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Coronary artery calcification and mortality in diabetic patients with proteinuria

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Vascular calcification is one of the mechanisms mediating the higher mortality risk associated with the hyperphosphatemia of chronic kidney disease. Though common, and often severe in non-dialyzed proteinuric diabetics, there are no studies on the prognostic significance of coronary artery calcification in early stage type 2 diabetic nephropathy. Here we determine this significance in 225 proteinuric diabetic patients (mean age 57 years, mean estimated glomerular filtration rate (eGFR) 52 ml/min per 1.73 m^2 and a median urine protein–creatinine ratio of 2.7). Coronary artery calcification, measured by electron beam computed tomography, was diagnosed in 86% of the patients, the severity of which correlated with older age, male gender, and white ethnicity. However, no association was found between eGFR, serum calcium, phosphorus, parathyroid hormone, or 25-hydroxy vitamin D. Over an average follow-up of 39 months, 54 patients died. A graded relationship between the severity of calcification and all-cause mortality was consistently demonstrated on both univariate and multivariate analyses. Patients in the highest quartile of calcification score had a 2.5-fold higher risk for death. Our results show the severity of coronary artery calcification early in the course of chronic kidney disease is an independent predictor of all-cause mortality. Additional studies need to determine whether altering the natural history of coronary artery calcification in early chronic kidney disease prolongs survival.

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Patients with chronic kidney disease (CKD), including those undergoing maintenance dialysis, suffer considerable morbidity and mortality.[1,2] Recent studies provide strong evidence for the role of disordered mineral metabolism, particularly elevated serum phosphorus levels, in explaining some of the substantial increase in cardiovascular risk.[3] In concert with this accumulating epidemiological data, a large body of cell culture and animal experiments has demonstrated the key role of phosphorus in inducing vascular calcification.[4,5] These emerging data support the thesis that the prevalence and severity of vascular calcification may be an appropriate intermediate outcome measure for observational and interventional studies in patients with CKD. Several studies have shown that vascular calcification begins early and is often severe during the course of CKD, particularly in those with diabetes mellitus.[6] Even though three randomized controlled trials have shown an attenuation of progression of vascular calcification with the use of non-calcium based binder sevelamer hydrochloride in CKD subjects, the effect of calcium avoidance on mortality of dialysis patients is contradictory.[7–11] It is conceivable that these apparently disparate findings suggest that there may be an advantage to begin intervention earlier in the course of CKD. However, to our knowledge, the association between coronary artery calcification (CAC) and mortality has heretofore not been studied in non-dialysis-dependent CKD subjects—a necessary prelude to any interventional studies that seek to use vascular calcification as an intermediate outcome. We undertook this study to test the hypothesis that a greater severity of CAC is associated with a higher risk for all-cause mortality in non-dialysis-dependent diabetic patients with overt proteinuria.

RESULTS

Subject characteristics and baseline CAC

The study cohort consisted of 225 proteinuric individuals with type 2 diabetes and presumed diabetic nephropathy (see Materials and Methods for definition). The baseline characteristics of study population are summarized in Table 1. CAC was present in 86% of study participants. Individuals in the lowest quartile of CAC score were younger and had lower serum 25-hydroxy vitamin D levels and non-Latino whites were more likely to be in the higher quartiles; there was no

Table 1 | Baseline patient characteristics, stratified by quartile of coronary artery calcification score

	1st Quartile (0–15)	2nd Quartile (16–149)	3rd Quartile (150–427)	4th Quartile (⩾428)	*P*-value	Entire cohort
Sample size (*n*)	56	57	56	56		225
Demographics						
Age (years)	54 ± 7	58 ± 8	58 ± 7	58 ± 6	0.009	57 ± 7
Gender (% males)	45	47	64	61	0.09	54
Race/ethnicity (*n* (%))					0.03[a]	
Non-Latino whites	3 (5)	2 (4)	10 (18)	11 (20)		26 (12)
Non-Latino blacks	15 (27)	11 (19)	8 (14)	10 (18)		44 (20)
Latino	38 (68)	41 (72)	37 (66)	34 (61)		150 (67)
Others	0 (0)	3 (4)	1 (2)	1 (2)		5 (2)
Clinical characteristics						
DM duration (years)	15 ± 5	16 ± 7	15 ± 7	16 ± 7	0.71	16 ± 6
History of CV disease (*n* (%))	12 (21)	16 (28)	16 (29)	22 (39)	0.19	66 (29)
Current smoker (*n* (%))	8 (14)	8 (14)	11 (20)	12 (21)	0.65	39 (17)
Body mass index (kg/m^2[b])	28 (6)	31 (12)	29 (11)	30 (10)	0.35	29 (10)
Systolic BP (mm Hg)	152 ± 28	157 ± 27	159 ± 27	157 ± 30	0.56	156 ± 28
Diastolic BP (mm Hg)	76 ± 12	76 ± 12	79 ± 14	78 ± 14	0.62	77 ± 13
Laboratory data						
Serum creatinine (mg/dl)	1.5 (0.8)	1.3 (1.2)	1.4 (1.0)	1.6 (1.0)	0.55	1.5 (1.0)
eGFR (ml/min per 1.73 m^2)	51 ± 25	54 ± 28	53 ± 26	49 ± 34	0.75	52 ± 26
HbA1c (%)	8.6 ± 2.4	8.4 ± 2.4	8.5 ± 2.1	8.3 ± 2.1	0.88	8.4 ± 2.2
Total cholesterol (mg/dl)	207 (95)	189 (64)	178 (59)	190 (73)	0.14	189 (69)
LDL-cholesterol (mg/dl)	109 (64)	112 (52)	102 (48)	113 (61)	0.47	109 (59)
Corrected serum calcium (mg/dl)	9.7 ± 0.5	9.8 ± 0.4	9.7 ± 0.4	9.8 ± 0.4	0.99	9.7 ± 0.4
Serum phosphorus (mg/dl[b])	4.5 (0.8)	4.2 (0.8)	4.3 (1.0)	4.4 (0.9)	0.39	4.3 (1.0)
Serum parathyroid hormone (pg/ml[b])	62 (69)	55 (96)	45 (52)	56 (84)	0.49	55 (68)
Serum 25-hydroxy vitamin D (ng/ml)	16 (12)	24 (18)	24 (14)	23 (15)	0.009	23 (14)
C-reactive protein (>0.4 mg/l, *n* (%))	21 (38)	33 (58)	29 (52)	28 (50)	0.21	111 (50)
Serum albumin (g/dl)	3.1 ± 0.5	3.3 ± 0.6	3.3 ± 0.5	3.2 ± 0.6	0.11	3.2 ± 0.6
Urine protein–creatinine ratio (mg/mg[b])	3.2 (4.7)	2.3 (3.3)	2.2 (3.1)	3.3 (6.2)	0.43	2.7 (4.7)
Baseline medical therapy[c]						
ACEIs or ARBs (*n* (%))	38 (68)	48 (84)	46 (82)	40 (71)	0.04	172 (76)
β Blocker (*n* (%))	23 (41)	26 (46)	30 (54)	35 (63)	0.11	114 (51)
Total number of anti-hypertensive agents[b]	2 (1)	2 (2)	2 (2)	2 (2)	0.32	2 (2)
Aspirin (*n* (%))	23 (41)	29 (51)	23 (41)	27 (48)	0.82	102 (45)
Lipid-lowering agents (*n* (%))	34 (61)	29 (51)	38 (68)	37 (66)	0.10	138 (61)
Phosphate binders (*n* (%))	1 (2)	3 (5)	1 (2)	2 (4)	0.71	7 (3)
Active vitamin D (%)	0 (0)	0 (0)	0 (0)	0 (0)		0 (0)
CAC score[a,b]	0 (3)	73 (66)	257 (153)	1112 (1020)		149 (415)

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blockers; BP, blood pressure; CAC, coronary artery calcification; CV, cardiovascular; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Data expressed as means ± s.d., except where indicated.
[a]Others not used to calculate significance of difference of proportions in each quartile.
[b]Data expressed as median and interquartile range.
[c]The data on medications were missing for 10 subjects.

significant difference between any of the other variables among the four groups (Table 1). Specifically, there was no significant difference in estimated glomerular filtration rate (eGFR, Figure 1a), or stage of CKD (Figure 1b) among the four quartiles of CAC scores. Furthermore, there was no correlation of measures of mineral metabolism with the severity of CAC scores (Spearman's correlation coefficients for the association of CAC with serum calcium, 0.02 ($P = 0.83$); phosphorus, −0.03 ($P = 0.71$); parathyroid hormone (PTH), 0.07 ($P = 0.37$); and 25-hydroxy vitamin D, 0.07 ($P = 0.37$)). Using multivariate linear regression analysis, increasing age ($P = 0.001$), male gender ($P = 0.01$), and non-Latino whites ($P = 0.003$) were independently associated with a higher log-transformed baseline CAC score.

Baseline CAC and all-cause mortality

Deaths were ascertained either by contact with next of kin and/or a screen of the National Death Index (NDI, available through 31 December 2007). Over a mean observation period of 39 ± 25 months, 54 deaths occurred, yielding a crude mortality rate of 5.7 per 100 patient years for the cohort. Of the 54 deaths, 40 occurred on or before 31 December 2007—29 were identified on both the NDI search and contact with next of kin, 5 were identified on NDI data screen, and 6 were

identified by contact with next of kin only (predominantly because of death occurring outside the United States of America). All the deaths after 31 December 2007 ($n = 14$) were ascertained by contact with next of kin. There

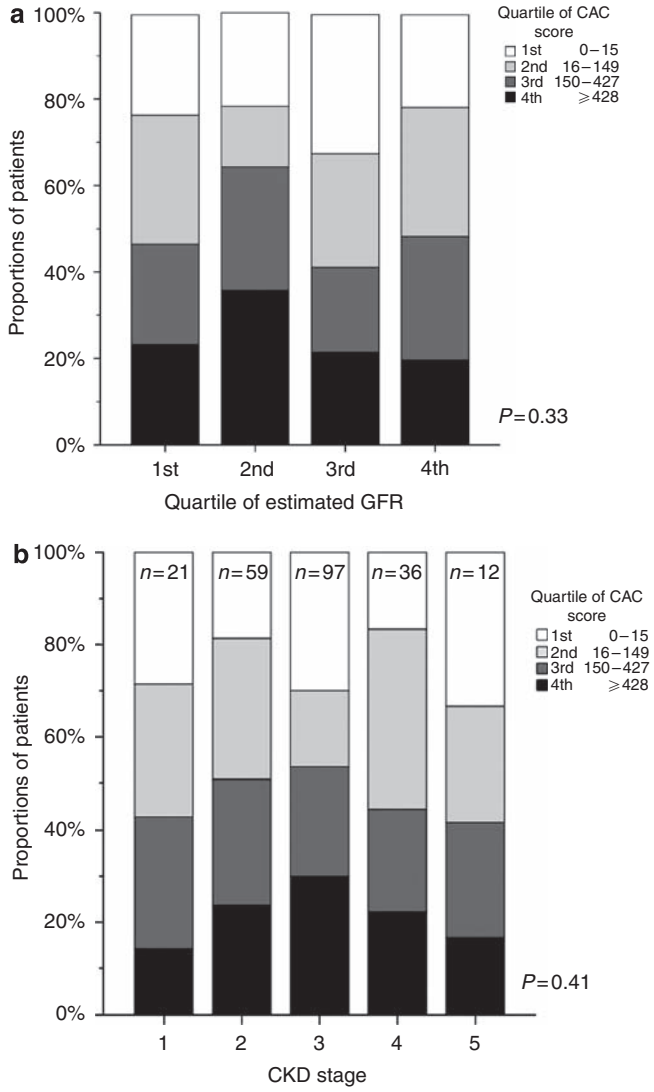

Figure 1 | Association of the severity of coronary artery calcification (CAC) with renal function. Proportions of patients in each quartile of CAC score, stratified either by (**a**) quartile of estimated glomerular filtration rate (eGFR), or (**b**) stage of chronic kidney disease. The ranges of eGFR in each quartile of GFR were: 1st quartile, ⩽31; 2nd quartile, 32–47; 3rd quartile, 47–71; and 4th quartile, ⩾71 ml/min per 1.73 m^2.

was a graded increase in the unadjusted risk for death with higher quartiles of CAC score (Table 2, and Figure 2; univariate log-rank sum test *P*-value, 0.015).

In addition to the baseline CAC score, race/ethnicity, estimated GFR, and serum albumin were significantly associated with a higher risk of death on univariate Cox's proportional hazards analysis (Table 3). The trend for an association of urine protein–creatinine ratio, and the use of β blockers with mortality did not reach statistical significance (Table 3).

Higher CAC score was an independent predictor of all-cause mortality in each one of the three multivariate models tested (data adjusted for race/ethnicity, estimated GFR, serum albumin, urine protein–creatinine ratio, and the use of β blockers)—in the first multivariate model, $\log_{10}$(CAC score + 1) was used as a continuous variable; in the second, quartile of CAC score was entered as categorical variables; and in the third, pre-determined cut-off ranges of CAC score were used (0, 1–99, 100–399, and ⩾400) (Table 4). Additional adjustment of data for age and gender did not change the hazard ratio for death with higher CAC scores in any of the three models.

Sensitivity analyses

Of the 171 subjects whose follow-up was censored, the last date of contact by the study staff for 21 participants occurred before the last day for which data were available from the NDI (31 December 2007). In order to exclude bias introduced by the censoring strategy, analyses were repeated such that follow-up was censored either at the time of death or last contact by study staff. In this sensitivity analysis, the mean observation period was 36 ± 24 months. Using the log-rank sum test, increasing quartiles of CAC score were significantly associated with a higher risk for death on univariate analyses ($P = 0.018$). The same additional univariate predictors of all-cause mortality were identified (race/ethnicity, estimated GFR, and serum albumin). Baseline CAC score remained an independent predictor of all-cause mortality in each one of the three multivariate models tested (hazard ratios for CAC score variable in model 1, $P = 0.005$; model 2, $P = 0.005$; and model 3, $P = 0.01$).

DISCUSSION

To our knowledge, this study is the first to report a significant association between CAC score obtained early in the course of CKD (baseline eGFR 52 ml/min per 1.73 m^2) and subsequent risk for death upon follow-up for a little over 3 years. Furthermore, this is the largest study that has evaluated

Table 2 | Unadjusted mortality rate, stratified by quartile of coronary artery calcification (CAC) score

	1st Quartile	2nd Quartile	3rd Quartile	4th Quartile	*P*-value	Entire cohort
CAC score range	0–15	16–149	150–427	⩾428		
Number of subjects	56	57	56	56		225
Observation period, months	42 ± 23	43 ± 27	37 ± 25	34 ± 25		39 ± 25
Death (*n* (%))	10 (18)	10 (18)	13 (23)	21 (38)		54 (24)
Unadjusted mortality rate per 100 patient years	5.1	5.0	7.5	13.9	0.015	5.7

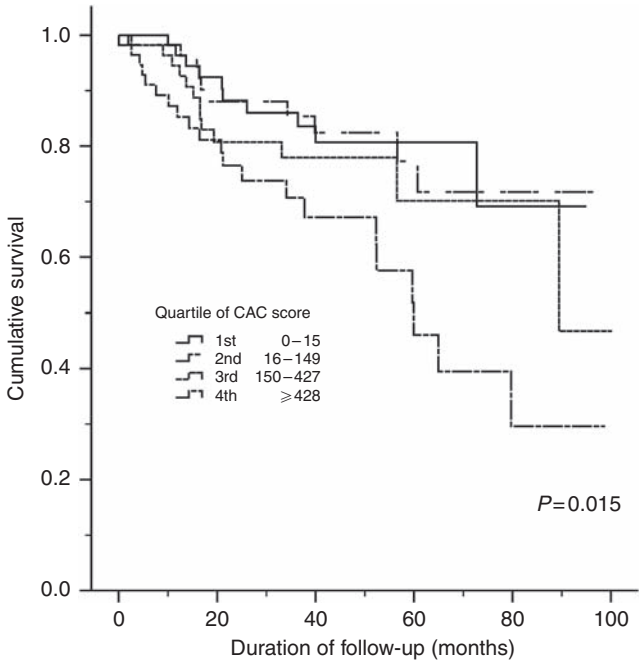

Figure 2 | Kaplan–Meier survival curve for all-cause mortality in the cohort, stratified by the quartile of coronary artery calcification (CAC) score. The *P*-values for the trend to predict all-cause mortality with quartile of CAC score were 0.02 by univariate and 0.005 by multivariate Cox proportional hazards model.

CAC in non-dialysis-dependent CKD subjects, and in this population enriched with individuals with substantial underlying renal disease and a near-universal prevalence of vascular calcification, we were unable to demonstrate a significant association between either eGFR or measures of mineral metabolism with the severity of CAC.

It has now long been known that maintenance dialysis patients have a very high burden of vascular calcification such that the CAC scores on electron beam computed tomography are substantially and markedly higher than age- and gender-matched controls.[12,13] Furthermore, the presence and/or severity of vascular calcification—whether ascertained by plain radiography, ultrasonography, or on computed tomography—has been shown to be associated with all-cause and cardiovascular mortality in patients undergoing maintenance dialysis.[11,14–16] The CAC scores reported in studies of subjects with non-dialysis-dependent CKD are substantially lower than those observed in populations of dialysis patients (reviewed by Mehrotra[6]). However, both the prevalence and severity of calcification in subjects with early-stage diabetic CKD, are significantly higher than in age- and gender-matched control diabetics and non-diabetics without kidney disease.[17,18] This is illustrated in our relatively large population of proteinuric diabetics, 86% of who had demonstrable coronary calcification and in more than one-quarter of patients, it exceeded 400 (severe calcification). It is now well-recognized that 'calcification begets calcification'; thus, the severity of calcification during early stages of CKD identifies those individuals, if alive by the time the disease reaches end-stage renal disease, who will have the greatest increase in systemic vascular calcification burden.[19–21] This is further highlighted by the demonstration of a consistent, and

Table 3 | Summary of baseline predictors of mortality on univariate analyses using the Cox proportional hazards model with *P*-value <0.10

Variable	Hazard ratio (95%CI)	*P*-value
CAC score (ref.: 1st quartile: 0–15)		0.02
2nd quartile (16–149)	0.97 (0.40, 2.33)	
3rd quartile (150–427)	1.46 (0.64, 3.34)	
4th quartile (⩾428)	2.61 (1.23, 5.54)	
Race/ethnicity (ref.: Latino)		0.003
Non-Latino whites	3.06 (1.41, 6.62)	
Non-Latino blacks	2.63 (1.45, 4.78)	
Others	—	
eGFR (ref.: 1st quartile: ⩽31.0 ml/min per 1.73 m²)		0.004
2nd quartile (32.0–46.9 ml/min per 1.73 m²)	0.78 (0.41, 1.49)	
3rd quartile (47.0–70.7 ml/min per 1.73 m²)	0.39 (0.18, 0.83)	
4th quartile (⩾70.8 ml/min per 1.73 m²)	0.19 (0.07, 0.56)	
Serum albumin (ref.: 1st quartile: ⩽2.9 g/dl)		0.004
2nd quartile (3.0–3.2 g/dl)	0.62 (0.32, 1.08)	
3rd quartile (3.3–3.5 g/dl)	0.41 (0.19, 0.88)	
4th quartile (⩾3.6 g/dl)	0.24 (0.10, 0.56)	
Urine protein–creatinine ratio (ref.: 1st quartile, ⩽1.23 mg/mg)		0.053
2nd quartile (1.24–2.658 mg/mg)	1.20 (0.45, 3.15)	
3rd quartile (2.659–5.86 mg/mg)	1.06 (0.40, 2.86)	
4th quartile (⩾5.87 mg/mg)	2.58 (1.09, 6.08)	
Missing[a]	1.76 (0.41, 3.47)	
Use of β-blocker, (ref.: no)		0.08
Yes	1.92 (1.09, 3.39)	
Missing	1.90 (0.43, 8.03)	

Abbreviations: CAC, coronary artery calcification; CI, confidence interval; eGFR, estimated glomerular filtration rate.
[a]Of 11 participants categorized as 'missing', data on 24-h urine protein excretion (without simultaneous measurement of creatinine) were available and used to determine eligibility for 7, and for 4 subjects, eligibility was determined at another hospital and data are not currently available.

Table 4 | Summary results of different multivariate analyses for the hazards ratio for death with CAC score

Predictors	Hazard ratio (95%CI)	*P*-value
Model 1[a]		
Log_{10} (CAC score+1), for every 1 increase	1.59 (1.17, 2.18)	0.004
Model 2[a]		
CAC score (ref.: 1st quartile)		0.005
2nd quartile	1.33 (0.54, 3.22)	
3rd quartile	1.41 (0.67, 3.61)	
4th quartile	3.54 (1.61, 7.77)	
Model 3[a]		
CAC score (ref.: score 0)		0.01
1–99	1.49 (0.42, 5.26)	
100–399	2.20 (0.63, 7.72)	
⩾400	4.32 (1.26, 14.78)	

Abbreviations: CAC, coronary artery calcification; CI, confidence interval.
[a]Adjusted for race/ethnicity, estimated glomerular filtration rate (eGFR), serum albumin, urine protein–creatinine ratio, and use of β-blockers.

independent-graded relationship between CAC score at an average eGFR of 52 ml/min per 1.73 m^2, and all-cause mortality in this study. Our findings are consistent with a recent study of 117 non-dialysis-dependent CKD subjects in whom a significant, univariate association of CAC with mortality ($n = 4$), cardiovascular events ($n = 15$), and hospitalization ($n = 19$) was observed. However, in that study the small number of events allowed for only limited multivariate adjustment and the authors were unable to confirm the independent association of CAC score with mortality.[22] Our findings are robust as they are based on a significant number of events (deaths, 54) over a relatively long period of follow-up (mean, 39 months). Furthermore, the higher risk for death with increasing CAC score was consistently seen in each one of the three *a priori* specified multivariate models (Table 4).

Vascular calcification can occur in either the intima (calcified atherosclerotic plaques), or the media of the blood vessels; the prevalence and severity of both intimal and medial calcification is increased with CKD.[23,24] Furthermore, plausible explanations have been put forth to explain how either intimal or medial calcification may lead to greater morbidity and mortality. Thus, the severity of intimal calcification is associated with coronary atherosclerosis and, possibly with ischemic cardiac damage.[25] On the other hand, medial calcification is associated with vascular stiffness, increased cardiac after-load, left ventricular hypertrophy, and congestive heart failure.[4] Limited evidence suggests that most of the calcification seen in the coronary artery is located in the intima, but electron beam computed tomography cannot distinguish intimal from medial calcification.[24,26] Moreover, we did not measure either the myocardial perfusion or vascular stiffness, or ventricular function and are unable to determine the mechanisms to explain the association of CAC score with all-cause mortality. Nevertheless, the effect size of increasing severity of vascular calcification was rather large such that subjects in the highest quartile of CAC score ($\geqslant 428$) had a 2.5-fold higher risk for mortality (ref., lowest quartile, CAC score 0–15). Analyzing the data differently, those with severe CAC (score $\geqslant 400$) had a 3.3-fold higher risk for death when compared with those with no detectable calcification (score 0). However, notwithstanding the clear trend of higher hazards ratio with increasing CAC scores, the risk for death was significantly higher only in individuals in the highest category of CAC score. This may be secondary to smaller number of events in the categories of lower CAC, or this may suggest that there is a threshold effect whereby the risk increases only when the CAC score exceeds about 400 U. Even though our data only demonstrate an association and not a causal relationship, it makes a strong argument to understand some of the pathophysiological mechanisms involved in the induction and progression of vascular calcification in early CKD. Understanding these mechanisms may provide us with insights into potential therapeutic interventions that may retard the progression of calcification and have a salutary effect on mortality.

As has been reported by others, increasing age, male gender, and non-Latino white race were associated with a greater severity of CAC.[27–29] We have previously demonstrated that compared with diabetes duration-matched normoalbuminuric controls, subjects with diabetic nephropathy have a significantly higher prevalence and greater severity of CAC.[18] Thus, the severity of vascular calcification burden in our study cohort reflects the effects of both diabetes mellitus and CKD. However, unlike some recent studies, we were unable to demonstrate an association between eGFR with either the prevalence or severity of CAC.[30,31] These apparently discordant findings should be interpreted with caution—the data on proteinuria were not available in most of the studies that have demonstrated an inverse association between eGFR and CAC scores. In contrast, overt proteinuria was a pre-requisite for enrollment in our study cohort. Furthermore, our study was limited only to subjects with type 2 diabetes, and should be extrapolated to non-diabetics with caution. This issue deserves further study using diabetic and non-diabetic cohorts with a wider range of urine protein excretion (from normoalbuminuria to overt proteinuria).

Similarly, unlike some recent studies, we were unable to demonstrate an association between any measure of mineral metabolism (serum calcium, phosphorus, PTH, and 25-hydroxy vitamin D levels) with the severity of CAC. The lack of association of the severity of vascular calcification with serum P was somewhat surprising as some recent studies have shown an association between serum phosphorus levels and incident and prevalent vascular calcification in individuals with and without CKD.[31–35] There is strong evidence from cell culture and animal experiments that supports the notion that phosphorus is very important in the induction and progression of vascular calcification.[4–5,36] However, serum phosphorus accounts for $<1\%$ of the total body phosphorus content and thus, may not be an accurate reflection of the total systemic phosphorus burden. Even though a higher 'phosphorus burden' is thought to underlie the increase in serum fibroblast growth factor-23 and PTH levels seen rather early during the course of CKD, neither post-absorptive nor post-prandial hyperphosphatemia are demonstrable until GFR declines to <30 ml/min per 1.73 m^2.[37,38] Furthermore, the association of serum fibroblast growth factor-23 levels with risk for death and the survival advantage with the use of phosphate binders in incident dialysis patients is independent of serum phosphorus levels;[39,40] this possibly also highlights the limitation of serum phosphorus as a marker of systemic phosphorus exposure. Consistent with these arguments, many previous studies have been unable to demonstrate any association between serum phosphorus levels and the severity of vascular calcification in either dialysis or non-dialysis CKD subjects.[12,14,18,41–49] Moreover, even though an association between serum phosphorus and the prevalence of vascular calcification was seen at baseline among participants in the Multi-Ethnic Study of Atherosclerosis, there was no

demonstrable association between serum phosphorus and the progression of CAC.[19,32] In addition to the limitation of the serum phosphorus level as a marker of systemic phosphorus exposure, there may be other reasons to explain this lack of association in our study. As pointed out earlier, our cohort had significant renal disease and the mean serum phosphorus levels were significantly higher than in many other previous studies. This may be secondary to tubular dysfunction from diabetic kidney disease that is not captured in the measurement of eGFR and/or a higher dietary phosphorus intake in our largely Latino population.

Use of the non-calcium phosphate binder, sevelamer hydrochloride, has been shown to attenuate the rate of progression of calcification in non-dialysis dependent CKD subjects.[9] Thus, it appears possible to modify the natural history of progression of calcification. However, virtually none of our patients were being treated with calcium or non-calcium phosphate binders at the time of initial evaluation. Furthermore, the effect of slowing the rate of progression of vascular calcification on the subsequent risk for death in non-dialysis-dependent CKD subjects is presently not known and needs to be further investigated.

Despite its considerable strength and novel findings, our study is not without limitations. First, our study was limited to proteinuric diabetics and the study participants were predominantly Latinos. Hence, extrapolation of our study results to non-diabetics or other racial populations should be done with caution. Furthermore, the association of urine protein excretion with mortality in our study cohort did not reach statistical significance. This may be a result of having included only individuals with overt proteinuria in our study cohort and as discussed earlier, the association needs to be re-examined in cohorts with a wider range of urine protein excretion. Second, ascertainment of risk factors was done only at baseline visit; those subjects who are still alive, are being periodically re-evaluated and thus, we will be able to evaluate the effect of change in risk factor profile over time on mortality in the future. Furthermore, our inability to demonstrate an association between the use of different classes of medications (like angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers, and statins) and mortality may be secondary to several reasons—their use was also ascertained only at baseline visit, and the adherence with the therapy or duration of use was not ascertained after the baseline visit. Third, concern has been raised that the NDI may not provide reliable information on the death of Latinos, as many of the sick and infirm may return to the country of their origin ('salmon effect');[50] to avoid bias arising from this, we carried out a sensitivity analysis by using an alternative method of censoring which yielded the same result. Fourth, the abbreviated four-variable Modification of Diet in Renal Disease equation used to estimate the glomerular filtration rate is not validated in Latino population—the predominant group in our study cohort. Finally, we did not have the information to analyze the association with cause-specific mortality.

To conclude, this is the first study to demonstrate a graded and consistent association between the severity of CAC early during the course of CKD with the risk for death. Future studies need to determine if altering the natural history of vascular calcification in early CKD will translate into a reduction in mortality in this high-risk population.

MATERIALS AND METHODS

Study subjects and baseline assessment

This is an analysis that pools participants from two prospective cohort studies of CAC in non-dialysis-dependent type 2 diabetics with nephropathy. The primary aim of each of the two studies was to evaluate racial/ethnic differences in prevalence and severity of CAC and some of the data from these two studies has been published previously.[18,51] The criteria used to define type 2 diabetes and diabetic nephropathy were similar in both studies and represented a modification from the National Institute of Diabetes and Digestive and Kidney Diseases-sponsored Family Investigation of Nephropathy in Diabetes study:[52] diagnosis of diabetes mellitus after the age of 30 years and treatment with either diet or oral hypoglycemic agents for at least 6 months, neither current nor previous treatment with maintenance dialysis or renal transplantation, urine protein–creatinine ratio $\geqslant$0.5 mg/mg either at the time of enrollment or at least once in the preceding 12 months before enrollment and one of the following two criteria (1) diabetes duration $\geqslant$10 years (or in individuals with retinopathy, $\geqslant$5 years), and/or (2) renal biopsy evidence of diabetic nephropathy. The study was approved by the Institutional Review Board at Los Angeles Biomedical Research Institute.

All study participants were scheduled for an outpatient clinic visit at the General Clinical Research Center. The subjects were asked to come in after an overnight fast and bring all the prescribed medications on the day of the study. Study procedures included relevant medical history, measurement of height and weight, recording blood pressure in duplicate, and collection of urine and blood samples. Data thus collected were used to determine the prevalence and/or severity of traditional (age, gender, hypertension, dyslipidemia, current smoking, obesity, and C-reactive protein), renal-related (urine protein–creatinine ratio, serum creatinine, albumin, calcium, phosphorus, intact PTH, and 25-hydroxy vitamin D) and diabetes-related (glycosylated hemoglobin (HbA1c) and duration of type 2 diabetes mellitus) risk factors. Clinical evidence of cardiovascular disease was defined as the presence of one of the following: angina on the Rose questionnaire, or history of either myocardial infarction, or previous revascularization, or cerebrovascular accident. Intact PTH concentrations were measured using immunochemiluminometric assay (Quest Diagnostic Nichols Institute, San Juan Capistrano, CA, USA; reference range 10–65 pg/ml), 25-hydroxy vitamin D levels using liquid chromatography, tandem mass spectroscopy (Quest Diagnostic Laboratory, San Juan Capistrano, CA, USA; analytic sensitivity 4 ng/ml), and albumin was measured using bromocresol purple method. GFR was estimated using the abbreviated four-variable Modification of Diet in Renal Disease equation.[53] Electron beam computed tomography scan was used to determine the CAC score on the day of the clinic visit, as reported previously.[18] The scans were read by experienced readers who were blind to the clinical information of the study participants.

Subject follow-up and ascertainment of outcomes

As per the study protocol, subjects and/or their next of kin were contacted by telephone at 6-month intervals to ascertain the vital status of each participant. If telephone contact was unsuccessful,

at least two certified letters were sent to the subject at their last known address, followed by a home visit by one of the members of the study staff. The information obtained from direct subject contact was supplemented by a screen of the NDI up until the last available data (through 31 December 2007). For a subject whose death could not be confirmed by direct contact with next of kin, probabilistic scores provided by the NDI were used to identify study participants as deceased. Subjects were followed up till either date of death, or last telephone contact, or 31 December 2007 (last date for which data are available from the NDI), whichever occurred later.

Statistical analysis

Continuous variables are expressed as mean and s.d., or median with inter-quartile range, as appropriate, and categorical variables as percentages. The significance of difference between continuous variables was tested using either *t*-test, one-way analysis of variance, Mann–Whitney rank-sum test, or Kruskal–Wallis test, as appropriate. The difference in the distribution of categorical variables was tested using the χ^2-test. Correlations were tested using Spearman's rank-sum test.

Time-to-event survival analysis was used to test the association between the baseline CAC score and all-cause mortality. Univariate and multivariate predictors of time-to-event were identified using Cox's proportional hazards model. In addition to the baseline CAC score, univariate associations with mortality were tested with the following variables: age, gender, race/ethnicity, current smoking, history of cardiovascular disease, duration of diabetes, body mass index, systolic and diastolic blood pressure, lipid profile, HbA1c, eGFR, corrected serum calcium, phosphorus, intact PTH, 25-hydroxy vitamin D, albumin, C-reactive and urine protein–creatinine ratio, use of aspirin, angiotensin-converting enzyme inhibitor and/or angiotensin-receptor blocker, β blocker, lipid-lowering agent, and a number of anti-hypertensive agents. All continuous variables were categorized into quartiles; a fifth category of missing data was created for variables, if indicated. All predictors with a *P*-value of <0.10 on univariate analyses were entered into the multivariate models. In order to determine the independent predictive value of baseline CAC score, three multivariate models (selected *a priori)* were built—log-transformed CAC score as continuous variable, and either quartiles of CAC score (0–15, 16–149, 150–427, and $\geqslant 428$) or categories based on pre-defined ranges of CAC score (0, 1–99, 100–399, and $\geqslant 400$) as categorical variables.[54]

Sensitivity analysis was carried out using an alternative censoring method such that length of follow-up was calculated as either up until time of death or last phone or in-person contact by study staff.

PASW Statistics 17.0 software (SPSS, Chicago, IL, USA) was used for all the statistic analyses above. A *P*-value of <0.05 was considered statistically significant.

DISCLOSURE

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