Association of diabetes mellitus with decline in ankle-brachial index among patients on hemodialysis: A 6-year follow-up study

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Abstract

Peripheral artery occlusive disease is common among diabetes mellitus (DM) and end-stage renal disease patients, and tends to progress faster and lead to worse outcomes. This study compared the association of DM with the decline in ankle-brachial index (ABI) among patients on hemodialysis (HD). This was a longitudinal analysis of ABI in HD patients from 2009 to 2015. Medical records and yearly ABI values were obtained. A longitudinal mixed-model analysis was used to evaluate ABI changing trends while accounting for within-patients correlation. There were 296 patients on HD in the period of 2009–2015. In a 6-year follow-up, those with DM had a more rapid ABI decline compared to non-DM patients (slopes: -0.014 vs. 0.010 per year, interaction p < 0.001). In DM patients, female sex, high pulse pressure, high triglyceride, low creatinine, and high uric acid were associated with a decrease in ABI. In non-DM patients, old age, high pulse pressure, high low-density lipoprotein cholesterol, and high uric acid were associated with a decrease in ABI. There were 49.6% of patients with a normal ABI experienced a decrease at least 0.1 of ABI from baseline, and 35.3% had a final ABI < 0.9 in patients with a baseline ABI ≥ 0.9 (n = 232). In this study, DM patients on HD tend to develop a more rapid decline in ABI than non-DM patients on HD. Age, sex, pulse pressure, lipid profile, creatinine, and uric acid are associated with a decreased in ABI.
Introduction

The incidence of non-traumatic lower-extremity amputation among patients with end-stage renal disease (ESRD) is high and the most common indication is peripheral artery occlusive disease (PAOD) [1]. Moreover, among patients with ESRD, PAOD is associated with increased cardiovascular mortality, morbidity, and hospitalization [2]. The ankle-brachial index (ABI) is a reproducible, non-invasive index used to screen and detect PAOD, with 90% sensitivity and 95% specificity [3]. Ono et al.[4] used ABI to evaluate all-cause and cardiovascular mortality in patients on hemodialysis (HD) and found that a reduction in ABI predicted poor survival. The hazard ratios of ABI < 0.9, ≥ 0.9 to < 1.0, and ≥ 1.0 to < 1.1 for all-cause mortality were 4.04, 3.24, and 1.92, respectively. Even those with modest reductions in ABI (≥ 0.9 to < 1.1) had increased risk of all-cause and cardiovascular mortality. Thus, identifying ESRD patients with decreasing ABI for aggressive treatment is important for attenuating the disease and improving survival.

Diabetes mellitus (DM) is the leading cause of chronic kidney disease worldwide, accounting for approximately 45% of ESRD cases in the Taiwan population on dialysis. Among patients with DM, PAOD is especially common, with a three-fold increased risk compared to the general population [5]. Furthermore, PAOD also tends to progress faster and lead to worse outcomes in DM patients [6]. The American Diabetes Association recommends measuring ABI for asymptomatic patients aged ≥ 50 years and for younger patients with DM or other vascular risk factors, and repeating the measurement every 5 years if the first result is normal [7]. Approximately 20% DM patients had a significant decrease in ABI > 0.1 [8], which was higher than that seen in the general population [9,10].

However, data on trends in ABI changes in HD patients with DM are limited. A decline in ABI may serve as an important marker for adverse PAOD outcomes. This study compared the association of DM with the decline in annual ABI over 6 years among patients on HD, and investigated the associated factors.

Patients and methods

Study design and patients

This study was conducted in a dialysis clinic in southern Taiwan. The inclusion criteria were: (1) maintenance HD therapy for at least 3 months; (2) age > 20 years; and (3) regular intake of anti-hypertensive or oral hypoglycemic agents for at least 1 month. Those with atrial fibrillation, bilateral below-knee amputation and hospitalization or antibiotic treatment in the last 4 weeks were excluded. Patients with only one ABI measurement during the follow-up period were also excluded. A total of 296 patients were enrolled from August 2009 to August 2015. For patients with first HD at or before 2009, their baseline measures were started at the year of 2009; for patients entering the study during 2010~2015, their baseline measures were taken at the entering year.

Each HD session was performed for 3.5–4.5 hours, with a blood flow rate of 250–300 mL/min and dialysate flow of 500 mL/min. Blood samples were taken before and after HD to calculate for the Kt/V [11].

Ethics statement

The study protocol was approved by the Institutional Review Board of Kaohsiung Medical University Hospital (KMUHIRB-E(I)-20150256). Written informed consent was obtained from the patients, and all clinical investigations were conducted according to the principles
expressed in the Declaration of Helsinki. The patients also consented to the publication of the clinical details.

Demographic and medical data, including age, sex, and co-morbidities were obtained from medical records and patient interviews. Laboratory data was measured from fasting blood samples using an AutoAnalyzer (Roche Diagnostics GmbH, D-68298 Mannheim COBAS Integra 400). Blood samples were taken within 1 month of ABI measurement. The Kt/V was evaluated as a dialysis marker and determined according to the Daugirdas procedure [11]. Information on patient medication, including intake of aspirin, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and HMG-CoA reductase inhibitors (statins) during the study period was obtained from medical records.

**Assessment of ABI**

The ABI was measured 10–30 minutes before HD using an ABI-form device that automatically and simultaneously measured blood pressure in both arms and ankles using an oscillometric method [12]. Briefly, occlusion and monitoring cuffs were placed tightly around the upper arm without blood access and on both lower extremities with the patient in a supine position. The ABI was calculated by the ratio of the ankle systolic blood pressure divided by the arm systolic blood pressure. After obtaining bilateral ABI values, the lower one was used for analysis. The ABI measurements were done for each patient every August.

**Statistical analysis**

Data were expressed as percentages, mean ± standard deviation, or median (25th-75th percentile) for duration of dialysis and levels of triglyceride and parathyroid hormone. A mixed-effect model analysis was used to evaluate ABI yearly changes between DM groups. This approach treated each ABI measure from each participant as a separate observation and was adjusted for within participant correlations. Subjects were treated as random effects so the analysis was adjusted to each individual’s own ABI levels. A first order autoregressive error structure was accounted for within-patient correlation. This model also explored the significance of risk factors at individual yearly measures for the ABI decline in DM and non-DM groups separately as well as between DM and non-DM groups using an interaction term of DM groups with years. Potential confounding factors were also included in the analysis model as covariates, which included age, sex, duration of dialysis, smoking history, hypertension, coronary artery disease, cerebrovascular disease, pulse pressure, laboratory data including albumin, triglyceride, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein cholesterol, hemoglobin, creatinine, total calcium, phosphorous, calcium-phosphorous product, uric acid, parathyroid hormone, Kt/V, and medications use. Survival curves for ABI decrease > 0.1 and progression to ABI < 0.9, in patients with a baseline ABI > 0.9, were obtained using Kaplan-Meier estimates. Statistical significance was set at \( p < 0.05 \). Statistical analysis was performed using the SAS statistical package version 9.4 (SAS Institutes, Cary, NC, USA).

**Results**

In the study period of 2009–2015, 296 patients were included for analysis. A total of 1412 ABI measurements were provided over the 6-year period. The frequency of ABI measures for each participant was 2–3 times (33.9%), 4–5 times (23.1%) and 6–7 times (43.1%). The characteristics of the study patients were shown in Table 1. In the 6-year period, 56 died, including 20 with baseline ABI < 0.9 (31.7%).
Association of baseline and change in ABI

In terms of ABI changes in HD patients with or without DM, the ABI declined from 0.959 to 0.867 over 6 years in patients with DM but only declined from 1.017 to 1.049 in non-DM patients. The ABI decline curve by follow-up years among DM and non-DM patients (Fig 1) revealed that HD patients with DM had a rapid decline in ABI, and no decline in ABI in non-DM patients. Using the estimated slopes by a mixed-effect model showed -0.023 (95% confidence interval [CI], -0.032 to -0.014; \( p < 0.001 \)) for DM patients and 0.002 (95% CI, -0.005 to 0.010; \( p = 0.535 \)) for non-DM patients. A comparison of the two slopes by an interaction term indicated significant difference (-0.025, 95% CI, -0.038 to -0.013; \( p < 0.001 \)) over the 6-year follow-up. When adjusting by all potential confounders in the mixed-effect model, the slopes

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**Table 1. Comparison of baseline characteristics between patients with and without diabetes mellitus (DM).**

| Characteristics                      | All (n = 296) | DM (n = 140) | Non-DM (n = 156) | \( p \) |
|--------------------------------------|--------------|--------------|------------------|--------|
| First ABI year                       |              |              |                  | 0.002  |
| 2009 or before                       | 177          | 69           | 108              |        |
| 2010–2015                            | 119          | 71           | 48               |        |
| Age at first ABI (year)              | 58.0 ± 11.8  | 60.8 ± 9.1   | 55.6 ± 13.4      | < 0.001|
| Male gender (%)                      | 51.0         | 54.3         | 48.1             | 0.286  |
| Duration of dialysis (years)         | 2.5 (0.8–7.0)| 1.3 (0.6–4.4)| 5.1 (1.3–9.2)    | < 0.001|
| Smoking history (%)                  | 33.4         | 41.7         | 26.3             | 0.005  |
| Hypertension (%)                     | 71.2         | 80.0         | 63.5             | 0.002  |
| Coronary artery disease (%)          | 18.9         | 22.9         | 15.4             | 0.101  |
| Cerebrovascular disease (%)          | 7.8          | 13.6         | 2.6              | < 0.001|
| Systolic blood pressure (mmHg)       | 151.6 ± 25.9 | 160.2 ± 25.4 | 143.9 ± 23.9     | < 0.001|
| Diastolic blood pressure (mmHg)      | 80.7 ± 14.5  | 82.0 ± 14.0  | 79.6 ± 14.9      | 0.154  |
| Pulse pressure (mmHg)                | 71.0 ± 17.6  | 78.5 ± 17.8  | 64.3 ± 14.5      | < 0.001|
| Laboratory parameters                |              |              |                  |        |
| Albumin (g/dL)                       | 3.9 ± 0.3    | 3.9 ± 0.3    | 4.0 ± 0.3        | 0.001  |
| Triglyceride (mg/dL)                 | 125.5 (89.3–192) | 140 (104–208) | 119 (83–181)    | 0.006  |
| Total cholesterol (mg/dL)            | 178.4 ± 42.2 | 182.5 ± 49.2 | 174.8 ± 34.5     | 0.135  |
| HDL-cholesterol (mg/dL)              | 40.3 ± 11.3  | 38.0 ± 10.0  | 42.5 ± 12.0      | < 0.001|
| LDL-cholesterol (mg/dL)              | 89.2 ± 31.3  | 91.8 ± 36.2  | 86.8 ± 26.0      | 0.182  |
| Hemoglobin (g/dL)                    | 10.1 ± 1.3   | 10.2 ± 1.2   | 10.0 ± 1.3       | 0.426  |
| Creatinine (mg/dL)                   | 10.2 ± 2.4   | 9.5 ± 2.7    | 10.7 ± 2.5       | < 0.001|
| Total calcium (mg/dL)                | 9.3 ± 0.9    | 9.2 ± 0.8    | 9.4 ± 0.9        | 0.054  |
| Phosphorous (mg/dL)                  | 4.9 ± 1.3    | 4.9 ± 1.3    | 4.8 ± 1.4        | 0.620  |
| Calcium-phosphorous product (mg²/dL²)| 45.2 ± 12.7  | 45.1 ± 12.1  | 45.4 ± 13.2      | 0.840  |
| Uric acid (mg/dL)                    | 7.8 ± 1.6    | 7.7 ± 1.6    | 7.8 ± 1.6        | 0.572  |
| PTH (pg/mL)                          | 312.6 (160.7–532.7) | 260.8 (150.5–421.6) | 351.2 (173.6–633.2) | 0.011 |
| Kt/V (Daugirdas)                     | 1.5 ± 0.3    | 1.5 ± 0.3    | 1.6 ± 0.3        | 0.035  |
| Medications                          |              |              |                  |        |
| Aspirin use (%)                      | 8.1          | 10.0         | 6.4              | 0.259  |
| ACEI and/or ARB use (%)              | 16.2         | 21.4         | 11.5             | 0.021  |
| Statin use (%)                       | 16.6         | 21.4         | 12.2             | 0.033  |
| ABI < 0.9 (%)                        | 21.0         | 30.9         | 12.2             | < 0.001|
| Death (%)                            | 19.0         | 22.5         | 15.9             | 0.153  |

Abbreviations. ABI, ankle-brachial index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PTH, parathyroid hormone; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

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of DM and non-DM as well as the coefficient of interaction were -0.014 (95% CI, -0.024 to -0.003; \( p = 0.010 \)), 0.010 (95% CI, 0.001 to 0.019; \( p = 0.027 \)) and -0.024 (95% CI, -0.036 to -0.012; \( p < 0.001 \)), respectively.

We have further performed sensitivity analysis using ABI change from either right or left sides. Using the estimated slopes from using right side ABI measures showed -0.015 (95% CI, -0.025 to -0.005; \( p = 0.002 \)) for DM patients and 0.002 (95% CI, 0.006 to 0.010; \( p = 0.681 \)) for non-DM patients. A comparison of the two slopes by an interaction term indicated significant difference -0.017 (95% CI, -0.030 to -0.004; \( p = 0.009 \)) over the 6-year follow-up. Besides, using the estimated slopes showed -0.024 (95% CI, -0.037 to -0.011; \( p < 0.001 \)) for DM patients and 0.000 (95% CI, -0.008 to 0.008; \( p = 0.991 \)) for non-DM patients from using left side. A comparison of the two slopes by an interaction term indicated significant difference -0.024 (95% CI, -0.037 to -0.011; \( p < 0.001 \)) over the 6-year follow-up. These estimates were similar to the estimate from using the minimum of ABI.

### Determinants of ABI decline in HD patients with DM

Using the mixed-effect model, the main effects of the variables on ABI change in HD patients with DM are female sex (coefficient: 0.1099; 95% CI, 0.0363 to 0.1835; \( p = 0.004 \)), high pulse pressure (coefficient: -0.0028; 95% CI, -0.0038 to -0.0019; \( p < 0.001 \)), high triglyceride (coefficient: -0.1116; 95% CI, -0.2189 to -0.0043; \( p = 0.042 \)), low creatinine (coefficient:0.0130; 95% CI, 0.0015 to 0.0245; \( p = 0.027 \)), and high uric acid (coefficient: -0.0110; 95% CI, -0.0219 to 0; \( p = 0.049 \)) were significantly associated with a decrease in ABI (Table 2).

### Determinants of ABI decline in HD patients without DM

The main effects of the variables on ABI change in HD patients without DM demonstrated that old age (coefficient: -0.0026; 95% CI, -0.0040 to -0.0012; \( p < 0.001 \)), high pulse pressure (coefficient: -0.0009; 95% CI, -0.0017 to -0.0002; \( p = 0.019 \)), high LDL-cholesterol (coefficient: -0.0011; 95% CI, -0.0021 to -0.0002; \( p = 0.015 \)), and high uric acid (coefficient: -0.0094; 95%
CI, -0.0170 to -0.0018; \( p = 0.015 \) were significantly associated with a decrease in ABI (Table 3).

A decrease in ABI by \( > 0.1 \) or a final ABI \( < 0.9 \)

We further perform subgroup analysis in patients with baseline ABI \( \geq 0.9 \) (\( n = 232 \)). There are 49.6% of patients with a normal ABI experienced a decrease at least 0.1 of ABI from baseline, and 35.3% have a final ABI \( < 0.9 \). Figs 2 and 3 illustrates the Kaplan-Meier survival curves for ABI decrease \( > 0.1 \) and progression to ABI \( < 0.9 \), respectively, in patients with baseline ABI \( \geq 0.9 \). DM patients had worse ABI decrease \( > 0.1 \)-free survival (log-rank test, \( p < 0.001 \)) and progression to ABI \( < 0.9 \)-free survival (log-rank test, \( p = 0.014 \)) than non-DM patients.

**Discussion**

There have been no published studies on serial measurements of ABI in HD patients. The present study is the first to evaluate changes in ABI yearly in HD patients over 6 years. We evaluated the associations of DM with decline in ABI yearly over 6 years in HD patients. The study was allowing us to describe the natural history of ABI in HD patients, and found the

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**Table 2. Main effects of the variables on ABI change over year in hemodialysis patients with DM.**

| Variables                              | ABI change | 95% confidence intervals | \( p \)  |
|----------------------------------------|------------|--------------------------|---------|
| Age (per 1 year)                       | -0.0024    | (-0.0050, 0.0002)        | 0.072   |
| Male vs. Female                        | 0.1099     | (0.0363, 0.1835)         | 0.004   |
| Duration of dialysis (per 1 year)      | -0.0058    | (-0.0143, 0.0028)        | 0.185   |
| Smoking history                        | -0.0645    | (-0.1332, 0.0043)        | 0.066   |
| Hypertension                           | 0.0002     | (-0.0515, 0.0519)        | 0.994   |
| Coronary artery disease                | -0.0186    | (-0.0705, 0.0334)        | 0.483   |
| Cerebrovascular disease                | -0.0083    | (-0.0716, 0.0549)        | 0.796   |
| Pulse pressure (per 1 mmHg)            | -0.0028    | (-0.0038, -0.0019)       | < 0.001 |
| Laboratory parameters                  |            |                          |         |
| Albumin (per 1 g/dL)                   | -0.0316    | (-0.1041, 0.0409)        | 0.392   |
| Triglyceride (per log 1 mg/dL)         | -0.1116    | (-0.2189, -0.0043)       | 0.042   |
| Total cholesterol (per 1 mg/dL)        | 0.0005     | (-0.0002, 0.0012)        | 0.132   |
| HDL-cholesterol (per 1 mg/dL)          | 0.0019     | (-0.0006, 0.0043)        | 0.128   |
| LDL-cholesterol (per 1 mg/dL)          | -0.0005    | (-0.0013, 0.0003)        | 0.183   |
| Hemoglobin (per 1 g/dL)                | -0.0084    | (-0.0212, 0.0043)        | 0.194   |
| Creatinine (per 1 mg/dL)               | 0.0130     | (0.0015, 0.0245)         | 0.027   |
| Total calcium (per 1 mg/dL)            | 0.0250     | (-0.0359, 0.0858)        | 0.420   |
| Phosphorous (per 1 mg/dL)              | 0.0430     | (-0.0628, 0.1488)        | 0.425   |
| Calcium-phosphorous product (per 1 mg²/dL²) | -0.0051 | (-0.0164, 0.0061)         | 0.372   |
| Uric acid (per 1 mg/dL)                | -0.0110    | (-0.0219, 0.0000)        | 0.049   |
| PTH (per log 1 pg/mL)                  | -0.0026    | (-0.0369, 0.0317)        | 0.880   |
| Kt/V (per 1)                           | 0.0353     | (-0.0459, 0.1165)        | 0.393   |
| Medications                             |            |                          |         |
| Aspirin use                            | -0.0356    | (-0.0958, 0.0246)        | 0.245   |
| ACEI and/or ARB use                    | -0.0323    | (-0.0772, 0.0126)        | 0.158   |
| Statin use                             | 0.0174     | (-0.0268, 0.0615)        | 0.440   |

Abbreviations are same as Table 1. Values expressed as ABI change and 95% confidence interval (CI).

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The most significant finding here is that HD patients with DM tend to portend a more aggressive course, with a rapid ABI decline, compared to non-DM patients (slopes: -0.014 vs. 0.010 per year, interaction \(p < 0.001\) over a 6-year follow-up. Some studies have looked at the progression of PAOD in selected patient populations via serial measurements of ABI in two readings [8,13–16]. The study by Bird [14] surveyed ABI changes in 508 PAOD patients and found a mean ABI change of -0.019 over a 4.6-year average follow-up (about -0.004 per year). Similarly, Hoe et al.[8] assessed ABI change in 82 DM patients over a mean follow-up of 27.6 months and demonstrated that one in five DM patients had a significant decrease in ABI. In addition, Jiwakanon et al.[16] evaluated ABI changes in 167 proteinuric DM patients after a mean interval of 23 ± 6 months and found that 17% of patients had either an ABI decrease of ≥ 0.1 or a final ABI < 0.9 [16]. Althouse et al.[13] likewise investigated ABI change for an average 4.6 years of follow-up in 1479 DM patients, and found that approximately 20% of participants with normal ABI had at least one PAOD-related incident. The PAOD-related incident included new ABI < 0.9 and a decrease of at least 0.1 from baseline, lower extremity revascularization, or lower extremity amputation [13]. In HD patients, Chen et al. assessed the progression of PAOD and revealed a mean ABI change of -0.04 in 3 years (about -0.013 per year).

### Table 3. Main effects of the variables on ABI change over year in hemodialysis patients without DM.

| Variables                              | ABI change | 95% confidence intervals | \(p\)  |
|----------------------------------------|------------|--------------------------|-------|
| Age (per 1 year)                       | -0.0026    | (-0.0040, -0.0012)       | < 0.001 |
| Male vs. Female                        | 0.0283     | (-0.0115, 0.0681)        | 0.163  |
| Duration of dialysis (per 1 year)      | -0.0027    | (-0.0057, 0.0003)        | 0.081  |
| Smoking history                        | 0.0253     | (-0.0110, 0.0615)        | 0.171  |
| Hypertension                           | -0.0138    | (-0.0426, 0.0149)        | 0.345  |
| Coronary artery disease                | -0.0125    | (-0.0599, 0.0350)        | 0.606  |
| Cerebrovascular disease                | -0.0051    | (-0.0843, 0.0741)        | 0.900  |
| Pulse pressure (per 1 mmHg)            | -0.0009    | (-0.0017, -0.0002)       | 0.018  |

#### Laboratory parameters

| Variables                        | ABI change | 95% confidence intervals | \(p\)  |
|----------------------------------|------------|--------------------------|-------|
| Albumin (per 1 g/dL)             | 0.0252     | (-0.0240, 0.0744)        | 0.315  |
| Triglyceride (per log 1 mg/dL)   | -0.0585    | (-1.383, 0.0212)         | 0.150  |
| Total cholesterol (per 1 mg/dL)  | 0.0006     | (-0.0002, 0.0014)        | 0.137  |
| HDL-cholesterol (per 1 mg/dL)    | -0.0001    | (-0.0017, 0.0015)        | 0.885  |
| LDL-cholesterol (per 1 mg/dL)    | -0.0011    | (-0.0021, -0.0002)       | 0.015  |
| Hemoglobin (per 1 g/dL)          | -0.0019    | (-0.0109, 0.0070)        | 0.673  |
| Creatinine (per 1 mg/dL)         | -0.0030    | (-0.0115, 0.0054)        | 0.482  |
| Total calcium (per 1 mg/dl)      | -0.0046    | (-0.0446, 0.0354)        | 0.823  |
| Phosphorous (per 1 mg/dL)        | 0.0004     | (-0.0752, 0.0759)        | 0.993  |
| Calcium-phosphorous product (per 1 mg2/dL2) | -0.0005  | (-0.0085, 0.0075)       | 0.900  |
| Uric acid (per 1 mg/dL)          | -0.0094    | (-0.0170, -0.0018)       | 0.015  |
| PTH (per log 1 pg/mL)            | 0.0016     | (-0.0217, 0.0250)        | 0.892  |
| Kt/V (per 1)                     | 0.0169     | (-0.0384, 0.0721)        | 0.549  |

#### Medications

| Variables                        | ABI change | 95% confidence intervals | \(p\)  |
|----------------------------------|------------|--------------------------|-------|
| Aspirin use                      | -0.0445    | (-1.011, 0.0121)         | 0.123  |
| ACEI and/or ARB use              | -0.0076    | (-0.0436, 0.0284)        | 0.679  |
| Statin use                       | 0.0240     | (-0.0093, 0.0574)        | 0.157  |

Abbreviations are same as Table 1. Values expressed as ABI change and 95% confidence interval (CI).
In the present study, the mean ABI decrease in HD patients with DM is about -0.017 per year, significantly higher compared to the findings of previous studies (ABI decline rates of -0.004 to -0.013 per year) [14,15]. Besides, our study showed there were 49.6% of patients with a normal ABI experienced a decrease at least 0.1 of ABI from baseline, and 35.3% had a final ABI < 0.9, which frequency were also higher than previous studies [8,13,16]. There is growing evidence that uremia itself promotes PAOD progression [17,18]. Such processes may include vascular calcification, inflammatory and coagulation pathways alterations, oxidative stress, malnutrition, or infection [19,20].

Another important finding of the present study is that high triglyceride, and high LDL-cholesterol are associated with a rapid ABI decline among HD patients. In previous cross-sectional studies, the identified risk factors of PAOD in HD patients are old age, hypertension, DM, previous coronary artery disease or cerebrovascular disease, wider pulse pressure, hyperlipidemia, malnutrition, and smoking [18]. Some longitudinal studies also support the role of lipid profile in PAOD progression [8,9,13]. High triglyceride and LDL-cholesterol are major risk factors of atherosclerosis [21,22], which was consistent with our results. Lipid-lowering agents may be effective in the primary prevention of coronary artery disease [23], but there is no evidence that these drugs are effective in preventing or treating PAOD. In a meta-analysis of seven prospective randomized trials of lipid-lowering agents of patients with existing PAOD, there is no significant improvement in pain, ABI, or skin necrosis [24]. Nonetheless, despite the paucity of...
of effective PAOD treatment, most clinicians still prescribe lipid-lowering agents because of their proven benefits of reducing coronary artery and cerebrovascular disease in patients on HD. This warrants further investigation in order to develop interventions that can slow the rapid progression of PAOD in high-risk populations. However, treatment with statins could potentially influence lipid parameters, and the effects of the variables of cholesterols and triglycerides are affected by medications. Therefore, the results related with these variables should be carefully estimated.

Approximately 45% of all Taiwanese dialysis patients with ESRD have DM, and it is the major cause of chronic kidney disease worldwide. In addition, the rate of elderly and diabetic patients receiving renal replacement therapy has dramatically increased in recent years, and they now account for a significant proportion of all patients undergoing dialysis [25,26]. The clinical outcomes of elderly patients with ESRD and diabetes would be expected to be worse compared to a younger population without diabetes [27,28]. In addition, functional activity and rehabilitation have been shown to be more severely affected in older patients with diabetes [29]. In large part, the differences in survival between patients with and without diabetes are attributable to poorer nutritional status in those with diabetes. Furthermore, a lower body mass index and malnutrition (due to dialysis-related protein loss, increased inflammation causing increased protein catabolism and a loss of appetite due to changes in taste and dietary
restrictions) is associated with worse outcomes in patients with ESRD. [30,31]. The present study shows that low creatinine is associated with PAOD progression in DM, not in non-DM patients. Compared to non-DM patients, DM patients had older age, lower albumin and creatinine, which may partly explain the role of creatinine in rapid PAOD progression in DM patients. Traditional risk factors like high blood pressure, obesity, and hypercholesterolemia also play important roles in cardiovascular mortality of the general population. The concept of reverse epidemiology has recently suggested that low body mass index, low blood pressure, hypcholesterolemia, low creatinine, and low homocysteine level are associated with high cardiovascular and total mortality in patients with ESRD [32,33]. Low creatinine level may be related to malnutrition and can cause poorer outcome by accelerating atherosclerosis and worsening inflammation [34]. In addition, creatinine level is not only a marker of nutrition but also of muscle mass, which is related to physical activity and performance. Skeletal muscular dysfunction severely affects mobility and physical performance, and increasing the risk of fractures, disability, hospitalization and mortality [35]. Impaired physical function is a major complication in patients with ESRD, and it is associated with both a low quality of life and an increased risk of all-cause mortality in dialysis patients [36,37].

The third important finding of this study is that in HD patients, regardless with or without DM, high uric acid level is associated with a rapid ABI decline. The mechanism by which uric acid is associated with atherosclerotic disease remains unclear. Uric acid may increase platelet adhesiveness [38,39], or urate crystals may be associated with increased platelet lysis [40]. Uric acid may also play a role in the formation of free radicals and in oxidative stress [41,42]. Moreover, hyperuricemia per se can induce endothelial dysfunction by inhibiting the synthesis and release of nitric oxide [43]. The renin-angiotensin system in vascular endothelial cells (a hormonal vasoconstriction system) is also activated by elevated uric acid [44]. A previous experiment has confirmed that the solubility of monosodium urate falls sharply with decreasing temperature [45]. Local temperature may partially explain the clinically observed distribution of gouty tophi and gouty arthritis. Presumably, urate deposition in the lower extremity of patients with PAOD during cold temperatures and promote urate-related atherosclerosis. We also observed that high uric acid level was associated with a decrease in ABI in HD patients. This may play a role in the clinically observed progression of atherosclerosis in HD patients with hyperuricemia.

We also found that the progression of PAOD was more strongly associated with the female patients with DM than the male patients with DM. Male sex is a known risk factor for most heart valve and vascular diseases, whereas females are known to be at higher risk of other disorders [46]. Cardiovascular diseases are the leading cause of death and disability in both men and women worldwide, but affect more women than men. Cardiovascular diseases are mostly caused by traditional risk factors, and whereas the effects of high blood pressure, overweight and obesity, and high cholesterol levels on cardiovascular outcomes are generally similar between men and women, the risk incurred by diabetes is significantly higher in women than in men [46,47]. Menopause plays a vital role in this difference, as it leads to adverse changes in cardiovascular structure due to hormonal changes. Oestrogens are known to be important modulators of lipid metabolism, inflammation and vascular homeostasis. Endogenous oestrogen plays a role in reducing the incidence of atherosclerotic vascular disease in premenopausal women with intact ovarian function, therefore the decrease in oestrogen production after menopause leads to an increase in the risk of cardiovascular diseases [47]. Women with ESRD are often associated with menstrual and fertility disorders due to disturbances in the endocrine system mediated by the kidneys [48]. This situation is characterized by an early decrease in the reserve of ovarian follicles, resulting in amenorrhea, infertility and the long-term reduction in estrogen and androgen [48]. In the present study, all of the women undergoing HD except for
seven without DM still had menopause. Therefore, factors specific to women undergoing HD may be risk factors for the progression of PAOD.

The strength of this study was its prospective serial follow-up of annual ABI measurements in 6 years in patients on HD, a high-risk group for PAOD. Moreover, a longitudinal mixed-model with risk factors of potential confounders at individual yearly measure was used to reflect the actual value change during the follow-up period. This tested for associations while accounting for within-patient correlation. Nonetheless, this study also has some limitations. First, the effect of medications on ABI change was not evaluated because this study was not a clinical trial aimed to investigate the effects of medication, which is lacking cumulative exposure duration and defined daily dose. Treatment with statins could potentially influence lipid parameters. However, because of ethical reason, we did not hold any drugs at the time of ABI evaluation. To decrease the influence of drugs, we had added statins in the multivariate analysis. Second, other important factors affecting PAOD progression like inflammatory markers were not evaluated. During follow-up, there were also a significant number of deaths that more likely had lower ABI. Overall, the effect of biased results might have caused an underestimation of the magnitude of PAOD progression in this study. Lastly, the sensitivity of ABI measurements for detection of PAOD among patients with ESRD has not been tested. This technique is probably less sensitive than in the general population, because of the high prevalence of arterial calcification in this population, especially with DM [4,49]. Thus, estimates of PAOD prevalence based on ABI testing might underestimate the true prevalence of PAOD in the ESRD population.

In conclusion, in this study, HD patients with DM tend to have a more rapid ABI decline compared with non-DM patients after 6 years of follow-up. Female sex, high pulse pressure, high triglyceride, low creatinine, and high uric acid were associated with a rapid ABI decrease in DM patients, and old age, high pulse pressure, high LDL-cholesterol, and high uric acid in non-DM patients.

Supporting information
S1 File. Relevant data including ABI change.
(XLS)

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References
1. Dumaine RL, Montalescot G, Steg PG, Ohman EM, Eagle K, Bhatt DL. Renal function, atherothrombosis extent, and outcomes in high-risk patients. Am Heart J 2009; 158:141–148. https://doi.org/10.1016/j.ahj.2009.05.011 PMID: 19540404
2. Rajagopalan S, Dellegrottaglie S, Furniss AL, Gillespie BW, Satayathum S, Lameire N, et al. Peripheral arterial disease in patients with end-stage renal disease: Observations from the dialysis outcomes and practice patterns study (DOPPS). Circulation 2006; 114:1914–1922. https://doi.org/10.1161/CIRCULATIONAHA.105.607390 PMID: 17060384
3. Criqui MH. Systemic atherosclerosis risk and the mandate for intervention in atherosclerotic peripheral arterial disease. Am J Cardio 2001; 88:43J–47J.
4. Ono K, Tsuchida A, Kawai H, Matsuo H, Wakamatsu R, Maetzawa A, et al. Ankle-brachial blood pressure index predicts all-cause and cardiovascular mortality in hemodialysis patients. J Am Soc Nephrol 2003; 14:1591–1598. PMID: 12761260
5. Selvin E, Erlinger TP. Prevalence of and risk factors for peripheral arterial disease in the united states: Results from the national health and nutrition examination survey, 1999–2000. Circulation 2004; 110:738–743. https://doi.org/10.1161/01.CIR.0000137913.26087.F0 PMID: 15262830
6. Jude EB, Oyibo SO, Chalmers N, Boulton AJ. Peripheral arterial disease in diabetic and nondiabetic patients: A comparison of severity and outcome. Diabetes Care 2001; 24:1433–1437. PMID: 11473082
7. American Diabetes Association: Peripheral arterial disease in people with diabetes. Diabetes Care 2003; 26:3333–3341. PMID: 14633825
8. Hoe J, Koh WP, Jin A, Sum CF, Lim SC, Tavintharan S. Predictors of decrease in ankle-brachial index among patients with diabetes mellitus. Diabet Med 2012; 29:e304–307. https://doi.org/10.1111/j.1464-5491.2012.03705.x PMID: 22587456
9. Aboyans V, Criqui MH, Denenberg JO, Kniske JD, Ridker PM, Fronot A. Risk factors for progression of peripheral arterial disease in large and small vessels. Circulation 2006; 113:2625–2629. https://doi.org/10.1161/CIRCULATIONAHA.105.608679 PMID: 16735675
10. Smith FB, Lee AJ, Price JF, van Wijk MC, Fowkes FG. Changes in ankle brachial index in symptomatic and asymptomatic subjects in the general population. J Vasc Surg 2003; 38:1323–1330.
11. Daugirdas JT. Simplified equations for monitoring Kt/V, PCRn, eKt/V, and ePCRn. Adv Ren Replace Ther 1995; 2:295–304. PMID: 8591121
12. Yamashina A, Tomiyama H, Takeda K, Tsuda H, Arat T, Hirose K, et al. Validity, reproducibility, and clinical significance of noninvasive brachial-ankle pulse wave velocity measurement. Hypertens Res 2002; 25:359–364. PMID: 12135313
13. Althouse AD, Abbott JD, Forker AD, Bertolet M, Barinas-Mitchell E, Thurston RC, et al. Risk factors for incident peripheral arterial disease in type 2 diabetes: Results from the bypass angioplasty revascularization investigation in type 2 diabetes (bARI 2d) trial. Diabetes Care 2014; 37:1346–1352. https://doi.org/10.2337/dc13-2903 PMID: 24595631
14. Bird CE, Criqui MH, Fronot A, Denenberg JO, Klauber MR, Langer RD. Quantitative and qualitative progression of peripheral arterial disease by non-invasive testing. Vasc Med 1999; 4:15–21. https://doi.org/10.1177/1358863X9900400103 PMID: 10359865
15. Chen YH, Lin KC, Tsai YF, Yu LK, Huang LH, Chen CA. Anti-platelet factor 4/heparin antibody is associated with progression of peripheral arterial disease in hemodialysis patients. Int Urol Nephrol 2015; 47:1565–1570. https://doi.org/10.1007/s11255-015-1056-3 PMID: 26198856
16. Jwakanon S, Adler S, Mehrotra R. Change in ankle-brachial index over time and mortality in diabetics with proteinuria. Clin Nephrol 2012; 78:335–345. https://doi.org/10.5414/CN107463 PMID: 22784559
17. Matsumae T, Abe Y, Murakami G, Ishihara M, Ueda K, Saito T. Determinants of arterial wall stiffness and peripheral artery occlusive disease in nondiabetic hemodialysis patients. Hypertens Res 2007; 30:377–385. https://doi.org/10.1291/hypres.30.377 PMID: 17587749
18. O’Hare AM, Hsu CY, Bacchetti P, Johansen KL. Peripheral vascular disease risk factors among patients undergoing hemodialysis. J Am Soc Nephrol 2002; 13:497–503. PMID: 11805180
19. O’Hare A, Johansen K. Lower-extremity peripheral arterial disease among patients with end-stage renal disease. J Am Soc Nephrol 2001; 12:2838–2847. PMID: 11729255
20. Webb AT, Franks PJ, Reaveley DA, Greenhalgh RM, Brown EA. Prevalence of intermittent claudication and risk factors for its development in patients on renal replacement therapy. Eur J Vasc Surg 1993; 7:523–527. PMID: 8405496
21. Masson W, Siniawski D, Lobo M, Molinero G, Huerin M. Association between triglyceride/HDL cholesterol ratio and carotid atherosclerosis in postmenopausal middle-aged women. Endocrinol Nutr 2016; pii: S1575-0922(16)30047-X.
22. Rose G, Hamilton PS, Keen H, Reid DD, McCartney P, Jarrett RJ. Myocardial ischaemia, risk factors and death from coronary heart-disease. Lancet 1977; 1:105–109. PMID: 64647
23. Pignone M, Phillips C, Mulrow C. Use of lipid lowering drugs for primary prevention of coronary heart disease: Meta-analysis of randomised trials. BMJ 2000; 321:983–986. PMID: 11039962
24. Leng GC, Price JF, JPegson RG. Lipid-lowering for lower limb atherosclerosis. Cochrane Database Syst Rev 2000;CD000123.
25. USRDS 2007 Annual Data Report: Atlas of Chronic Kidney Disease And End-Stage Renal Disease in the United States. Volume II Reference Tables. Bethesda, MD: National Kidney and Urologic Diseases Information Clearinghouse, 2007.
26. USRDS 2007 Annual Data Report: Atlas of Chronic Kidney Disease And End-Stage Renal Disease in the United States. Volume 1. Bethesda, MD: National Kidney and Urologic Diseases Information Clearinghouse, 2007.
27. Mooraki A, Kliger AS, Juergensen P, Gorban-Brennan N, Finkelstein FO. Selected outcome criteria and adequacy of dialysis in diabetic and elderly patients on CAPD therapy. Adv Perit Dial 1994; 10: 89–93. PMID: 7999872
28. Toriyama T, Yokoya M, Nishida Y, Kawajiri K, Takahashi H, Kawahara H. [Increased incidence of coronary artery disease and cardiac death in elderly diabetic nephropathy patients undergoing chronic hemodialysis therapy]. J Cardiol 2000; 36: 165–171. PMID: 11026525
29. Ifudu O, Mayer J, Matthew J, Tan CC, Cambridge A, Friedman EA. Dismal rehabilitation in geriatric inner-city hemodialysis patients. JAMA 1994; 271: 29–33. PMID: 8256893
30. Kalantar-Zadeh K, Kopple JD, Regidor DL, Jing J, Shinaberger CS, Aronovitz J, et al. A1C and survival in maintenance hemodialysis patients. Diabetes Care 2007; 30: 1049–1055. https://doi.org/10.23777/dc06-2127 PMID: 17337501
31. Raffaitin C, Lasseur C, Chauveau P, Barthe N, Gin H, Combe C, et al. Nutritional status in patients with diabetes and chronic kidney disease: a prospective study. Am J Clin Nutr 2007; 85: 96–101. PMID: 17209183
32. Chen JH, Chen SC, Liu WC, Su HM, Chen CY, Mai HC, et al. Determinants of peripheral arterial stiffness in patients with chronic kidney disease in southern Taiwan. Kaohsiung J Med Sci 2009; 25:366–373. https://doi.org/10.1016/S1607-551X(09)70529-7 PMID: 19605328
33. Kopple JD. The phenomenon of altered risk factor patterns or reverse epidemiology in persons with advanced chronic kidney failure. Am J Clin Nutr 2005; 81:1257–1266. PMID: 15941874
34. Levin NW, Handelman GJ, Coresh J, Port FK, Kayser GA. Reverse epidemiology: A confusing, confounding, and inaccurate term. Semin Dial 2007; 20:586–592. https://doi.org/10.1111/j.1525-139X.2007.00366.x PMID: 17991209
35. Studenski S, Perera S, Patel K, Rosano C, Faulkner K, Inzitari M, et al. Gait speed and survival in older adults. JAMA 2011; 305: 50–58. https://doi.org/10.1001/jama.2010.1923 PMID: 21205966
36. Johansen KL, Chertow GM, Ng AV, Mulligan K, Carey S, Schoenfeld PY, et al. Physical activity levels in patients on hemodialysis and healthy sedentary controls. Kidney Int 2000; 57: 2564–2570. https://doi.org/10.1046/j.1523-1755.2000.00116.x PMID: 10844626
37. DeOreo PB. Hemodialysis patient-assessed functional health status predicts continued survival, hospitalization, and dialysis-attendance compliance. Am J Kidney Dis 1997; 30: 204–212. PMID: 9261030
38. Emmerson BT. Atherosclerosis and urate metabolism. Aust N Z J Med 1979; 9:451–454. PMID: 389225
39. Newland H. Hyperuricemia in coronary, cerebral and peripheral arterial disease: An explanation. Med Hypotheses 1975; 1:152–155. PMID: 1196166
40. Ginsberg MH, Kozin F, O’Malley M, McCarty DJ. Release of platelet constituents by monosodium urate crystals. J Clin Invest 1977; 60:999–1007. https://doi.org/10.1172/JCI108880 PMID: 908764
41. Vasquez-Vivar J, Santos AM, Junqueira VB, Augusto O. Peroxynitrite-mediated formation of free radicals in human plasma: EPR detection of ascorbyl, albumin-thiyl and uric acid-derived free radicals. Biochem J 1996; 314 (Pt 3):869–876.

42. Anker SD, Leyva F, Poole-Wilson PA, Kox WJ, Stevenson JC, Coats AJ. Relation between serum uric acid and lower limb blood flow in patients with chronic heart failure. Heart 1997; 78:39–43. PMID: 9290400

43. Khosla UM, Zharikov S, Finch JL, Nakagawa T, Roncal C, Mu W, et al. Hyperuricemia induces endothelial dysfunction. Kidney Int 2005; 67:1739–1742. https://doi.org/10.1111/j.1523-1755.2005.00273.x PMID: 15840020

44. Kanellis J, Feig DI, Johnson RJ. Does asymptomatic hyperuricaemia contribute to the development of renal and cardiovascular disease? An old controversy renewed. Nephrology (Carlton) 2004; 9:394–399.

45. Loeb JN. The influence of temperature on the solubility of monosodium urate. Arthritis Rheum 1972; 15:189–192. PMID: 5027604

46. Masjedi S, Ferdous Z, Understanding the Role of Sex in Heart Valve and Major Vascular Diseases. Cardiovasc Eng Technol 2015; 6: 209–219. https://doi.org/10.1007/s13239-015-0226-x PMID: 26577355

47. Peters SA, Huxley RR, Woodward M. Diabetes as a risk factor for stroke in women compared with men: a systematic review and meta-analysis of 64 cohorts, including 775,385 individuals and 12,539 strokes. Lancet 2014; 383: 1973–1980. https://doi.org/10.1016/S0140-6736(14)60040-4 PMID: 24613026

48. Ginsburg ES, Owen WF Jr., Greenberg LM, Shea BF, Lazarus JM, Walsh BW. Estrogen absorption and metabolism in postmenopausal women with end-stage renal disease. J Clin Endocrinol Metab 1996; 81: 4414–4417. https://doi.org/10.1210/jcem.81.12.8954051 PMID: 8954051

49. Orchard TJ, Strandness DE Jr. Assessment of peripheral vascular disease in diabetes. Report and recommendations of an international workshop sponsored by the American Heart Association and the American Diabetes Association 18–20 September 1992, New Orleans, Louisiana. Diabetes Care 1993; 16:1199–1209. PMID: 8375253