suPAR and CVD

We analyzed the association between suPAR and CVD in patients undergoing maintenance dialysis, with a mean follow-up period of 33 months. During the study period, 36 of the 99 patients (36.4%) experienced at least one cardiovascular event. The quartiles of the suPAR levels were used as the cut-off points for the subgroup analysis, and a clear association was observed between higher suPAR and new-onset CVD in these patients (Table 2). In a univariate Cox regression analysis, high suPAR was a risk factor for CVD (hazard ratio [HR] = 2.573 [1.708–3.876], $P < 0.001$).

In the univariate Cox analysis, high suPAR was directly associated with cardiovascular events, as were greater age, longer period of dialysis, higher CRP, CACS, and ALP (Table 3). In the COX proportional hazards model after adjustment for multiple factors, suPAR remained an independent risk factor for CVD (HR = 2.960 [1.638–5.355], $P < 0.001$). In a multivariate

Fig. 1. Comparison of suPAR in diabetic and nondiabetic patients undergoing maintenance hemodialysis.

Fig. 2. Correlation between suPAR and CACS. A significant positive correlation was detected between CACS and suPAR ($r = 0.315$, $P = 0.001$).

Cox analysis, suPAR was independently associated with cardiovascular events after adjustments were made for multiple factors ($HR = 3.629$ [1.823 – 7.225], $P < 0.001$).

In the univariate Cox regression analysis, high suPAR was a risk factor for mortality ($HR = 2.806$ [1.139 – 6.915], $P = 0.025$). In the Cox proportional hazards model after adjustment for multiple factors, suPAR was no longer an independent factor for mortality ($HR = 2.401$ [0.666 – 8.66], $P = 0.181$) (Table 4).

Table 2. Comparison of new-onset CVD and death in each quartile of suPAR

Variable	CVD (n, %)	Death (n, %)
Quartile 1	4(16%)	1 (4%)
Quartile 2	9(37.5%)	2 (8.3%)
Quartile 3	10(41.7%)	2 (8.3%)
Quartile 4	13(50%)	5 (19.2%)
p	<0.001	0.001

suPAR and mortality

We investigated the link between suPAR and overall mortality. During the study period, 10 (10.1%) patients died, eight (80%) from cardiovascular death, and the other two from systemic failure attributable to infection secondary to CVD. A definite association was observed between high suPAR and mortality in patients treated with maintenance hemodialysis ($P = 0.001$). A Kaplan–Meier analysis showed clear differences in the rate of CVD occurrence in the four groups (log rank 32.482, $P < 0.001$). The survival curves for the quartiles of suPAR demonstrated a specific association between suPAR and overall mortality ($P = 0.001$) (Fig. 3). In a univariate Cox analysis, high suPAR was directly associated with mortality, as were greater age, CACS, longer period of dialysis, and diabetes. We used a Spearman rank analysis to assess the risk factors for suPAR, but found known positive association with age, female sex, period of dialysis, serum phosphorus, ALP, or hs-CRP (all $P < 0.05$) (Table 5).

Discussion

The mortality risk for patients with ESRD is substantially greater than that for the general population. The higher CVD rate is due, in part, to traditional risk factors in the general population, such as old age, hypertension, diabetes, and dyslipidemia [14]. However, typical cardiovascular risk factors are insufficient to accurately predict the cardiovascular risk in patients with CKD and vascular calcification is one of the principal contributors to CVD in ESRD. Several studies have demonstrated that CACS is associated with CVD and mortality in patients with CKD undergoing dialysis [15–18]. Our study has also shown that CACS is independently associated with CVD and all-cause mortality in patients treated with hemodialysis.

Table 3. Cox proportional hazard analysis of the time to the first cardiovascular event. Abbreviations: suPAR, soluble urokinase plasminogen activator receptor; CACS, coronary artery calcification score; ALP, alkaline phosphatase; hs-CRP, high sensitivity C-reactive protein; Hb, hemoglobin; iPTH, intact parathyroid hormone; CVD, cardiovascular disease

Variable	Hazard ratio(95%CI)	P
1.Unadjusted suPAR(Ln)	2.573(1.708-3.876)	<0.001
2.CACS(Ln)	2.082(1.574-2.752)	<0.001
3.CACS(Ln), age dialysis vintage, Alp, hs-CRP(Ln)	2.960(1.638-5.355)	<0.001
4.Full model: age, gender, dialysis vintage, calcium, phosphorus, Hb, albumin, ALP, iPTH(Ln), CACS(Ln), hsCRP(Ln), diabetes, hypertension, prior CVD	3.629(1.823-7.225)	<0.001

Table 4. Cox proportional hazard survival analysis. Abbreviations: suPAR, soluble urokinase plasminogen activator receptor; CACS, coronary artery calcification score; ALP, alkaline phosphatase; hs-CRP, high-sensitivity C-reactive protein; Hb, hemoglobin; iPTH, intact parathyroid hormone; CVD, cardiovascular disease

Variable	Hazard ratio(95%CI)	P
1.Unadjusted suPAR (Ln)	2.806(1.139-6.915)	0.025
2.CACS(Ln)	1.904(1.169-3.10)	0.01
3.CACS(Ln),age, diabetes	2.411(0.917-6.495)	0.07
4. Full model: age, gender, dialysis vintage, calcium, phosphorus, Hb, albumin, Alp, iPTH(Ln),CACS(Ln), hsCRP(Ln),diabetes, hypertension, prior CVD	2.401(0.666-8.66)	0.181

Fig. 3. Event-free cardiovascular disease and survival stratified by suPAR quartiles. Kaplan-Meier curves of the time to a cardiovascular event and the number of surviving patients as a function of suPAR concentration quartiles. The survival curves for the quartiles of suPAR show a specific association between suPAR (log rank 32.482, $P < 0.001$) and overall mortality (log rank 6.555, $P = 0.001$).

suPAR originates from the proteolytic cleavage of uPAR. suPAR is composed of three domains (D1–D3) and arises from cleavage of uPAR at the glycosylphosphatidylinositol anchor. suPAR is found in the serum, plasma, and other body fluids, and because its mass is ~56 kDa, it is not usually eliminated by ordinary dialyzers [19]. Previous studies have found higher suPAR concentrations in older patients (> 50 years) and female patients, which is consistent with our results. We also found that suPAR was positively associated with the period of dialysis and iPTH, phosphorous, and ALP levels, suggesting that suPAR probably provides prognostic information on the CKD population.

As well as CKD-specific abnormalities, such as anemia, disturbances of the bone and mineral metabolism, and the retention of organic solutes, it has become increasingly clear that chronic inflammation plays a vital role in the development of atherosclerosis and CVD,

especially in ESRD [20]. suPAR, an emerging inflammatory biomarker, seems to be linked to endothelial dysfunction and the presence of atherosclerotic plaques, and therefore potentially reflects the later processes that occur in the development of CVD. Lynqbaek et al. [21] demonstrated that suPAR behaves differently from CRP and outperforms CRP in predicting CVD. Therefore, the combination of both factors should significantly improve the prognostic information on CVD risk. suPAR is also a novel indicator of risk in the CKD population. A recent large study involving 3683 patients demonstrated that elevated suPAR levels were independently associated with the incidence of CKD and predicted an accelerated decline in the estimated glomerular filtration rate in groups [22].

suPAR is also associated with the development, severity, and outcome of coronary artery disease. In a study that analyzed 296 patients with ST-elevation MI undergoing a primary percutaneous coronary intervention, suPAR remained associated with mortality and the risk of MI recurrence during the 5.76-year follow-up period [21]. Another study [23] that followed 1126 healthy middle-aged people for a 10-year period suggested that suPAR is associated with CACS independently of the evaluated systematic coronary risk, and provides prognostic information on CVD risk in the general population beyond the Framingham risk score. suPAR levels also reflect low-grade inflammation and are associated with lifestyle factors such as smoking, alcohol intake, and sedentary lifestyle. suPAR levels are also higher in women than in men. We found that suPAR is an independent risk factor for CVD in both univariate and multivariate Cox analyses. suPAR may also be a marker of established CVD. After adjustment for a past history of CVD, suPAR remained an independent determinant of cardiovascular events.

One limitation of this study is that we were unable to provide a definite mechanistic explanation for the observed strong association between suPAR and cardiovascular events. Several plausible mechanisms may explain our findings, including chronic inflammation and/or the overproduction of suPAR by the diseased vasculature. Although suPAR is associated with several aspects of CVD, we do not know whether suPAR plays a causal role in cardiovascular pathogenesis or merely reflects the disease process. Our study sample size was small and the follow-up period short, so additional studies with greater numbers of patients and long-term follow-up are required. However, our group is the first to establish an association between suPAR and cardiovascular morbidity and mortality in patients undergoing maintenance dialysis.

Conclusion

suPAR is associated with CACS and shows a clear, positive association with the risk of new-onset CVD in patients undergoing maintenance dialysis.

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Table 5. Relationships between suPAR and risk factors

Variables	r	p
Age	0.129	0.002
Gender(female vs. male)	0.11	0.05
Dialysis vintage	0.255	0.011
iPTH	0.323	0.001
Calcium	0.161	0.112
Phosphorus	0.264	0.023
Hb	0.02	0.884
Alb	-0.108	0.286
Alp	0.338	0.001
hs-CRP	0.327	0.001

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Disclosure Statement

The authors declare no conflict of interest regarding the publication of this article.

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