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# The association of profilin-1 levels with survival in chronic kidney disease

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**Abstract**

**Background:** Profilin-1 is a ubiquitous, actin-binding protein that plays an important role in the regulation of actin polymerization and cytoskeleton remodelling and contributes to vascular dysfunction. We conducted this study to investigate the association of serum profilin-1 levels with fatal and nonfatal CVE in a cohort of patients with stage 1-5 CKD.

**Materials and methods:** Serum concentrations of profilin-1 levels were determined by enzyme-linked immunosorbent assay. Endothelium-dependent vasodilatation (flow-mediated dilatation [FMD]) and endothelium-independent vasodilatation (nitroglycerine-mediated dilatation [NMD]) of the brachial artery were assessed noninvasively, using high-resolution ultrasound.

**Results:** Both fatal and nonfatal CVE were significantly higher in patients with high profilin-1 levels. Kaplan-Meier survival curves showed that patients with profilin-1 below the median value (114 pg/mL) had higher cumulative survival compared with patients who had profilin-1 levels above the median value (log-rank test, $P < .001$).

**Conclusions:** This is the first study that demonstrates the serum profilin-1 is independently associated with endothelial dysfunction, cardiovascular events and survival in patients with CKD.

**KEYWORDS**
cardiovascular events, chronic kidney disease, endothelial dysfunction, profilin-1, survival

## 1 | INTRODUCTION

Cardiovascular disease (CVD) is the main cause of morbidity and mortality in patients with chronic kidney disease (CKD).[1] Disproportionately elevated oxidative stress and inflammation are considered among the important causes of this increased risk. Vascular inflammation and endothelial dysfunction are the main processes in the development of cardiovascular disease.[2,3] However, the mechanism of cardiovascular damage has not been well clarified by traditional risk factors in this population.[4] Therefore, new biomarkers are needed for explaining that this process is of particular importance. Thus, better understanding of the relationship between CKD and CVD is essential for guiding future strategies for screening and treatment.

Profilin-1 is a ubiquitous, small (12-15 kD) actin-binding protein that plays an important role in the regulation of actin polymerization and cytoskeleton remodelling, by activating hypertrophic signalling cascades, to thus contribute to vascular hypertrophy.[5,6] Profilin-1 was increased in the endothelium of diabetic animals, and its overexpression in cultured endothelial cells triggered indicators of endothelial

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dysfunction.[7] Several studies have found strong evidence for the important role of profilin-1 in vascular inflammation and vascular remodelling.[8] Also, it has been well established that profilin-1 plays a role in the regulation of multiple signalling pathways and contributes to the occurrence of hypertension and cardiovascular diseases in human studies.[9,10]

The aim of this study was threefold: (i) to evaluate profilin-1 levels in stage 1-5 CKD (vs non-CKD) patients and their relation with eGFR; (ii) to evaluate the relationship between profilin-1 and different markers of inflammation and endothelial dysfunction in the same CKD population; and (iii) to test the ability of profilin-1 as a novel predictor of all-cause mortality and cardiovascular events (CVE) in CKD patients.

## 2 | METHODS

### 2.1 | Patients and study design

Between November 2011 and July 2015, 490 patients were referred to the Renal Unit of the Gulhane School of Medicine Medical Center, Ankara, Turkey, for the first time because of suspected or manifest CKD. All patients included in the study were diagnosed as having CKD according to the National Kidney Foundation K/DOQI Guidelines.[11] Exclusion criteria including acute infections and unwillingness to participate in the study were applied (n = 126). Ninety-nine eligible patients dropped out for the following reasons: lost to follow-up (n = 69) and withdrew consent (n = 30). The estimated GFR (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[12] Two hundred and 65 patients were included in the final analysis. None of the patients in stage 5 CKD were on haemodialysis or peritoneal dialysis. Hypertension was considered to be present if the average systolic pressure was ≥140 mm Hg and/or average diastolic pressure was ≥90 mm Hg for whole day, or if the individual was taking antihypertensive medication.[13] We further recruited in the control group 45 patients, matched to the CKD patients for age and gender, from our outpatient department whose primary reason for referral did not include diabetes or cardiovascular or renal disease.

All included patients were followed up for time-to-event analysis until occurrence of fatal or nonfatal cardiovascular events. Fatal and nonfatal cardiovascular events including death, stroke, myocardial infarction and complicated peripheral vascular disease were recorded. Hospital records and death certificates were reviewed by three of the investigators unaware of baseline parameters. If information could not be obtained, the patient was assumed to be lost to follow-up starting from the date of the last actual visit. The last visit of the study was in December 2016. Local ethical committee of Gulhane School of Medicine approved the study protocol, and all patients were included in the study after signing informed consent forms.

### 2.2 | Biochemical analyses

All blood samples were obtained from patients in the morning after 12 hours of fasting, for measurement of fasting plasma glucose (FPG), serum albumin, total serum cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol at the beginning of the study. Total plasma cholesterol, TG and HDL cholesterol were measured by enzymatic colorimetric method with Olympus AU 600 Autoanalyzer using reagents from Olympus Diagnostics, GmbH (Hamburg, Germany). Low-density lipoprotein (LDL) cholesterol was calculated by Friedewald's formula.[14] For the measurement of high-sensitive C-reactive protein (hs-CRP), serum samples were diluted with a ratio of 1/100 with the diluents solution. Calibrators, kit controls and serum samples were all added on each microwell with an incubation period of 30 minutes. After 3 washing intervals, 100 μL of enzyme conjugate (peroxidase-labelled anti-CRP) was added on each microwell for additional 15 minutes incubation in room temperature in dark. The reaction was stopped with a stop solution, and photometric measurement was performed at the 450-nm wavelength. The amount of serum samples was calculated as mg/L with a graphic that was made by noting the absorbance levels of the calibrators. The serum basal insulin value was determined by the coated tube method (DPC-USA). An insulin resistance score homeostasis model assessment-insulin resistance (HOMA-IR) was computed by the following formula: HOMA-IR = fasting plasma glucose (mg/dL) x immunoreactive insulin (IRI) (μIU/mL)/405.[15] Proteinuria was quantified using 24-hour timed urine collection.

Serum total calcium was measured by the cresolphthalein complex-one method using Menagent Calcium 60sec kits (Menarini Diagnostics, Florence, Italy). Serum phosphorus was measured by the ammonia molybdate complex method using Menagent Phosphofix kits (Menarini Diagnostics). Intact parathormone was measured by IRMA, using a commercial kit (Immulite Intact PTH) from Diagnostic Product Corporation (Los Angeles, CA, USA) with a sensitivity of 1 pg/mL.

### 2.3 | Serum profilin-1 measurement

Profilin-1 levels were determined by sandwich ELISA kit. Profilin-1 concentrations were assayed according to the profilin-1 ELISA protocol of manufacturer's instruction (Human profilin-1 ELISA Kit) (Cusabio Biotech Co., Ltd. Wuhan, China). Briefly, 100 μL of standards and samples

was added to 96-microwell plate wells and incubated 2 hours at 37°C. After incubation, liquid of each well was aspirated, and 100 μL biotin antibody was added to each well. The plate was incubated 1 hour at 37°C. After incubation, each well was washed three times with 200 μL wash buffer, and 100 μL HRP-avidin was added to each well. The plate was incubated at 37°C for 1 hour, and each well was washed five times with 200 μL wash buffer. Ninety microlitres of TMB substrate solution was added to each well, and the plate was incubated in the dark at 37°C for 20 minutes. Then, 50 μL of stop solution was added to each well, and the plate was measured with the absorbance at 450 nm with a Synergy HT plate reader (BioTek Instruments, Winooski, VT, USA). From the standard curve, the profilin-1 levels in each test sample were quantitated. Intra-assay coefficient of variation was <8%, and limit of detection was 7.8 pg/mL according to the manufacturer's product information sheet.

Intact parathormone was measured by IRMA, using a commercial kit (Immulite Intact PTH) from Diagnostic Product Corporation (Los Angeles, CA, USA) with a sensitivity of 1 pg/mL.

## 2.4 | Assessment of endothelial function

Endothelium-dependent vasodilatation (flow-mediated dilatation [FMD]) and endothelium-independent vasodilatation (nitroglycerine-mediated dilatation [NMD]) of the brachial artery were assessed noninvasively, using high-resolution ultrasound as described by Celermajer et al[16] Measurements were made by a single observer using an ATL 5000 ultrasound system (Advanced Technology Laboratories Inc., Bothell, WA., USA) with a 12-Mhz probe. The vascular assessment method was in agreement with the criteria set forth by the International Brachial Artery Reactivity Task Force.[17] All vasoactive medications were withheld for 24 hours before the procedure. The subjects remained at rest in the supine position for at least 15 minutes before the examination started. Each subject's right arm was comfortably immobilized in the extended position to allow consistent recording of the brachial artery 2-4 cm above the antecubital fossa. Three adjacent measurements of end-diastolic brachial artery diameter were made from single 2D frames. All ultrasound images were recorded on Super Video Home System (S-VHS) videotape for subsequent blinded analysis. The FMD and NMD were then calculated as the per cent change in diameter compared with baseline resting diameters. The intra-observer coefficient of variation for FMD was 5.9%.

## 2.5 | Statistical analyses

All statistical analyses were performed with SPSS 19.0 (SPSS Inc., Chicago, IL, USA) statistical package. Non-normally distributed variables were expressed as median (range), and normally distributed variables were as mean ± SD, as appropriate. A $P$ value <.05 was considered to be statistically significant. Between-group comparisons were assessed for nominal variables with the Chi-square test, and by Kruskal-Wallis test (ANOVA) for the rest of variables. Spearman's rank correlation was used to determine correlations between paired variables. Stepwise multivariate regression analysis was used to assess the predictors for FMD. Survival and time-to-event analyses of cardiovascular outcomes were performed using Cox proportional hazards model, including adjustment for potential confounding factors. Data are presented in the form of hazard ratios (HR) and 95% confidence intervals (CI).

# 3 | RESULTS

## 3.1 | Patient characteristics

In total, 53 patients with stage 1 CKD, 53 patients with stage 2 CKD, 52 patients with stage 3 CKD, 48 patients with stage 4 CKD and 59 patients with stage 5 CKD were included in the study. Demographic and clinical characteristics of the entire study cohort are depicted in Table 1. There was no difference among the groups in terms of age and sex distribution. Laboratory values, including inflammatory cytokines and vascular measurements, are shown in Table 1. As expected, serum calcium and albumin decreased, whereas serum phosphorus and intact parathyroid hormone levels increased across CKD stages 1-5. There was a significant declining trend for serum haemoglobin from stage 1 to stage 5 ($P < .001$). There were significant increases in serum levels of hs-CRP, Homa-IR index and uric acid across increasing CKD stages. In a similar way, profilin-1 levels were significantly increased as eGFR decreased (Figure 1). Both endothelium-dependent vasodilation (expressed as FMD) and endothelium-independent vasodilation (expressed as NMD) were decreased from stage 1 CKD to stage 5 CKD. Antihypertensive medication in study group according to CKD stages is as follows; stage 1: 4 patients with angiotensin converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB), 1 patient with calcium-channel blocker (CCB); stage 2: 6 patients with ACEI or ARB, 2 patients with CCB; stage 3: 5 patients with ACEI or ARB, 2 patients with CCB, 1 patients with diuretic; stage 4: 4 patients with ACEI or ARB, 2 patients with CCB, 2 patients with ACEI or ARB plus CCB, 2 patients with ACEI or ARB plus diuretic; and stage 5: 10 patients with ACEI or ARB plus CCB, 6 patients with ACEI or ARB plus diuretic.

**TABLE 1** Clinicodemographic characteristics, biochemical parameters and vascular assessments according to chronic kidney disease (CKD) stages

| Variables | Groups | | | | | | P |
|---|---|---|---|---|---|---|---|
| | Control (n = 45) | Stage 1 (>90 mL/min; n = 53) | Stage 2 (60-89 mL/min; n = 53) | Stage 3 (30-59 mL/min; n = 52) | Stage 4 (15-29 mL/min; n = 48) | Stage 5 (<15 mL/min; n = 59) | |
| Age (years) | 49.02 ± 11.35 | 49.38 ± 11.38 | 47.64 ± 10.03 | 49.27 ± 13.50 | 51.69 ± 12.03 | 48.86 ± 12.64 | .690 |
| Gender (male,%) | 19 (42.2) | 27 (50.9) | 26 (49.1) | 25 (48.1) | 25 (52.1) | 28 (47.5) | .952 |
| Haemoglobin (g/L) | 14.00 ± 0.67[a] | 12.71 ± 2.55[b] | 12.05 ± 1.65[bc] | 11.15 ± 2.58 [cd] | 10.85 ± 2.09[d] | 10.64 ± 1.72[d] | <.001 |
| SBP(mm Hg) | 130.36 ± 5.16 | 134.70 ± 8.93 | 135.08 ± 9.89 | 132.79 ± 9.22 | 133.00 ± 12.58 | 134.49 ± 10.66 | .165 |
| DBP(mm Hg) | 82.89 ± 2.69[a] | 83.30 ± 4.08[ab] | 83.74 ± 3.44[ab] | 85.25 ± 4.04[b] | 84.50 ± 5.56[ab] | 83.63 ± 3.85[ab] | .049 |
| BMI (kg/m$^2$) | 25.91 ± 1.98[ab] | 26.80 ± 2.32[a] | 26.88 ± 2.82[a] | 25.40 ± 2.62[ab] | 26.20 ± 2.95[ab] | 25.30 ± 2.63[b] | .003 |
| eGFR(mL/min/1.73 m$^2$) | 119.27 ± 4.66[a] | 95.64 ± 3.50[b] | 77.51 ± 9.97[c] | 48.13 ± 9.67[d] | 22.56 ± 3.84[e] | 9.29 ± 2.94[f] | <.001 |
| Total cholesterol (mg/dL) | 191.96 ± 15.75 | 192.68 ± 16.56 | 196.25 ± 16.79 | 197.19 ± 21.60 | 196.08 ± 17.75 | 191.56 ± 17.69 | .407 |
| Triglyceride, mg/dL | 139.18 ± 10.65 | 136.04 ± 13.95 | 140.30 ± 10.16 | 137.06 ± 16.36 | 140.54 ± 13.16 | 133.73 ± 19.59 | .107 |
| LDL cholesterol (mg/dL) | 121.02 ± 11.33 | 126.74 ± 16.13 | 125.04 ± 15.36 | 123.65 ± 13.90 | 124.00 ± 15.01 | 119.78 ± 19.99 | .209 |
| HDL cholesterol (mg/dl) | 42.22 ± 6.60 | 44.36 ± 4.74 | 43.04 ± 5.37 | 41.00 ± 5.17 | 42.19 ± 6.85 | 42.51 ± 6.47 | .103 |
| Total protein | 7.10 (6.80-7.20)[a] | 7.00 (6.70-7.20)[a] | 6.70 (6.20-7.20)[a] | 7.00(6.60-7.40)[a] | 7.05 (6.85-7.15)[a] | 6.30 (6.00-6.90)[b] | <.001 |
| Albumin(g/dL) | 4.26 ± 0.42[a] | 4.01 ± 0.27[b] | 3.98 ± 0.33[b] | 4.22 ± 0.40[ab] | 3.95 ± 0.32[bc] | 3.80 ± 0.30[c] | <.001 |
| Uric acid (mg/dL) | 4.36 ± 0.79[a] | 4.77 ± 1.58[ab] | 5.18 ± 1.72[b] | 6.81 ± 1.29[c] | 7.11 ± 1.12 [cd] | 7.91 ± 1.06[d] | <.001 |
| Calcium (mg/dL) | 9.13 ± 0.47[a] | 9.06 ± 0.52[b] | 8.74 ± 0.56[b] | 8.30 ± 0.48[c] | 8.08 ± 0.37 [cd] | 8.15 ± 0.38[d] | <.001 |
| Phosphorus (mg/dL) | 3.79 ± 0.44[a] | 4.19 ± 0.38[a] | 4.09 ± 0.67[a] | 4.81 ± 0.87[b] | 6.03 ± 1.37[c] | 6.73 ± 1.62[d] | <.001 |
| PTH (pg/mL) | 46.00 ± 11.63[a] | 53.53 ± 24.40[a] | 61.28 ± 29.02[a] | 133.50 ± 64.09[b] | 154.13 ± 20.84[b] | 321.46 ± 160.88[c] | <.001 |
| NMD, % | 13.08 ± 0.60[a] | 12.97 ± 0.49[a] | 13.10 ± 0.33[a] | 12.82 ± 0.48[a] | 13.00 ± 0.37[a] | 11.79 ± 0.89[b] | <.001 |
| Profilin (pg/mL) | 29.62 (25.06-33.52)[a] | 33.28 (29.82-38.00)[ab] | 70.00 (60.00-78.70)[b] | 114.96 (107.70-168.6)[bc] | 179.22 (124.40-276.5)[c] | 288.00 (121.00-588.00)[c] | <.001 |
| FMD, % | 9.00 (8.20-9.20)[a] | 8.40 (8.10-9.00)[ab] | 7.20 (7.10-7.40)[bc] | 7.20 (6.20-7.30)[c] | 6.20 (5.85-7.20)[c] | 5.00 (4.50-5.70)[d] | <.001 |
| hs-CRP (mg/L) | 2.00 (1.60-3.00)[a] | 7.00 (5.50-8.80)[bc] | 11.00 (9.20-11.60)[c] | 17.00 (14.00-18.00)[d] | 20.50 (11.00-24.00)[de] | 26.00 (21.00-31.00)[e] | <.001 |
| Homa-IR index | 1.34 (1.17-1.79)[a] | 1.38 (1.19-1.89)[b] | 1.57 (1.37-2.04)[b] | 1.52 (1.33-2.13)[b] | 1.49 (1.29-2.15)[b] | 1.54 (1.36-2.22)[b] | <.001 |
| Glucose (mg/dL) | 85.00 (78.00-95.00)[a] | 82.00 (79.00-92.00)[a] | 92.00 (82.00-100.00)[b] | 91.00 (77.00-103.00)[b] | 92.00 (80.50-106.00)[b] | 90.00 (80.00-101.00)[b] | .030 |
| DM (present) | - | 9 (17.0) | 10 (18.9) | 12 (23.1) | 14 (29.2) | 13 (22.0) | .634 |
| HT (present) | - | 5 (9.4) | 8 (15.1) | 9 (17.3) | 5 (10.4) | 16 (27.1) | .083 |
| CVD (present) | - | 8 (15.1)[a] | 11 (20.8)[a] | 16 (30.8)[ab] | 11 (22.9)[a] | 26 (44.1)[b] | <.006 |
| Death | - | 0 (0.0) | 3 (5.7) | 6 (11.5) | 2 (4.2) | 9 (15.3) | .096 |

SBP, systolic blood pressure; DBP, diastolic blood pressure; NMD, nitroglycerine-mediated dilatation; FMD, flow-mediated dilatation; CVD, cardiovascular; HOMA-IR, homeostasis model assessment-insulin resistance; BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein.

Values are expressed as n(%), mean ± SD or median(1st-3rd quartiles). Different superscripts in a same row indicate statistically significant difference among groups.

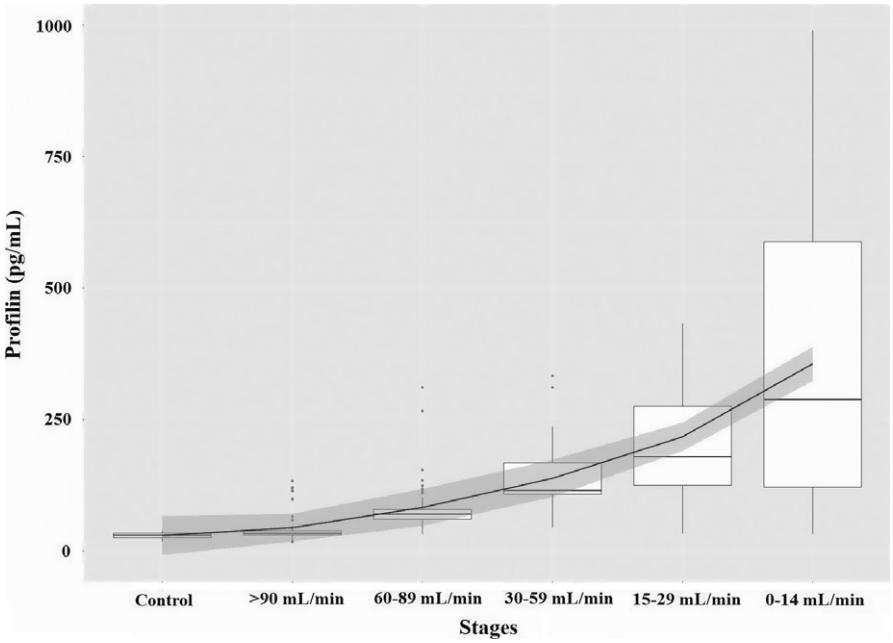


**FIGURE 1** The association between profilin-1 levels and estimated GFR (eGFR)

## 3.2 | Phenotypical characteristics

Associated with high profilin-1 concentrations, we stratified the entire study cohort into two groups according to the median serum profilin-1 level (Table 2). All of the studied inflammatory cytokines were significantly higher in the group with profilin-1 concentrations above the median value compared with the group with profilin-1 concentrations below the median value. Diabetes mellitus was more frequent in patients with high serum profilin-1, and FMD was significantly lower. The number of both fatal and nonfatal CV events was significantly higher in patients with high profilin-1 levels.

In multivariate determinants of profilin-1, eGFR, hs-CRP, uric acid and the FMD were significantly related to profilin-1 in univariate analysis. Additionally, the multivariate analysis showed that only the FMD and serum hs-CRP levels were associated with profilin-1 (Table 3). In addition, urine albumin creatinine ratio was positively correlated with serum profilin levels ($r$:.297, $P$:.02).

## 3.3 | CV outcomes

Cardiovascular outcomes were determined from patient inclusion in the study onwards, with a mean follow-up period of 29 (range 2-52) months. Twenty patients died, 16 of which due to presumable cardiovascular causes.

Cardiovascular mortality was defined as death due to coronary heart disease (n = 8), sudden death (n = 2), stroke (n = 5) or complicated peripheral vascular disease (n = 1). A total of 56 additional nonfatal major adverse cardiovascular events took place during the follow-up period. These included stroke (n = 29), myocardial infarction (n = 22) and peripheral vascular disease (n = 5).

The predictors for time-to-CV event (fatal and nonfatal CV events = 72) were studied by univariate and multivariate Cox regression analyses. We included all significant parameters derived from the univariate analysis and well-known risk factors for CV disease (such as age and sex) into the multivariate Cox model. The multivariate Cox analysis showed that haemoglobin, profilin-1 and the presence of diabetes mellitus were associated with the risk of CV events (Table 4). In addition, Kaplan-Meier survival curves showed that patients with profilin-1 below the median value (114 pg/mL) had higher cumulative survival compared with patients who had profilin-1 levels above the median value (log-rank test, $P < .001$) (Figure 2).

# 4 | DISCUSSION

This study provides a comprehensive analysis of the prognostic value of profilin-1 in a real-life cohort of CKD patients. We show for the first time that this biomarker is independently associated with endothelial dysfunction, cardiovascular disease and survival in this population. However profilin-1 is correlated with renal function decline, we demonstrated that eGFR is not associated with profilin-1 levels in multiple regression analysis.

It is well known that CKD patients suffer a several-fold higher risk of CVD and mortality.[18] Although multiple possible explanations exist for the association between CKD and increased risks of CVD and death, studies are still ongoing to explain further potential culprits. It has been shown that CKD progression is associated with both

**TABLE 2** Biochemical parameters, vascular assessment results and composite CV events in patient groups, which were stratified by median profilin-1 levels

| | Profilin | | |
|---|---|---|---|
| **Variables** | **<114 pg/m (n = 132)** | **≥114 pg/mL (n = 133)** | ***P*** |
| Age (years) | 48.91 ± 11.71 | 49.71 ± 12.24 | .585 |
| Gender (male,%) | 67 (50.8) | 64 (48.1) | .668 |
| Haemoglobin (g/L) | 11.74 ± 2.32 | 11.21 ± 2.20 | .058 |
| SBP (mm Hg) | 132.48 ± 8.99 | 135.59 ± 11.22 | .013 |
| DBP (mm Hg) | 83.82 ± 3.74 | 84.30 ± 4.69 | .356 |
| BMI (kg/m$^2$) | 26.46 ± 2.63 | 25.74 ± 2.80 | .030 |
| eGFR(mL/min/1.73 m$^2$) | 72.13 ± 28.36 | 28.50 ± 22.17 | <.001 |
| Total cholesterol (mg/dL) | 195.14 ± 17.48 | 194.15 ± 18.86 | .657 |
| Triglyceride, mg/dL | 137.95 ± 14.38 | 136.84 ± 16.09 | .556 |
| LDL cholesterol (mg/dL) | 125.30 ± 16.15 | 122.20 ± 16.54 | .124 |
| HDL cholesterol (mg/dL) | 43.36 ± 4.92 | 41.80 ± 6.53 | .020 |
| Albumin(g/dL) | 4.00 ± 0.34 | 3.97 ± 0.37 | .545 |
| Uric acid (mg/dL) | 5.28 ± 1.71 | 7.46 ± 1.17 | <.001 |
| Calcium (mg/dL) | 8.70 ± 0.62 | 8.23 ± 0.47 | <.001 |
| Phosphorus (mg/dL) | 4.48 ± 0.94 | 5.90 ± 1.64 | <.001 |
| PTH (pg/mL) | 82.45 ± 63.30 | 214.34 ± 145.33 | <.001 |
| NMD, % | 12.91 ± 0.59 | 12.51 ± 0.83 | <.001 |
| FMD, % | 7.40 (7.10-8.20) | 6.20 (5.00-7.00) | <.001 |
| hs-CRP (mg/L) | 10.00 (8.40-12.40) | 19.00 (12.50-26.00) | <.001 |
| Homa-IR index | 1.44 (1.23-1.96) | 1.59 (1.33-2.30) | .003 |
| Glucose (mg/dL) | 86.50 (80.00-95.00) | 91.00 (81.00-101.00) | .008 |
| DM (present) | 17 (12.9) | 41 (30.8) | <.001 |
| HT (present) | 13 (9.8) | 30 (22.6) | .005 |
| CVD (present) | 7 (5.3) | 65 (48.9) | <.001 |
| Death | 4 (3.0) | 16 (12.0) | <.001 |

Values are expressed as n(%), mean ± SD or median(1st-3rd quartiles.

**TABLE 3** Univariate and multiple linear regression analyses to identify the association between profilin 1 and other variables

| | Univariate | | Multiple | |
|---|---|---|---|---|
| **Variables** | **Beta** | ***P*** | **Beta** | ***P*** |
| eGFR(mL/min/1.73 m$^2$) | −0.621 | <.001 | - | - |
| hs-CRP (mg/L) | 0.538 | <.001 | 0.252 | <.001 |
| BMI (kg/m$^2$) | −0.098 | .112 | - | - |
| Uric acid (mg/dL) | 0.407 | <.001 | - | - |
| FMD, % | −0.685 | <.001 | −0.556 | <.001 |

Model Adjusted $r^2$:.515.

endothelial dysfunction, hypertension and increased prevalence of atherosclerosis, also these parameters are associated with mortality in this cohort.[19,20] An initial step in vascular dysfunction leading to atherogenesis is the adhesion cascade that involves the rolling, tethering, adherence and subsequent transmigration of leucocytes through the endothelium. Recruitment and accumulation of leucocytes to the endothelium are mediated by an upregulation of adhesion molecules such as vascular cell adhesion molecule-1 and intracellular cell adhesion molecule-1.[21,22]

Identifying biomarkers that would serve as indicators of vascular dysfunction and atherosclerosis or as surrogates for endothelial cell activation and/or dysfunction is of particular importance in CKD population. Profilin-1, an ubiquitous actin-binding protein, is highly expressed in endothelial cells (ECs) in patients with diabetes and atherosclerotic diseases.[7-9] Profilin-1 upregulation was required for oxide LDL-induced adhesion molecule expression in ECs.[7,23] Also, profilin-1 overexpression in ECs resulted in increased adhesion and migration.[24] Dardik et al[25] showed that together homocysteine and turbulent

**TABLE 4** Univariate and multivariate Cox analyses predicting fatal and nonfatal CV events

| | Univariate | | Multiple | |
|---|---|---|---|---|
| **Variables** | **HR(95%CI)** | ***P*** | **HR(95%CI)** | ***P*** |
| Haemoglobin (g/L) | 1.26 (1.14-1.38) | <.001 | 1.20 (1.09-1.32) | <.001 |
| SBP(mm Hg) | 1.04 (1.02-1.06) | <.001 | - | - |
| DBP(mm Hg) | 0.99 (0.91-1.05) | .714 | - | - |
| Age (years) | 0.99 (0.98-1.01) | .477 | - | - |
| eGFR(mL/min/1.73 $m^2$) | 0.99 (0.99-1.00) | .05 | - | - |
| LDL Cholesterol (mg/dL) | 1.00 (0.99-1.01) | .959 | - | - |
| Albumin(g/dL) | 1.45 (0.69-3.02) | .325 | - | - |
| Uric acid (mg/dL) | 1.37 (1.17-1.60) | <.001 | - | - |
| Calcium (mg/dL) | 1.06 (0.71-1.61) | .768 | - | - |
| Phosphorus (mg/dL) | 1.35 (1.17-1.55) | <.001 | - | - |
| PTH (pg/mL) | 1.01 (1.00-1.02) | <.001 | - | - |
| NMD,% | 0.78 (0.60-1.02) | .069 | - | - |
| Profilin (pg/mL) | 1.01 (1.00-1.02) | <.001 | 1.03 (1.00-1.08) | <.001 |
| hs-CRP (mg/L) | 1.02 (1.01-1.03) | .02 | - | - |
| Homa-IR index | 1.73 (1.49-2.00) | <.001 | - | - |
| DM (present) | 4.12 (2.59-6.54) | <.001 | 3.82 (2.36-6.18) | <.001 |
| HT (present) | 2.49 (1.53-4.04) | .001 | - | - |
| Gender (male) | 0.73 (0.30-1.82) | .504 | - | - |

HR, hazard ratio; CI, confidence interval.

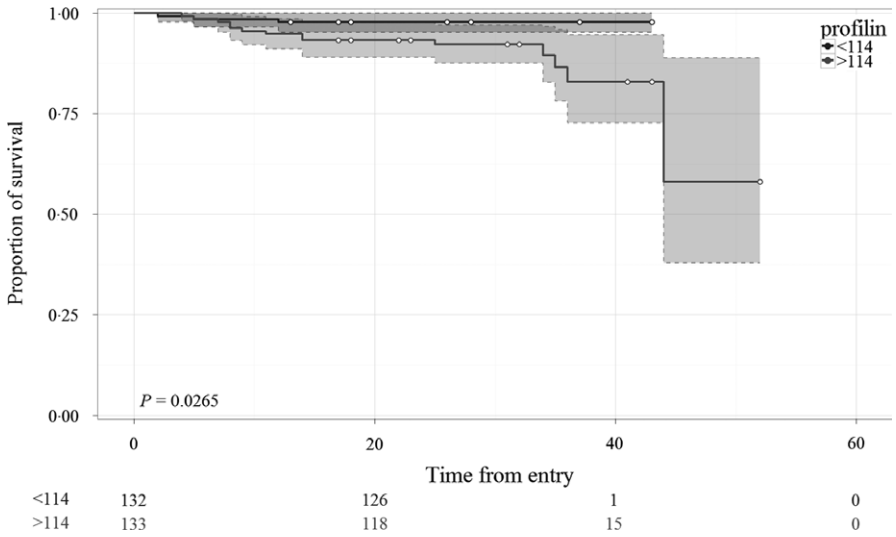


**FIGURE 2** Kaplan-Meier survival curves according to patients with profilin-1 below the median value (114 pg/mL)

flow conditions, both promoting factors for atherosclerosis, enhanced profilin-1 gene expression in ECs. Thus, these studies suggest that biomechanical and metabolic abnormalities relevant to vascular disease elicit the upregulation of profilin-1, which in turn could integrate these signs into remodelling of EC cytoskeleton. More recently, studies focused on the association between profilin-1 and atherosclerotic vascular diseases including hypertension and coronary artery disease.[9,10,26] Caglayan et al[9] showed that profilin-1 expression is significantly enhanced in human atherosclerotic plaques compared to normal vessel wall and the serum levels of profilin-1 correlated with the degree of atherosclerosis in humans. Moreover, Ramaiola et al[10] studied profilin-1 in patients with myocardial infarction and

reported that profilin-1 is secreted from fully activated platelets in the thrombotic mass in coronary arteries and detected in systemic circulation that may be a potential marker of thrombosis. In addition, Liang et al demonstrated that reduced profilin-1 provides to stabilize blood pressure and improvement in myocardial hypertrophy in a hypertensive mouse model.[26] However, the data about profilin-1 in CKD population are lacking. The molecular weight of profilin-1 is nearly 12-15 kD, and its clearance rate has not been studied yet in the current literature. Profilin-1 nearly has a radius of 1.6 nm according to the calculation of radius by molecular weight of proteins.[27] The crucial structure accounting for the size selectivity of the filtration barrier appears to be slit diaphragm in nephron unit. Uncharged macromolecules up to an effective radius of 1.8 nm pass freely through the filter. Larger components restricted at the effective radii of more than 4 nm.[28,29] Thus, it is possible that profilin-1 may filter the filtration barrier due to its low molecular weight. However, there are no data about the clearance of profilin-1 in the kidney. Therefore, we investigated the role of profilin-1 levels in our CKD cohort and showed that profilin-1 level is inversely correlated with kidney function deterioration, endothelial dysfunction which is monitored by FMD and consequently cardiovascular events and all-cause mortality.

Endothelial dysfunction has now emerged as a relevant risk factor for major cardiovascular events in the general population and in chronic kidney disease.[30,31] The FMD test, the standard noninvasive tool used to assess endothelial function,[16] well-established surrogate of atherosclerosis, has been noted for their capacity to predict future CVE in the CKD population.[32] We utilized the FMD measurements for monitoring the endothelial dysfunction. The present study demonstrated the close relation between profilin-1 and endothelial dysfunction which may be a harbinger of CVD in this population for future investigations. In addition, the potential role of albuminuria on endothelial dysfunction has been demonstrated in hypertensive patients studies focused on the relationship between albuminuria and cardiovascular outcomes.[33] It has been shown that albuminuria is independently related with cardiovascular disease and mortality in general population, hypertension, diabetes mellitus and chronic kidney disease.[34-37] Based upon these results, we further showed that albuminuria is also correlated with profilin-1 levels in our CKD cohort.

In conclusion, we show that profilin-1 levels are significantly associated with endothelial dysfunction in CKD patients. In addition, our study indicates that baseline profilin-1 values are independently associated with future CVE and, for the first time, that profilin-1 measurement can improve the prediction accuracy for CVE in a nondialysed CKD population, above and beyond established traditional and renal-specific cardiovascular risk factors.

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## CONFLICT OF INTEREST

The authors report no conflict of interests.

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