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Original Article

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The Relationship of Fetuin-A with Coronary Calcification, Carotid Atherosclerosis, and Mortality Risk in Non-Dialysis Chronic Kidney Disease

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The authors have no conflicts of interest to declare.

ABSTRACT

Objective: This study investigated the relationship of fetuin-A with coronary calcification, carotid atherosclerosis, and mortality risk in non-dialysis chronic kidney disease (CKD).
Methods: The study included 135 adult patients with CKD at stages 3–5, who were divided into coronary artery calcification (CAC) and non-CAC groups. We excluded current smokers and individuals with diabetes mellitus, inflammatory conditions, liver diseases, acute kidney failure, chronic hemodialysis, and cancer. We conducted kidney function tests, complete blood counts, and measured serum levels of fetuin-A, tumor necrosis factor-alpha (TNF-α), high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), total cholesterol (TC), total triglycerides (TG), high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol. Cardiac spiral computed tomography was used to calculate the CAC score, employing the Agatston method. Carotid ultrasonography was performed to assess carotid intima-media thickness (CIMT) and to detect the presence of plaques.
Results: CAC patients had considerably higher levels of TNF-α (p<0.001), IL-6 (p<0.001), hs-CRP (p=0.006), TC, TG, parathyroid hormone (PTH) (p<0.001) and phosphorus (p<0.001) than non-CAC patients. They also had significantly lower levels of fetuin-A (p<0.001). Fetuin-A was considerably lower in CKD subgroups as CKD progressed. Fetuin-A (p=0.046), age (p=0.009), TNF-α (p=0.027), IL-6 (p=0.005), TG (p=0.002), PTH (p=0.002), and phosphorus (p=0.004) were significant predictors of CAC. CAC and fetuin-A were strong predictors of all-cause mortality and cardiovascular (CV) mortality. Fetuin-A was a significant predictor of CIMT (p=0.045).
Conclusion: Fetuin-A reliably predicted CAC and CIMT. Fetuin-A and CAC emerged as significant risk factors for all-cause and CV mortality in non-dialysis CKD.

Keywords: Atherosclerosis; Dyslipidemia; Inflammation; Hyperparathyroidism

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Data Availability Statement

All data and materials related to this article are presented in the main text and in the supplemental materials. Data files are available from Harvard Dataverse: https://doi.org/10.7910/DVN/LWAIS0.

Author Contributions

Conceptualization: Hassan IG, Alakkad AF; Data curation: Mohamed ON, Hassan IG; Formal analysis: Osman A; Investigation: Osman A, Issa AS, Setouhi A; Supervision: Mohamed ON, Mohamed MRM; Writing - original draft: Alakkad AF, Hassan IG; Writing - review & editing: Mohamed ON, Hassan IG, Setouhi A.

INTRODUCTION

Chronic kidney disease (CKD) is defined as defects in kidney structure or function that have affected an individual's health and have persisted for at least 3 months.[1] Diabetes, hypertension (HTN), cardiovascular (CV) diseases (CVDs) such as heart failure, and repeated episodes of acute kidney injury are the most prevalent risk factors for CKD. Other risk factors include obesity, nephrotoxic medications, systemic lupus erythematosus, exposure to environmental pollutants, low birth weight, family history of renal disorders, preeclampsia, and genetics.[2] The Cancer of the Kidney Global Assessment categorization system for CKD is a useful tool for assessing CKD and determining an individual's risk and severity of the disease. Two crucial components in this system are albuminuria (A1–A3) and the estimated glomerular filtration rate (eGFR) (G1–G5).[3]

Patients with CKD have an elevated risk of premature mortality, predominantly due to CV causes. The association between accelerated atherosclerosis and CKD was identified many years ago. Recent evidence suggests that the risk for atherosclerosis begins to rise in the early stages of CKD.[4] Traditional risk factors for atherosclerosis in CKD patients include male sex, age, diabetes, HTN, dyslipidemia, smoking, family history of CVD, physical inactivity, and menopause. Additionally, CKD-related risk factors such as systemic inflammation, albuminuria, vascular calcification, uremic toxins, abnormal calcium-phosphate metabolism, oxidative stress, endothelial dysfunction, and abnormal lipid modification also contribute to the disease.[5] Atherosclerosis is linked to an increase in the intima-media thickness (IMT) of the arteries, which can lead to luminal obstruction and subsequent ischemic events, including stroke and myocardial infarction (MI). Arteriosclerosis, meanwhile, causes arterial stiffening and an increase in pulse wave velocity and pulse pressure, which can result in reduced coronary perfusion and left ventricular hypertrophy. Both arteriosclerosis and atherosclerosis tend to manifest early in CKD patients and progress more rapidly, heightening the risk of CV death.[6]

Fetuin-A, also known as α-Heremans-Schmid glycoprotein, is a protein synthesized and secreted by the liver, with a widespread presence in extracellular fluid.[7] Its main function is to regulate bone and vascular calcification by forming temporary soluble colloidal spheres composed of fetuin-A, calcium, and phosphate. These spheres prevent the crystallization of hydroxyapatite and abnormal calcification within tissues. Consequently, fetuin-A is primarily recognized as an inhibitor of arterial calcification.[8]

The effects of fetuin-A are believed to be directly associated with the onset and progression of coronary heart disease (CHD). However, there are currently significant inconsistencies in the clinical research findings regarding the relationship between serum fetuin-A levels and CHD risk.[9] Recent studies suggest that serum fetuin-A levels are inversely related to valvular calcification[10] and are lower in older hemodialysis patients than in controls.[11] Nevertheless, a recent study involving adult hemodialysis patients found no link between fetuin-A levels and the extent of abdominal aortic calcification and coronary artery calcification (CAC), as determined by computed tomography (CT) scans.[12] A significant association was observed between lower fetuin-A levels and coronary artery lesions, as objectively measured by coronary angiography, in a cohort of individuals with coronary artery disease and mild-to-moderate renal impairment.[13] In contrast, the study by Zheng et al. reported that patients in the coronary artery disease group had significantly higher fetuin-A levels than those in the control group.[9] High fetuin-A levels were associated with an increased risk of ischemic

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stroke and MI in a population-based study, while several studies have indicated that lower serum fetuin-A levels were associated with CV events and mortality in cohorts with end-stage renal disease (ESRD). Low fetuin-A levels have been linked to carotid plaque in patients with diabetes, but data are scarce on patients with moderate renal impairment or normal kidney function who also have advanced atherosclerosis.[14] Therefore, the precise relationship between the risk of atherosclerotic disease and serum fetuin-A levels remains unclear. Consequently, our study aims to investigate the association of fetuin-A with CAC, carotid atherosclerosis, and the risk of mortality in non-dialysis patients with CKD.

MATERIALS AND METHODS

1. Study design and population

Our prospective, cross-sectional, 3-year follow-up study was conducted at the Renal Unit of Nephrology and Urology at Minia University Hospital in Minia, Egypt, from October 2019 to October 2020. We included a total of 135 patients with advanced CKD in the study, who were also followed for 3 years from 2020 to 2023. The diagnosis and staging of CKD were based on the Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guideline for the evaluation and management of CKD. CKD is defined as structural kidney damage or an eGFR of <60 mL/min/1.73 m^2 for a period longer than 3 months. Estimated GFR was used to classify the CKD patients into the CKD-3 group (eGFR: 30–60 mL/min/1.73 m^2) CKD-4 group (eGFR: 15–30 mL/min/1.73 m^2) and CKD-5 group (eGFR <15 mL/min/1.73 m^2). The CKD Epidemiology Collaboration (CKD-EPI) equation was created to provide a more accurate formula for estimating GFR.[15] The study included patients with CKD stages 3–5 who were over 18 years old and without common CVDs such as MI, unstable angina, arrhythmia, stroke, and heart failure. The exclusion criteria were acute kidney failure, patients on chronic hemodialysis, pregnancy, diabetes mellitus (DM), smokers, acute or chronic inflammatory states, severe liver disease, malignancy, any disease that could reduce life expectancy to less than one year, and the inability to perform cardiac CT. The patients were divided into two groups based on the presence of CAC: the CAC group and the non-CAC group. Out of the 313 individuals invited to participate, a study sample of 135 participants was used for the CAC analysis. Of those invited, 35 were ineligible due to common CVDs, 65 declined the invitation, 55 had moved out of the area or could not be scheduled, and 23 lacked fetuin-A measurements from the previous visit. All participants provided written informed consent. The hospital's Research Ethics Committee approved the study protocol, with the Institutional Review Board of Minia University, Faculty of Medicine approval number being 317-4-2022.

2. Clinical and laboratory characteristics

Each patient had a comprehensive history taken before undergoing a clinical examination. This assessment included consideration of factors such as age, gender, smoking history, history of HTN, and DM. The clinical examination entailed a thorough evaluation of the patient's vital signs and body mass index (BMI), as well as detailed assessments of the abdomen, chest, heart, and nervous system.

Following an overnight fast, a sterile venipuncture was performed on each patient in a highly aseptic environment to collect 6 mL of venous blood from a peripheral vein in the morning. For the complete blood count, 1 mL of blood was placed into a tube containing ethylenediaminetetraacetic acid. The remaining 5 mL was left to clot in a plain tube for 30 minutes before being centrifuged for 15 minutes at approximately 3,000 rpm. The serum

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obtained was stored at −70°C for further analysis. The serum was used to conduct common laboratory tests, including assessments for viral hepatitis markers, renal function, liver biochemistry, fasting blood glucose levels, postprandial blood glucose levels at two hours, total triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C).

The quantitative analysis of fetuin-A, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and high-sensitivity C-reactive protein (hs-CRP) was conducted using the enzyme-linked immunosorbent assay (ELISA). This analysis employed the Sandwich-ELISA technique. The ELISA kit included a microplate pre-coated with specific antibodies for fetuin-A, TNF-α, or IL-6. Standards or samples were added to the wells of the microplate, followed by the addition of a biotinylated detection antibody specific to fetuin-A, TNF-α, or IL-6. This was then combined with an Avidin-Horseradish Peroxidase conjugate. After the incubation period, the reaction was stopped by adding a stop solution, resulting in a color change to yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm±2 nm. The OD for each well was determined using a microplate reader set to 450 nm. The hs-CRP ELISA was based on a solid-phase assay that utilized a unique monoclonal antibody targeting a specific antigenic determinant on the CRP molecule. Absorbance was spectrophotometrically measured at 450 nm. The microtiter plate reader recorded the absorbance at 450 nm within 15 minutes.

3. Imaging investigations

At a follow-up visit, cardiac spiral CT and carotid IMT (CIMT) measurements were performed after blood samples were taken for fetuin-A measurements. Cardiac spiral CT was used to calculate CAC.[16] A skilled radiologist used a Toshiba 640-slice CT scanner (Toshiba) to perform the CT scan. The Agatston scoring method, as supplied by the KDIGO, was used to score CAC. The lesion score was determined by multiplying the lesion area (mm^2) by a density factor.[17] The weighted density scores were graded as follows: 130–199 hounsfield units (HU): 1, 200–299 HU: 2, 300–399 HU: 3, and ≥400 HU: 4. The scores for every lesion were added to obtain the overall CAC score. A score of 0 indicated no risk of coronary calcification, while scores of 1–100, 101–400, and >400 represented mild, moderate, and high risk, respectively.[18]

Every participant in the study underwent bilateral carotid examinations using Xario-200 Toshiba ultrasonography equipment (Toshiba). The patient was positioned either supine or semi-supine, with their head turned 45 degrees away from the side being examined and slightly extended. High-frequency linear transducers (12 MHz) were used to measure the morphology of the plaque and the thickness of the intima-media. For Doppler studies, linear transducers with a lower frequency of 7 MHz were preferred. Gray-scale imaging was employed to assess the dimensions, location, and characteristics of the atherosclerotic plaque in the internal carotid artery (ICA) and common carotid artery (CCA). To ensure a comprehensive scan of the vasculature, the transducer was angled caudally in the supraclavicular region and cephalically at the level of the mandible. Color Doppler imaging was used to identify regions of abnormal blood flow that warranted further Doppler spectral analysis. Pulsed wave Doppler spectral analysis was essential for determining blood flow velocity in the mid-CCA, proximal ICA, and in areas immediately adjacent to, at, and just distal to the plaques. Plaque characteristics were evaluated and classified as smooth, irregular, heterogeneous, or homogeneous. IMT was measured at the near or far walls of the bulb, ICA, and CCA, involving only the media (echo-poor layer) and the intima (echo-rich layer). The diagnostic criteria for arteriosclerosis were met if any of the following were

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present: 1) roughness and thickening of the carotid intima with a CIMT greater than 1 mm; 2) the presence of plaque or stenosis in the carotid lumen.[19]

4. Mortality and survival analysis

The period from enrollment to death from any cause, regardless of whether it occurred in an inpatient or outpatient setting, was utilized to determine all-cause mortality. Records were also kept of major adverse CV events, encompassing MI, heart failure, stroke, and CV death, whether as individual or composite endpoints. The definition of CV mortality included deaths attributable to MI, cardiac arrhythmia, congestive heart failure, peripheral vascular disease, sudden cardiac death, or stroke.

5. Statistical analysis

SPSS version 25 was utilized for data analysis. Categorical data were presented as numbers and percentages, while continuous data were represented by means with standard deviations or medians with interquartile ranges. The Mann-Whitney test was used to assess non-parametric continuous variables in two-group comparisons. The chi-square test was employed for the comparison of categorical data. The Spearman correlation coefficient was used to determine the relationship between continuous and categorical variables or between two categorical variables, whereas the Pearson correlation coefficient was used for analyzing the relationship between two continuous variables. MedCalc statistical software was used to create the receiver operating characteristic (ROC) curve. This software was also used to estimate the cut-off value, the area under the curve (AUC), and the specificity and sensitivity of laboratory variables for predicting CAC. Cox regression and logistic regression analyses were conducted to identify predictors of CAC and mortality. Kaplan-Meier analysis was applied to compare patient survival based on the CAC score.

RESULTS

1. Demographic and laboratory characteristics of the patients

The median age of patients with CAC was 47 years, comprising 41 males (48.8%) and 43 females (51.2%). In contrast, the median age of non-CAC patients was 41 years, with 29 males (56.9%) and 22 females (43.1%). There were no significant differences between patients with and without CAC in terms of BMI, HTN, serum creatinine, and blood urea levels. However, the CAC group had a significantly lower hemoglobin level (p=0.03) than the non-CAC group. Serum levels of TNF-α, IL-6, and hs-CRP were significantly higher in CAC patients compared to non-CAC patients (p<0.001, p<0.001, and p=0.006, respectively). Additionally, CAC patients exhibited significantly higher serum levels of TC, TG, and LDL-C than non-CAC patients (p=0.007, p=0.03, and p=0.02, respectively). In terms of bone mineral parameters, CAC patients had significantly higher levels of PTH and phosphorus (p<0.001 for both), and a significantly lower serum level of total calcium (p<0.001) compared to non-CAC patients. Furthermore, CAC patients had a significantly lower serum level of fetuin-A (p<0.001) and a significant increase in CIMT (p<0.001) compared to non-CAC patients. During a 36-month follow-up, 21 patients in the CAC group and 2 patients in the non-CAC group died. Of these, 11 patients died from CV causes. The overall mortality and CV mortality were significantly higher in the CAC group compared to the non-CAC group (p=0.002 and p=0.007, respectively) (**Table 1**).

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Table 1. Demographic and laboratory parameters of the CAC and non-CAC groups

Variables	CAC group (n=84)	Non-CAC group (n=51)	*p*-value
Age (yr)	47 (35–57)	41 (25–50)	**<0.001**
Sex (frequency/%)			0.36
Male	41 (48.8)	29 (56.9)	
Female	43 (51.2)	22 (43.1)	
HTN (frequency/%)	48 (57.1)	32 (62.7)	0.52
BMI (kg/m^2)	22.4 (20.7–29.4)	22.2 (20.8–24.65)	0.4
Serum creatinine (mg/dL)	3.95 (1.8–7)	4 (1.6–6.4)	0.06
Blood urea (mg/dL)	74 (55–100)	76 (49–97)	0.7
Hb (g/dL)	9 (6–12.6)	10.1 (6.2–15.4)	**0.03**
TNF-α (pg/mL)	34.25 (11–98)	21 (10.8–85)	**<0.001**
IL-6 (pg/mL)	27.1 (4–101)	13.7 (3.3–54)	**<0.001**
hs-CRP (mg/L)	44 (12–102)	30 (12–102)	**0.006**
TC (mg/dL)	190 (155–255)	180 (150–250)	**0.007**
TG (mg/dL)	175 (125–285)	160 (140–260)	**0.03**
LDL-C (mg/dL)	118.5 (90–173)	108 (63–182)	**0.02**
HDL-C (mg/dL)	38 (28–45)	39 (30–55)	0.5
iPTH (pg/mL)	360 (137–520)	145 (125–230)	**<0.001**
P (mg/dL)	6.2 (4.8–8)	5.9 (4.8–6.2)	**<0.001**
Total calcium (mg/dL)	7.9 (7–8.5)	8 (7.8–8.4)	**<0.001**
CIMT (mm)	1.2 (0.5–1.6)	0.7 (0.5–0.9)	**<0.001**
Fetuin-A (g/L)	354 (250–478)	423 (275–769)	**<0.001**
All-cause mortality (frequency/%)	21 (25)	2 (3.9)	**0.002**
Cardiovascular mortality (frequency/%)	11 (13.1)	0 (0)	**0.007**

Continuous variables are expressed as median and range, while categorical variables are expressed as number and percentage. Bold are significant *p*-values ($p<0.05$).
CAC, coronary artery calcification; HTN, hypertension; BMI, body mass index; Hb, hemoglobin; TNF-α, tumor necrosis factor alpha; IL-6, interleukin-6; hs-CRP, high-sensitivity C-reactive protein; TC, total cholesterol; TG, total triglyceride; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol; iPTH, intact parathyroid hormone; P, phosphorus; CIMT, carotid intima-media thickness.

2. Fetuin-A and CAC in the patients' groups

The CKD patients were further divided based on their eGFR into the CKD-3, CKD-4, and CKD-5 subgroups. Patients in the CKD-4 and CKD-5 groups had significantly lower serum levels of fetuin-A compared to those in the CKD-3 group ($p=0.016$ and $p<0.001$, respectively). Additionally, CAC was found to be significantly higher in the CKD-5 group compared to the CKD-3 ($p=0.001$) and CKD-4 groups ($p=0.01$). Mild to moderate CAC was detected in 18 patients in the CKD-3 group, 16 patients in the CKD-4 group, and 6 patients in the CKD-5 group. While severe CAC was not observed in the CKD-3 group, it was present in 13 patients in the CKD-4 group and 31 patients in the CKD-5 group (**Table 2**).

3. Predictors of CAC

Univariate logistic regression identified age (odds ratio [OR], 1.27; $p<0.001$), TNF-α (OR, 1.04; $p=0.001$), hs-CRP (OR, 1.02; $p=0.01$), IL-6 (OR, 1.05; $p<0.001$), TC (OR, 1.02; $p=0.009$), TG (OR, 1.01; $p=0.015$), and LDL-C (OR, 1.02; $p=0.018$) as predictors of CAC. Additionally,

Table 2. Fetuin-A and coronary artery calcification in the CKD subgroups of the studied patients

Variables	CKD-3 (group I) (n=32)	CKD-4 (group II) (n=45)	CKD-5 (group III) (n=58)	I vs. II	I vs. III	II vs. III
Fetuin-A (g/L)	414 (275–769)	355 (275–650)	370 (250–750)	**0.016**	**<0.001**	0.18
CAC	207.5 (0–320)	280 (0–840)	517.5 (0–910)	0.054	**0.001**	**0.01**
No CAC	14 (43.8)	16 (35.6)	21 (36.2)			
Mild to moderate	18 (56.2)	16 (35.6)	6 (10.3)			
Severe CAC	0 (0)	13 (28.9)	31 (53.4)			

Continuous variables are expressed as median and range, while categorical variables are expressed as number and percentage. Bold are significant *p*-values ($p<0.05$).
CKD, chronic kidney disease; CAC, coronary artery calcification.

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our research demonstrated that intact PTH (iPTH) (OR, 1.03; p<0.001), phosphorus (OR, 3.96; p<0.001), and calcium (OR, 0.02; p<0.001) were independent risk factors for CAC. Furthermore, fetuin-A emerged as a strong risk factor for the development of CAC (OR, 0.98; p<0.001). Multivariate logistic regression found that age (OR, 1.47; p=0.009), TNF-α (OR, 0.83; p=0.027), IL-6 (OR, 1.36; p=0.005), TG (OR, 0.88; p=0.002), iPTH (OR, 1.09; p=0.002), and phosphorus (OR, 61.87; p=0.004) were significant predictors of CAC. It also confirmed that fetuin-A was a significant risk factor for CAC (OR, 0.97; p=0.046) (**Table 3**). The ROC curve analysis indicated that fetuin-A (AUC, 0.85; p<0.001) (**Fig. 1**), age (AUC, 0.81), iPTH (AUC, 0.92; p<0.001), and phosphorus (AUC, 0.82; p<0.001), along with inflammatory markers such as TNF-α, IL-6, and hs-CRP, and lipid markers including TC, TG, and LDL-C, accurately predicted CAC in our patients (**Table 4**).

4. Predictors of overall and CV mortality

Univariate Cox regression analysis demonstrated that serum creatinine (p=0.03), inflammatory markers including TNF-α (p<0.001) and hs-CRP (p=0.001), as well as TG and bone mineral markers such as iPTH (p<0.001), calcium (p=0.003), and phosphorus (p=0.001) were predictive factors associated with all-cause mortality. Additionally, the analysis revealed that both fetuin-A and CAC significantly predicted overall mortality (p<0.001 for each). Multivariate Cox regression analysis identified CAC (hazard ratio [HR], 1.00; p=0.009) and

Table 3. Logistic regression analysis to determine predictors of CAC in non-dialysis CKD patients

Variables	Univariate logistic regression				Multivariate logistic regression			
	p-value	OR	95% CI		*p*-value	OR	95% CI	
			Lower	Upper			Lower	Upper
Age	**<0.001**	1.27	1.16	1.38	**0.009**	1.47	1.10	1.96
Hb	**0.02**	0.79	0.64	0.96	**0.02**	0.34	0.14	0.85
TNF-α	**0.001**	1.04	1.02	1.07	**0.027**	0.83	0.71	0.98
hs-CRP	**0.01**	1.02	1.00	1.04	0.18	0.94	0.86	1.03
IL-6	**<0.001**	1.05	1.02	1.08	**0.005**	1.36	1.09	1.67
TC	**0.009**	1.02	1.00	1.03	0.20	1.14	0.93	1.41
TG	**0.015**	1.01	1.00	1.02	**0.002**	0.88	0.81	0.95
LDL-C	**0.018**	1.02	1.00	1.04	0.15	0.87	0.72	1.05
iPTH	**<0.001**	1.03	1.01	1.04	**0.002**	1.09	1.03	1.14
P	**<0.001**	3.96	2.03	7.75	**0.004**	61.87	3.86	992.60
Total Ca	**<0.001**	0.02	0.00	0.12	0.84	1.78	0.01	502.95
Fetuin-A	**<0.001**	0.98	0.98	0.99	**0.046**	0.97	0.95	1.00

Bold are significant p-values (p<0.05).
CAC, coronary artery calcification; CKD, chronic kidney disease; OR, odds ratio; CI, confidence interval; Hb, hemoglobin; TNF-α, tumor necrosis factor alpha; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; TC, total cholesterol; TG, total triglyceride; LDL-C, low density lipoprotein-cholesterol; iPTH, intact parathyroid hormone; P, phosphorus; Ca, calcium.

Table 4. ROC curve analysis to determine the potential predictors of CAC in the studied CKD patients

Variables	Cut-off value	AUC	Sensitivity (%)	Specificity (%)	95% CI	*p*-value
Fetuin-A (g/L)	<413	0.85	88.1	72.4	0.79–0.90	<0.001
TNF-α (pg/mL)	>25	0.80	66.7	80.2	0.73–0.86	<0.001
IL-6 (pg/mL)	>14.9	0.79	73.8	75.0	0.72–0.85	<0.001
hs-CRP (mg/L)	>40	0.74	52.4	80.6	0.67–0.81	<0.001
TC (mg/dL)	>167	0.74	95.2	43.4	0.67–0.81	<0.001
TG (mg/dL)	>157	0.72	72.6	61.8	0.65–0.79	<0.001
LDL-C (mg/dL)	>115	0.74	59.5	81.6	0.66–0.80	<0.001
iPTH (pg/mL)	>230	0.92	64.3	100	0.86–0.95	<0.001
P (mg/dL)	>6.1	0.82	59.5	94.7	0.75–0.87	<0.001
Age (yr)	>44	0.81	75.0	75.0	0.74–0.87	<0.001

ROC, receiver operating characteristic; CAC, coronary artery calcification; CKD, chronic kidney disease; AUC, area under the curve; CI, confidence interval; TNF-α, tumor necrosis factor alpha; IL-6, interleukin-6; hs-CRP, high-sensitivity C-reactive protein; TC, total cholesterol; TG, total triglyceride; LDL-C, low density lipoprotein-cholesterol; iPTH, intact parathyroid hormone; P, phosphorus.

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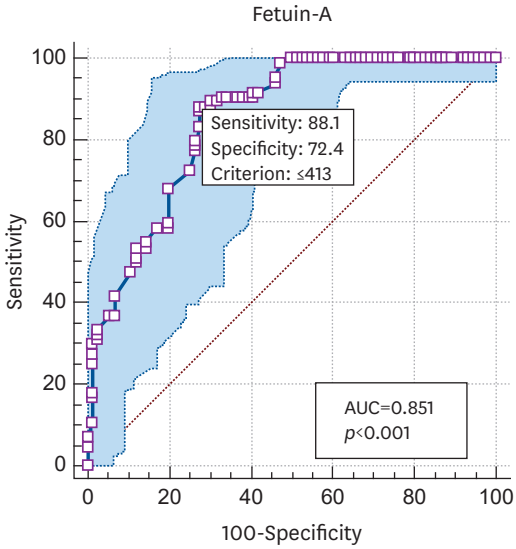

Fig. 1. Receiver operating characteristic curve showing the accuracy of low levels of serum fetuin-A in predicting coronary artery calcification.
AUC, area under the curve.

fetuin-A (HR, 0.99; p=0.04) as strong predictors of all-cause mortality (**Table 5**). We also found that serum creatinine, TG, HDL-C, inflammatory markers (TNF-α, IL-6, and hs-CRP), and bone mineral parameters (iPTH, phosphorus, and calcium) predicted CV mortality in univariate Cox regression analysis. Our study indicated that fetuin-A and CAC were reliable predictors of CV mortality (p<0.001 for each). Furthermore, multivariate Cox regression demonstrated that fetuin-A (HR, 0.99; p=0.029) and CAC (HR, 1.00; p=0.016) were significant predictive factors for CV mortality (**Table 6**). According to Kaplan-Meier analysis, patients with CKD and severe CAC had a significantly higher overall mortality rate compared to those with mild to moderate CAC (log-rank p<0.001) (**Fig. 2**).

5. Predictors of carotid atherosclerosis

Multivariate linear regression analysis revealed that fetuin-A (p=0.04), HDL-C (p=0.04), and PTH (p<0.001) are strong independent risk factors for carotid atherosclerosis (**Table 7**). Furthermore, the ROC curve analysis indicated that fetuin-A is a significant predictive factor for carotid atherosclerosis, with an AUC of 0.86 (95% confidence interval, 0.799–0.911;

Table 5. Cox regression analysis to detect risk factors of all-cause mortality in CKD patients

Variables	Univariate Cox regression				Multivariate Cox regression			
	p-value	HR	95% CI		*p*-value	HR	95% CI	
			Lower	Upper			Lower	Upper
Creatinine	**0.03**	1.34	1.02	1.75	0.63	0.88	0.51	1.50
TNF-α	**<0.001**	1.03	1.01	1.04	0.22	0.98	0.94	1.01
hs-CRP	**0.001**	1.02	1.01	1.04	0.20	1.02	0.99	1.04
TG	**0.003**	1.01	1.00	1.02	0.99	1.00	0.98	1.02
iPTH	**<0.001**	1.01	1.00	1.01	0.61	1.00	0.99	1.01
P	**0.001**	2.58	1.51	4.41	0.23	1.65	0.73	3.72
Total Ca	**0.003**	0.19	0.07	0.57	0.97	1.03	0.26	4.05
Fetuin-A	**<0.001**	0.98	0.98	0.99	**0.04**	0.99	0.98	1.00
CAC	**<0.001**	1.00	1.00	1.01	**0.009**	1.00	1.00	1.01

Bold are significant p-values (p<0.05).
CKD, chronic kidney disease; HR, hazard ratio; CI, confidence interval; TNF-α, tumor necrosis factor alpha; hs-CRP, high-sensitivity C-reactive protein; TG, total triglyceride; iPTH, intact parathyroid hormone; P, phosphorus; Ca, calcium, CAC, coronary artery calcification.

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Table 6. Cox regression analysis of risk factors for cardiovascular mortality in CKD patients

Variables	Univariate Cox regression				Multivariate Cox regression			
	p-value	HR	95% CI		*p*-value	HR	95% CI	
			Lower	Upper			Lower	Upper
Creatinine	**0.02**	1.65	1.07	2.53	0.66	0.88	0.50	1.55
TNF-α	**<0.001**	1.04	1.02	1.07	0.36	0.98	0.95	1.02
IL-6	**0.027**	1.03	1.00	1.05	0.06	0.97	0.93	1.00
hs-CRP	**0.003**	1.03	1.01	1.05	0.10	1.02	1.00	1.05
TG	**0.004**	1.02	1.01	1.04	0.68	1.00	0.99	1.02
HDL-C	**0.018**	0.85	0.74	0.97	0.89	1.01	0.87	1.17
iPTH	**0.001**	1.01	1.00	1.02	0.78	1.00	0.99	1.01
P	**0.001**	3.37	1.60	7.09	0.11	2.01	0.85	4.72
Total Ca	**0.01**	0.14	0.03	0.64	0.64	1.46	0.29	7.17
Fetuin-A	**<0.001**	0.97	0.95	0.98	**0.03**	0.99	0.97	1.00
CAC	**<0.001**	1.01	1.00	1.01	**0.02**	1.00	1.00	1.01

Bold are significant *p*-values ($p<0.05$).
CKD, chronic kidney disease; HR, hazard ratio; CI, confidence interval; TNF-α, tumor necrosis factor alpha; IL-6, interleukin-6; hs-CRP, high-sensitivity C-reactive protein; TG, total triglyceride; HDL-C, high density lipoprotein-cholesterol; iPTH, intact parathyroid hormone; P, phosphorus; Ca, calcium; CAC, coronary artery calcification.

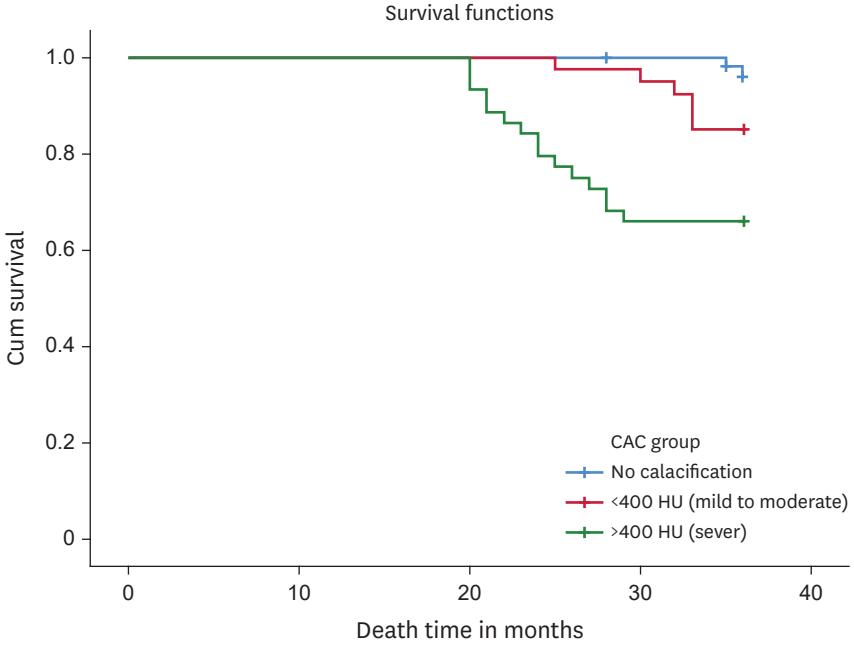

Fig. 2. Kaplan Meier showing that patients with CACs ≥400 HU had significantly higher all-cause mortality than those with CACs <400 HU.
CAC, coronary artery calcification; HU, hounsfield units.

$p<0.001$) (**Fig. 3**). Additionally, we observed a positive correlation between CIMT and fetuin-A levels ($p<0.001$) (**Fig. 4**).

DISCUSSION

In the present study, we observed a significant association between fetuin-A, CAC, and carotid atherosclerosis in non-dialysis CKD patients. Additionally, we found that fetuin-A was a significant predictor of both all-cause and CV mortality. CAC is present in a range of CVDs,

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Table 7. Multivariate linear regression analysis to assess predictors of carotid atherosclerosis

Variables	Unstandardized coefficients		Standardized coefficients	T	*p*-value
	B	SE	Beta		
Fetuin-A	0.00	0.00	−0.074-	−2.030-	**0.04**
Age	−0.002-	0.002	−0.042-	−1.056-	0.29
Creatinine	0.01	0.01	0.049	1.062	0.29
Urea	0.00	0.001	−0.007-	−0.214-	0.83
TNF-α	0.00	0.001	−0.009-	−0.145-	0.88
IL-6	0.00	0.001	0.016	0.346	0.73
hs-CRP	8.31E-6	0.001	0.001	0.012	0.99
TC	0.002	0.002	0.169	1.075	0.28
TG	0.00	0.001	0.032	0.436	0.66
HDL-C	−0.008-	0.004	−0.105-	−2.037-	**0.04**
LDL-C	−0.002-	0.002	−0.122-	−0.866-	0.39
iPTH	0.002	0.00	0.742	13.475	**<0.001**
P	0.03	0.02	0.056	1.527	0.13
Total Ca	−0.069-	0.04	−0.065-	−1.665-	0.09

Bold are significant *p*-values ($p<0.05$).
SE, standard error; TNF-α, tumor necrosis factor alpha; IL-6, interleukin-6; hs-CRP, high-sensitivity C-reactive protein; TC, total cholesterol; TG, total triglyceride; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; iPTH, intact parathyroid hormone; P, phosphorus; Ca, calcium.

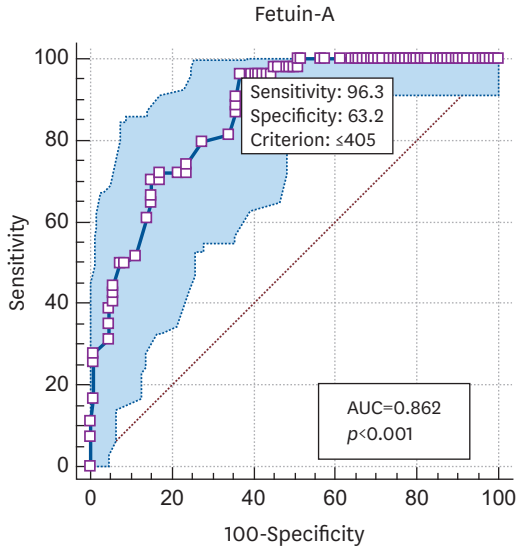

Fig. 3. Receiver operating characteristic curve analysis revealing the accuracy of low fetuin-A levels in predicting carotid atherosclerosis.
AUC, area under the curve.

from asymptomatic early coronary artery disease to severe CVD.[20] It is an important marker of subclinical atherosclerosis[21] and is associated with mortality and major CV events.[22]

Multiple studies have suggested a link between higher levels of phosphorus and iPTH and an increased propensity for accelerated vascular calcification.[23-25] Furthermore, hyperphosphatemia and elevated iPTH levels are associated with a higher risk of mortality and CV events.[26-29] Disorders in calcium and phosphorus metabolism, including hyperphosphatemia and hypocalcemia, along with elevated iPTH levels, can induce vascular wall smooth muscle cells to exhibit osteoblast-like activity, secrete bone morphogenetic proteins, and enhance the process of vascular calcification.[30,31]

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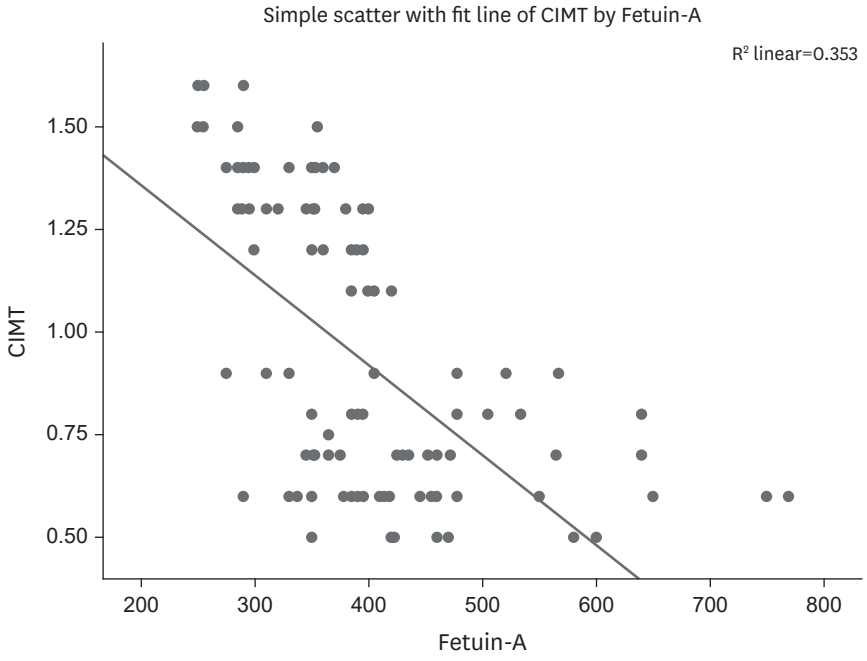

Fig. 4. Simple scatter plot showing the correlation of CIMT with fetuin-A.
CIMT, carotid intima-media thickness.

The studies conducted by Amdur et al.[32] and Knight et al.[33] have revealed that inflammatory biomarkers are independently associated with the occurrence of atherosclerotic vascular disease episodes and mortality in patients with CKD. Inflammation is widely recognized as a key contributor to the development of atherosclerosis. Uremic toxins prompt a pro-inflammatory response in macrophages, vascular cells, and parenchymal cells, which is a critical step in the pathogenesis of atherogenesis. Additionally, these toxins increase interactions between endothelial cells and macrophages, facilitating the infiltration of inflammatory cells into the vascular wall.[4] Furthermore, macrophage pro-inflammatory responses contribute to vascular calcification by releasing calcifying extracellular vesicles and by promoting osteogenic differentiation in vascular smooth muscle cells.[34]

Several studies have found a strong correlation between the risk of CV events and elevated TG, low HDL-C, and high LDL-C levels.[35-38] Dyslipidemia increases the risk of developing arteriosclerotic plaques and atheromas. These lesions can lead to changes in blood pressure by thickening the vessel walls and reducing their elasticity. Additionally, the vessels may form aneurysms, which heighten the risk of potentially fatal internal hemorrhage. Consequently, dyslipidemia-related CVD continues to be a significant factor contributing to higher mortality and morbidity rates in patients with CKD.[39]

In our research on the serum levels of fetuin-A in patients with CKD and its association with CAC, we observed a gradual decline in fetuin-A levels as CKD progressed. This finding is consistent with a study that included CKD patients at stages 3, 4, and 5, comprising both diabetics and non-diabetics who were not on dialysis, where fetuin-A levels decreased as renal function worsened.[40] Additionally, Deepa et al. found similar results.[41] However, these findings contrast with those of Anderson et al., who reported no significant differences in serum fetuin-A levels between stages 2, 3, and 4 of CKD.[42] Pro-inflammatory cytokines, such as TNF-α and IL-6, have been identified as negative regulators of fetuin-A synthesis.[43]

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Turkmen et al. found a statistically significant difference in serum fetuin-A levels between group 1 (low CAC) and group 2 (high CAC) in hemodialysis patients, with levels of 303.8±22.6 and 234.2±38.3, respectively (p<0.001). Additionally, fetuin-A levels were significantly correlated with the CAC score in these patients (r=−0.86, p<0.001).[44] Serum fetuin-A levels were inversely associated with coronary artery calcium scoring parameters (r=−0.881, p<0.001). Both univariate and multivariate analyses revealed a highly significant association between serum fetuin-A and CAC.[45] Vascular calcification was negatively correlated with fetuin-A in univariate analysis and was found to be independently associated with fetuin-A upon multivariate regression analysis.[46] A multicenter cohort study, with 7 years of follow-up in approximately 1,000 dialysis patients with CKD, reported that lower serum fetuin-A expression was linked to an increased risk of CV mortality.[47] As CKD progresses, fetuin-A levels decline, and a deficiency in this protein is associated with a higher risk of systemic calcification and a poorer prognosis.[48]

Fetuin-A levels are directly associated with CAC and are significantly higher in patients with non-dialysis-dependent diabetic nephropathy than in control subjects with diabetes. This apparent contradiction might be explained by the possibility that overexpression of fetuin-A acts as a protective mechanism against early vascular calcification.[49] However, other studies have reported varying findings. One study found no relationship between vascular calcification and fetuin-A levels in patients undergoing regular hemodialysis.[50] Furthermore, no correlation was observed between the CAC score and fetuin-A levels in a cohort of 85 patients with diabetic kidney disease.[51] Another study concluded that fetuin-A did not affect vascular calcification in hemodialysis patients.[52]

Our data demonstrated that a low fetuin-A level was a strong predictor of overall and CV mortality. However, the conclusion that low fetuin-A levels are associated with an increased risk of CV or all-cause death is not supported by data from studies that included a larger number of patients with non-dialysis CKD.[42,53] Additionally, Alderson et al. found that fetuin-A is not associated with the risk of mortality in non-dialysis CKD.[42] The study by Ix et al. revealed multiple plausible reasons for the absence of a correlation between fetuin-A and both CV and all-cause mortality.[53] First, it is unclear whether vascular calcification is linked to CV and all-cause mortality among individuals with stage 3–4 CKD. Second, in this study, kidney function was comparable across all fetuin-A tertiles. This finding is consistent with earlier research showing that serum fetuin-A concentrations remain constant from stage 3 CKD to normal kidney function. Therefore, fetuin-A concentrations may not be linked to an increased risk of death until they decrease below a certain threshold, which may not be common among populations without ESRD. Third, the mechanisms by which lower serum levels develop within ESRD populations are unclear, given that the molecular size of fetuin-A is 64 kDa, which is too large to be eliminated by standard hemodialysis membranes.[54]

Similar findings have been observed in various studies investigating the role of fetuin-A in CV mortality. Lower levels of fetuin-A have been associated with an increased risk of both CV and all-cause mortality.[55] Patients with ESRD undergoing renal replacement therapy have shown higher rates of CV mortality when their fetuin-A levels are low.[49] It has been demonstrated that a single hemodialysis session lowers fetuin-A levels,[56] suggesting that the renal replacement therapy may be the cause of the observed inverse association in this patient group. Weikert et al. reported that plasma fetuin-A levels could predict the risk of MI and ischemic stroke, independent of traditional CVD risk factors such as hs-CRP, DM, HTN, dyslipidemia, and lifestyle factors like physical inactivity or smoking. These findings lend

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support to the hypothesis that fetuin-A is involved in the pathophysiology of CVD and may represent a novel pathway that contributes to the development of MI and ischemic stroke, independent of traditional risk factors.[57]

Fetuin-A is recognized for its negative regulation of calcium metabolism and bone. It acts as a potent endogenous inhibitor of pathological mineralization and calcification, both locally and systemically, which is associated with an increased risk of various CVDs. In mice deficient in fetuin-A, abnormal systemic ectopic calcification of soft tissues has been observed, underscoring the role of fetuin-A in regulating unwanted calcification and bone osteogenesis. Fetuin-A interacts with insoluble calcium phosphate (or hydroxyapatite) to form highly soluble, stable, and inactive colloidal complexes in the bloodstream, known as calciprotein particles. These particles play a crucial role in preventing extraosseous calcification by limiting the precipitation of calcium and phosphorus—fetuin-A is responsible for inhibiting approximately 50% of this precipitation, highlighting its importance as a serum factor in the prevention of vascular calcification.[58] There are three primary effects of fetuin-A that have both physiological and pathophysiological significance: the inhibition of ectopic calcification, the exacerbation of insulin resistance, and anti-inflammatory actions. Each of these effects significantly influences the risk of CV events and the pathophysiology of atherosclerosis. The most well-documented advantage of fetuin-A regarding blood vessels is its ability to suppress ectopic calcification.[22]

Concerning the relationship of fetuin-A with carotid atherosclerosis, our study found a significant association between CIMT and low levels of fetuin-A, iPTH, and HDL-C. The study by Sevinc et al. demonstrated that patients with atherosclerosis had substantially lower levels of fetuin-A, as indicated by CIMT measurements, and that fetuin-A was a reliable predictor of CIMT.[59] Sommer et al. found that higher levels of fetuin-A predicted reduced progression of atherosclerotic plaques (p=0.01).[60] Guarneri et al.[61] demonstrated a negative relationship between CIMT and fetuin-A levels in individuals with HTN. Another study indicated that, even in the absence of known atherogenic factors, serum fetuin-A levels in healthy individuals were linked to carotid artery stiffness.[62] In contrast, a study by Bakan and Ecder[63] revealed that patients with amyloid A amyloidosis did not show a relationship between fetuin-A levels and CIMT. Additionally, research by Ix et al.[64] found that lower serum fetuin-A levels were associated with CAC, but not with peripheral arterial disease (PAD) or CIMT. The inverse relationship between fetuin-A and CAC, but not with PAD and CIMT, was unexpected because it was hypothesized that lower fetuin-A levels would be linked to a higher prevalence of PAD and CIMT. Since fetuin-A inhibits calcium buildup in the arteries, the amount of calcium in each unique atherosclerotic lesion can vary. Therefore, low fetuin-A may be associated with increased calcium deposition but not necessarily with the overall burden or progression of atherosclerosis itself. [64]

Costa et al. demonstrated a significant link between serum iPTH and CIMT (r=0.31, p=0.03).[65] The study by Abajo et al.[66] revealed a strong and independent association between iPTH levels and accelerated CIMT progression in patients with moderate to ESRD without a previous history of CVD. After 9.4 years of follow-up, the study by Blondon et al.[67] examined the relationship between iPTH and the progression of CIMT in 3,251 participants from the Multi-Ethnic Study of Atherosclerosis. They did not find a relationship between iPTH and progression.[67] Similarly, Reis et al.[68] did not discover a relationship between CIMT and iPTH in a 654-person community-based cohort study. The differences in our results may be partially explained by the fact that both studies used general population-based cohorts

with significantly lower iPTH levels than those found in our CKD patients.[68] Arzpeyma et al.[69] showed that CIMT was negatively linked with HDL-C. Additionally, Mohamed et al. demonstrated that CIMT had a negative relationship with HDL-C (r=−0.43, p<0.001). They also found that HDL-C was a strong predictor of CIMT (AUC=0.69, p<0.001).[3]

According to our research, CAC was prevalent in CKD patients and increased as the disease worsened. Our CAC patients were older than non-CAC patients, and age was a strong predictor of CAC. A similar prospective study that included 117 patients with non-dialysis-dependent CKD found that nearly half (48%) of the participants had CAC, with 21% exhibiting severe CAC (≥400 HU).[70] Another study reported the presence of CAC in varying degrees across different stages of CKD: 1.4% in stage 3, 2.7% in stage 4, 13.7% in stage 5 without dialysis, 56.8% in stage 5 with hemodialysis, and 25.3% in stage 5 with peritoneal dialysis patients, respectively.[71] From CKD stages 1 to 3, there was a significant increase in the percentage of subjects with CAC. A negative correlation was observed between renal function and the severity of CAC. Additionally, patients with a CAC score greater than 400 HU were older than those with a score below 400 HU.[38] Age was shown to be a significant predictor of CAC.[72] Among non-dialysis CKD patients, CAC was significantly and independently associated with an increased incidence of CV events and all-cause mortality.[22,70] CKD patients with a CAC score greater than 400 HU are at an increased risk of hospitalization due to CVD and all-cause mortality.[71] Furthermore, age and a CAC score greater than 400 HU were identified as predictive factors for CV death in CKD patients.[38]

The environment associated with CKD fosters the development of vascular calcification, particularly CAC. Vascular calcification is influenced by a variety of factors. As CKD progresses, the kidneys' capacity for excretion and metabolic processing progressively diminishes. This decline leads to an imbalance of several electrolytes, notably hyperphosphatemia and hypocalcemia, which in turn can cause secondary hyperparathyroidism and vitamin D deficiency. Both of these conditions are closely linked to vascular calcification. Additional CKD-related risk factors include chronic inflammation, the accumulation of uremic toxins, the upregulation of factors that promote calcification (such as FGF-23, osteoprotegerin, and matrix Gla protein), and the downregulation of factors that inhibit calcification (including fetuin-A and pyrophosphate).[73]

In conclusion, CAC was commonly observed in patients with CKD, and its prevalence increased with the progression of the disease. Levels of fetuin-A were significantly lower across all CKD subgroups, particularly as CKD advanced. Patients with CAC were older and exhibited notable signs of inflammation, secondary hyperparathyroidism (characterized by hyperphosphatemia, hypocalcemia, and elevated levels of iPTH), and hyperlipidemia (evidenced by high levels of TC, TG, and LDL-C) compared to those without CAC. Multivariate logistic regression and ROC curves identified fetuin-A, TG, advanced age, inflammatory markers including TNF-α and IL-6, and bone mineral parameters such as iPTH and phosphorus as significant risk factors for CAC. Both fetuin-A and CAC emerged as strong predictors of all-cause and CV mortality. Moreover, fetuin-A was a significant predictor of carotid atherosclerosis. Therefore, we propose that fetuin-A serves as a reliable biomarker for CAC and carotid atherosclerosis. It also has the potential to predict all-cause and CV mortality in patients with non-dialysis-dependent CKD.

A strength of our study lies in the availability of several parameters related to coronary atherosclerosis and the measurement of numerous potential confounding variables.

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Additionally, the absence of CVD in the study sample and the inclusion of two CVD measures, namely CAC and CIMT, strengthen the robustness of our findings. However, our study faced certain limitations. It was conducted at a single center, and its cross-sectional design may influence the results. The levels of fetuin-A, which are associated with the long-term development of CAC and bone mineral parameters, were measured only once instead of at regular intervals. Similarly, CIMT was measured just once, precluding the ability to observe changes over time. The sample size was relatively small. Moreover, establishing a correlation between serum fetuin-A levels and vascular calcification may be challenging in a cross-sectional study. This is because vascular calcification is a slowly progressive disease that develops over an indeterminate period, while serum fetuin-A levels can vary due to inflammatory episodes.

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