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Original Article

Prevalence of subclinical atheromatosis and associated risk factors in chronic kidney disease: the NEFRONA study

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ABSTRACT

Background. The causes of the high cardiovascular mortality observed in chronic kidney disease (CKD) are unknown. Here, we report data on prevalence of subclinical atherosclerosis in the NEFRONA population and a stratified multivariate logistic analysis of factors associated with the presence of plaque.

Methods. We analysed 2445 patients with an estimated glomerular filtration rate (eGFR) <60 mL/min (CKD 3: 937; CKD 4–5: 820; CKD 5D: 688) and 559 non-CKD subjects (eGFR >60 mL/min), 18–75 years old, without previous cardiovascular events. An itinerant team of professionals performed carotid and femoral arterial ultrasound.

Results. The already high prevalence of plaques in CKD 3 is even higher in more severe CKD. Multivariate logistic analysis showed that, at any CKD stage, age and being male are independently associated with the presence of plaques. In CKD 3, there was a significant interaction of the smoking status and triglycerides levels which were independently associated with the presence of plaque. Furthermore, being diabetic was also associated with the presence of subclinical atherosclerosis. In stage 4–5 there was a significant association with smoking, high phosphate and hsCRP levels. In dialysis patients, being diabetic, having low levels of 25(OH)-vitamin D_3 and smoking status also showed a significant association with the presence of plaque. Furthermore, the association of phosphate levels with the presence of subclinical atheromatosis showed a U-shaped curve.

Conclusions. This analysis demonstrates the magnitude of subclinical atheromatous disease in a large CKD population. The patient characteristics associated with the presence of plaque differ in every CKD stage.

Keywords: subclinical, atheromatosis, cardiovascular disease prevention, chronic kidney disease, ultrasound

INTRODUCTION

Chronic kidney disease (CKD) patients have an unacceptable high risk for cardiovascular (CV) death [1–3]. The role of accelerated atheromatosis has recently been questioned, given that most CV deaths in dialysis patients are due to sudden death and heart failure [4]. Furthermore, the common association between cholesterol, atheromatosis and coronary heart disease is weak or even absent in CKD [5, 6], and recent trials have shown a lack of efficacy of statins in reducing CV disease in dialysis patients [7, 8]. The case against a role for atheromatosis has been reinforced by reports favouring the involvement of other mechanisms, such as arterial stiffness, vascular calcification, 'myocyte/capillary mismatch', congestive cardiomyopathy and sudden cardiac death [9].

Many other studies suggest an involvement of atheromatosis in the CV health of CKD patients. The JUPITER and SHARP trials demonstrated that treatments aiming to reduce lipid burden were effective in reducing CV events, mainly in the early stages of CKD [10, 11]. Furthermore, the low atherothrombotic event rate observed among dialysis patients could be the result of bias related to the high atherothrombotic mortality in the earlier stages, as shown in several studies that revealed a higher ischaemic event rate in CKD patients not on dialysis [12–16]. Additionally, several studies have reported an increase in the incidence and severity of coronary heart disease as the GFR decreases [17–19]. Last but not least, some of the events labelled as sudden deaths could be undiagnosed silent myocardial infarctions, as these are often found in this population [20].

Although this issue is of maximum importance, there are no strong data regarding the prevalence of subclinical atheromatosis in earlier stages of CKD. The studies found in the literature are methodologically heterogeneous, with a low number of patients, and comparisons between them are not possible. Thus, to evaluate the atheromatous burden in patients without a previous event, the use of non-invasive techniques, like carotid/femoral ultrasound, is mandatory [21].

We present here the first cross-sectional analysis of the NEFRONA study [22, 23], regarding the prevalence of atheromatous plaques and the risk factors associated, compared with a non-CKD population.

METHODS

Study design and participants

NEFRONA was designed as a multicentre prospective observational cohort study to evaluate the subclinical atherosclerosis burden and the predictive value of carotid/femoral ultrasound in a group of Spanish CKD patients [22, 23]. Briefly, 2445 CKD patients (937 in CKD stage 3, 820 in CKD stage 4–5 and 688 in dialysis) who were 18–75 years of age were enrolled from 81 Spanish hospitals between October 2010 and June 2012. A non-CKD group of 559 subjects was selected within several Spanish primary care centres using a sex- and age-matched design. The exclusion criteria were previous CV events, active infections (HIV, tuberculosis), pregnancy, having received any organ transplantation or having a life expectancy of <1 year.

The study was approved by each local ethics committee, and subjects were included after providing informed consent.

Clinical and biochemical data

At recruitment, the patients were asked to complete a questionnaire including clinical history of diabetes, hypertension and dyslipidaemia; CV risk factors (such as smoking habit) and medication use. Biochemical parameters were obtained from a routine fasting blood test performed around 3 months from the vascular exploration.

Since NEFRONA is a multicentre study, particular attention was paid to those parameters measured with different methods. In particular, PTH for the dialysis group was corrected using a well-established conversion method [24]. For patients not on dialysis, the correction was not applied because a residual or normal renal function decreases the formation of the 7–84 PTH fragments which, at high levels, are responsible for the high inter method differences. Furthermore, levels of hsCRP and 25 (OH)-vitamin D_3 were measured in the same laboratory to avoid possible interferences due to the use of different methods. hsCRP was determined by a immunoturbidimetric method (Roche/Hitachi MODULAR *ANALYTICS)* and 25(OH)-vitamin D_3 levels by Elisa (IDS, UK).

The ultrasound explorations (carotid and femoral) were performed according to a standardized protocol by three itinerant teams belonging to the UDETMA (Unit for Detection and Treatment of Atherothrombotic Diseases, Hospital Universitari Arnau de Vilanova, Lleida, Spain). The itinerant teams also collected the anthropometric parameters as well as blood samples, which were stored within 24 h at the centralized biobank of the Spanish Network for Nephrological Research (REDinRen).

Carotid and femoral ultrasound

B-mode ultrasound of the carotid and femoral arteries was performed using the Vivid BT09 apparatus (General Electric instrument) equipped with a 6–13 MHz broadband linear array probe. The analysis of the presence of atheromatous plaques was performed by a unique reader in a blinded fashion, using the semi-automatic software EchoPAC Dimension (General Electric Healthcare). To assess the quality of the reading and the intraobserver reliability, a sample of 20 individuals was measured 3–5 times on different days. A kappa coefficient of 1 was obtained, indicating excellent intraobserver reliability.

Carotid ultrasound. Ultrasound imaging was performed for both carotid arteries with the subjects in a supine position and the head turned 45° contralateral to the side of the probe. The presence of atheromatous plaques at each site was defined as carotid intima-media thickness (CIMT) ≥1.5 mm protruding into the lumen, which is the criterion given in the ASE Consensus Statement [25] and the Mannheim CIMT Consensus [26]. This criterion was used in the ARIC study [27] and in the Framingham Offspring Study [28], two important studies showing the predictive value of atheromatous plaques in CV risk.

Femoral ultrasound. B-mode ultrasound imaging was performed in the right and left femoral arteries. Subjects were examined in a supine position, and the presence of atheromatous plaques was explored in the common and superficial femoral arteries.

Statistical analysis

Descriptive analysis included the absolute and relative frequencies for qualitative variables as well as the mean and standard deviation for quantitative variables. The Pearson chi-square test was used to compare the distribution of qualitative variables between the CKD and non-CKD patients as well as among the three CKD stages. The non-parametric Mann–Whitney test was

used to compare the distribution of quantitative variables between the CKD and non-CKD patients. Meanwhile, the non-parametric Kruskal–Wallis test was used to compare the distribution of quantitative variables between CKD stages. The presence of atheromatous plaques in any territory was estimated by fitting a multivariate logistic regression model to measure the statistical contribution of CKD stage after adjusting for sex, age and diabetes. The non-linear contribution of age on the presence of atheromatous plaques was modelled by adding its quadratic term. Interaction effects between explanatory variables were tested and included in the model only if statistically significant. The contribution of phosphate and corrected total calcium serum levels in explaining the presence of plaque were analysed in a stratified analysis by CKD stage, given the completely different distribution of values among them. A logistic regression model was independently fitted to each CKD stage: one for stage 3, one for stages 4–5 and one for 5D. Besides adjusting for age, sex and diabetes in constructing the model for each CKD stage, the possible contribution of systolic and diastolic blood pressures, body mass index, tobacco use, cholesterol (total, LDL, ratio (total/HDL), non-HDL), triglycerides, hsCRP, 25(OH)-vitamin D_3, phosphate, corrected total calcium and PTH was analysed. The model for patients 5D included the assessment of the contribution of the time in dialysis. For the quantitative variables, a linear as well non-linear contribution was tested. Linear relationships were tested by checking the monotonous increase or decrease of the coefficients associated to the variable once recoded into three intervals of equal length. Non-linear relationships were checked by including the recoded version of the variable into three intervals defined using tertiles. The quadratic effect of age was also assessed. Variables were only included in models when proving a statistically significant contribution according to the Likelihood Ratio test. The statistical level of significance was fixed at 0.05. The R programme was used for all statistical analyses.

RESULTS

Demographic and clinical data are shown in Table 1. The percentage of men and the average age were higher among the CKD patients than the non-CKD patients. Although the percentage of smokers was lower, the CKD patients showed a higher frequency of diabetes, hypertension and dyslipidaemia. Systolic and diastolic blood pressure were higher in the CKD group than in the non-CKD group. Total cholesterol, LDL and HDL were lower while triglycerides were higher in the CKD group. Serum phosphate levels were higher and corrected serum calcium lower in CKD patients. Body mass index (BMI) was similar between both groups. 25(OH)-vitamin D_3 levels were lower and hsCRP higher in CKD patients.

Table 1. Baseline demographic and biochemical parameters

	Non-CKD	CKD	CKD 3	CKD 4–5	CKD 5D	P non-CKD versus CKD	P between CKD groups
N	559	2445	937	820	688		
Sex [men/women (%)]	53.3/46.7	61.7/38.3	66.3/33.7	58.3/41.7	59.4/40.6	<0.001	<0.001
Age [years; mean (SD)]	54.6 (11.5)	57.9 (12.8)	60.9 (11.4)	58.7 (12.3)	53.2 (13.8)	<0.001	<0.001
Clinical history (%)							
Smoking (former/current)	40.8/19.5	35.3/19.4	36.6/18.9	34/19.3	34.9/20.2	0.029	0.78
Diabetes	10.9	25.7	27.5	30	18	<0.001	<0.001
Hypertension	35.2	89.3	88.3	93.4	85.8	<0.001	<0.001
Dyslipidemia	35.1	64.9	70.1	69.1	52.8	<0.001	<0.001
Atheromatous plaque (%)[a]	51	69.4	59.8	68.7	69.8	<0.001	0.87
Body mass index [kg/m^2; mean (SD)]	28.1 (4.5)	28.3 (5.2)	29.2 (4.9)	28.8 (5.5)	26.5 (4.9)	0.83	<0.001
Systolic blood pressure [mm Hg; mean (SD)]	133.2 (17.7)	142.9 (22)	142.9 (20.2)	146.7 (22.1)	138.4 (23.4)	<0.001	<0.001
Diastolic blood pressure [mm Hg; mean (SD)]	80 (9.7)	81.3 (11.9)	81.6 (10.2)	82.2 (11.4)	80 (14.2)	0.010	0.005
Creatinine [mg/dL; mean (SD)]	0.8 (0.2)	4.1 (3.3)	1.6 (0.4)	3.3 (1.3)	8.4 (2.9)	<0.001	<0.001
Glomerular filtration rate[a] [mL/min; mean (SD)]	91.8 (16.8)	na	43.5 (8.7)	20.6 (7.5)	na	na	na
Total cholesterol [mg/dL; mean (SD)]	203.4 (35.6)	178.3 (39.7)	187.4 (36.5)	179.1 (37.9)	164.9 (42.4)	<0.001	<0.001
LDL cholesterol [mg/dL; mean (SD)]	127.3 (32.8)	101.8 (33.7)	109.6 (31.6)	101.9 (32.7)	91.2 (34.8)	<0.001	<0.001
HDL cholesterol [mg/dL; mean (SD)]	53.9 (15.4)	49.3 (15.4)	50.9 (15.4)	49.2 (15.1)	47.3 (15.6)	<0.001	<0.001
Non-HDL cholesterol [mg/dL; mean (SD)]	150.4 (36.1)	129.3 (37.4)	136.8 (34.5)	130.6 (36.3)	117.8 (39.7)	<0.001	<0.001
Triglycerides [mg/dL; mean (SD)]	115.5 (67.5)	145 (82.3)	144.7 (83)	149.8 (85.7)	139.7 (76.1)	<0.001	0.06
Corrected calcium [mg/dl; mean (SD)]	9.4 (0.4)[b]	9.2 (0.6)	9.5 (0.5)	9.3 (0.6)	9.1 (0.7)	0.0003	<0.001
Phosphorus [mg/dL; mean (SD)]	3.5 (0.5)[b]	4.1 (1.1)	3.4 (0.6)	4.1 (0.8)	4.9 (1.3)	<0.001	<0.001
PTH [pg/mL; mean (SD)]	na	181.7 (193.5)	85.6 (69)	173.5 (123.8)	297.1 (273.2)	na	<0.001
hsCRP [mg/L; mean (SD)]	3.4 (7.9)	4.7 (9.3)	3.9 (6.1)	4.8 (9.9)	5.6 (11.7)	<0.0001	0.02
25(OH)-vitamin D_3 [ng/L; mean (SD)]	20.1 (7.9)	16.1 (7.5)	16.7 (8)	16.2 (6.8)	15.1 (7.4)	<0.0001	<0.0001
Time on dialysis [months; mean (SD)]	na	na	na	na	28 (37.2)		
Treatments (%)							
Antihypertensives	34.5	88.1	91.8	95.6	74.3	<0.001	<0.001
Dyslipidaemia	27.4	64.4	67.6	70.7	52.5	<0.001	<0.001
Phosphate binders	na	35.7	5.7	31.1	82	na	<0.001
Vitamin D	na	38.7	18	50.4	53.1	na	<0.001

na, not applicable.
[a]Presence of plaque at any territory; glomerular filtration rate: MDRD4.
[b]Data only in a 36% of participants.

Table 2. Presence of plaque at any territory [*n*(%)] stratified by age, sex, CKD stage and diabetic condition

	Men					Women				
Age (years)	Non-CKD	CKD 3	CKD 4–5	CKD 5D	P trend	Non-CKD	CKD 3	CKD 4–5	CKD 5D	P trend
Non-diabetics										
≤35	1 (5.0)	1 (3.8)	2 (9.5)	6 (13.0)	0.171	1 (4.0)	0 (0)	1 (5.6)	8 (25.8)	0.008
36–45	10 (27.0)	11 (31.4)	9 (25.0)	37 (51.4)	0.009	4 (11.1)	3 (13.6)	9 (29.0)	13 (31.7)	0.015
46–55	43 (60.6)	30 (65.2)	37 (59.7)	60 (81.1)	0.019	24 (34.3)	18 (45.0)	23 (44.2)	35 (59.3)	0.007
56–65	65 (77.4)	105 (73.4)	94 (89.5)	77 (92.8)	<0.001	28 (42.4)	38 (58.5)	49 (68.1)	36 (75.0)	<0.001
66–75	39 (78.0)	167 (89.8)	91 (89.2)	55 (96.5)	0.009	24 (61.5)	69 (67.6)	60 (82.2)	45 (88.2)	<0.001
Diabetics										
≤35	0 (0)	0 (0)	0 (0)	1 (33.3)	na[a]	0 (0)	0 (0)	0 (0)	1 (50.0)	na[a]
36–45	0 (0)	8 (61.5)	6 (60.0)	8 (80.0)	na[a]	1 (100)	3 (60.0)	1 (11.1)	5 (55.6)	0.704
46–55	2 (33.3)	11 (73.3)	16 (84.2)	10 (100.0)	0.002	6 (100)	5 (62.5)	10 (76.9)	3 (50.0)	0.138
56–65	15 (93.8)	62 (87.3)	35 (81.4)	25 (96.2)	0.827	5 (71.4)	18 (69.2)	14 (77.8)	14 (93.3)	0.141
66–75	11 (78.6)	78 (94.0)	69 (95.8)	27 (100.0)	0.017	6 (54.5)	29 (90.6)	41 (80.4)	15 (93.8)	0.114

[a]na stands for not applicable groups, i.e. those empty groups (with nobody) for whom any plaque percentages cannot be estimated.

Additionally, Table 1 shows the differences among the different stages of CKD. CKD patients in stage 3 displayed a higher prevalence of men and a higher average age when compared with the other two CKD groups. Moreover, patients in stage 3 showed higher prevalence rates of diabetes, hypertension and dyslipidaemia than patients on dialysis. Moreover, levels of BMI, total cholesterol, LDL cholesterol, HDL cholesterol, corrected calcium and 25(OH)-vitamin D_3 decreased progressively with the decrease in renal function. Serum phosphorus, PTH and hsCRP were higher in severe CKD patients. The absolute and relative prevalences of subclinical atheromatous plaque are summarized in Table 2 (*n*; %) and stratified by CKD stage, age group, sex and diabetic status. In non-diabetic men, the higher prevalence of atheromatous plaques was parallel to CKD severity (trend $P < 0.001$ in all age groups). For women without diabetes, the prevalence of atheromatous plaques was lower compared with men in any age group or CKD stage. Furthermore, there was a higher plaque prevalence in more severe CKD stages (trend $P < 0.001$ in all age groups). For men with diabetes, the presence of atheromatous plaques was already high at early ages, and the positive trend along the CKD stages was statistically significant at ages lower than 55 years and higher than 65 years ($P = 0.043$ and $P = 0.02$, respectively). Lastly, for women with diabetes, the presence of atheromatous plaques was also already high at early ages, although no significant trend was observed. Importantly, regardless of sex, age group or presence of diabetes, a higher prevalence of atheromatous plaques was always observed in the dialysis group.

Figure 1 shows the estimated prevalence of atheromatous plaques based on the results of the multivariate logistic regression model shown in Table 3. As illustrated by the figure, age showed a marked quadratic effect ($P = 0.0004$) and interacted significantly with sex. In agreement with the curves, the presence of atheromatous plaques was higher with age until it plateaued, although this association was weaker for women than for men (interaction between age and sex, $P = 0.0023$). The presence of atheromatous plaques was markedly and significantly higher among CKD patients on dialysis ($P < 0.0001$) or patients in CKD stage 4–5 ($P = 0.0001$) than among non-CKD patients. Furthermore, diabetic patients also showed a higher prevalence of atheromatous plaques than non-diabetic patients ($P < 0.001$). Considering CKD, the estimated prevalence of atheromatous plaques is positively associated with the severity of CKD for both sexes and both diabetic and non-diabetic patients.

The logistic model (Table 3) estimates an odds ratio (IC95) for having a plaque of 1.3 (1.0–1.7) in CKD stage 3 when compared with non-CKD subjects. This odds ratio increased to 1.7 (1.3–2.2) among CKD 4–5 patients and to 3.7 (2.8–5.0) among dialysis patients.

In Table 4–6 we depict the multivariate logistic models to identify variables significantly associated to the presence of plaque, stratified by CKD stage. Table 4 shows the parameters of the adjusted model for patients in CKD stage 3. Higher age, and being male or diabetic significantly associated with the presence of plaques. The association with age also presented a quadratic effect. There is a significant interaction between smoking status and triglycerides. Thus, the significant effects were observed between current smokers (both in the central and in the higher tertile of triglycerides) or former smokers in the highest tertile of triglycerides with respect to the reference group of non-smokers with triglycerides in the lowest tertile. In addition, levels of phosphate in the highest tertile also showed a tendency to associate with the presence of atheromatous plaque ($P = 0.07$).

In patients in CKD stages 4–5 (Table 5), being male and older were also statistically associated with having a carotid plaque. The interaction between smoking status and lipids was lost, but both being former smoker or current smoker showed a statistically significant association with the presence of subclinical atheromatosis. Having levels of phosphate or hsCRP in the highest tertile was also significantly associated with the presence of plaque. The levels of total cholesterol showed a tendency, but did not reach statistical significance in this group of patients.

In dialysis patients (Table 6) the effect of age, sex and diabetes was still present. The results show again a significant association between non-smoker and former smokers but not with current smokers probably due to the low sample size in the current smokers group.

The phosphorus levels display a U-shaped curve. Thus, being in the lower tertile or in the higher tertile were both

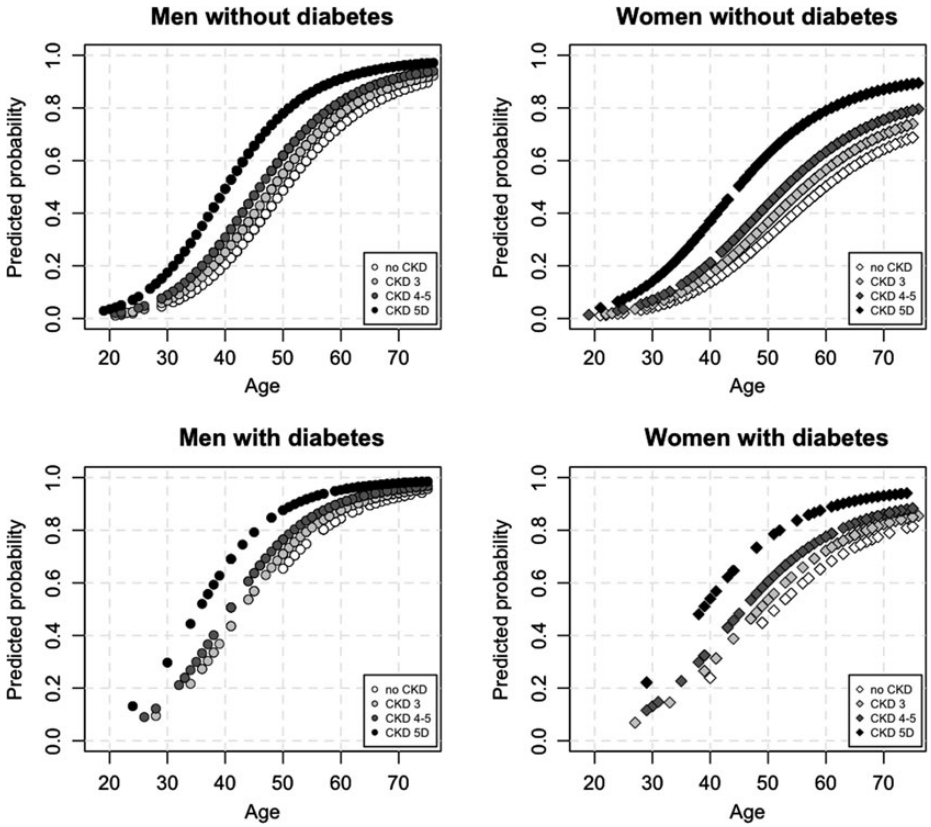

FIGURE 1: Estimated prevalence of plaque at carotid or femoral arteries by the multivariate logistic regression model including gender, diabetes status, CKD stage and the quadratic effect of age (see Table 3), including a significant interaction between gender and age.

Table 3. Coefficients (standard errors) and P-values for the multivariate logistic regression to model presence of plaque at any territory

	Estimate (SE)	P-value
Intercept	−8.8385 (0.9666)	<0.0001
Group CKD 3	0.2566 (0.1321)	0.0588
Group CKD 4–5	0.5407 (0.1348)	0.0001
Group CKD 5D	1.3178 (0.1466)	<0.0001
Age	0.2338 (0.0357)	<0.0001
Age^2	−0.0012 (0.0003)	0.0004
Gender	0.4876 (0.4776)	0.2670
Diabetes	0.6917 (0.1246)	<0.0001
Gender: Age	−0.0248 (0.0084)	0.0023

significantly associated with the presence of plaque when compared with the central tertile as the reference. Furthermore, being in the highest tertile of 25(OH)-vitamin D_3 levels was significantly associated with the absence of plaque.

DISCUSSION

The present study is the first to demonstrate the prevalence of atheromatous plaques in asymptomatic CKD patients throughout all stages of CKD and to compare it with a non-CKD population. The data showed that the absolute and adjusted prevalence rates of atheromatous plaques were higher among CKD patients than in a non-CKD population.

Table 4. Odds ratio, 95% confidence interval and P-values for the multivariate logistic regression to model presence of plaque at any territory: patients in CKD stage 3.

	P	OR	IC 95%
Age, years	0.0015	1.30	1.11, 1.55
Age^2	0.05	0.998	0.997, 1
Sex, women versus men	0.0004	0.49	0.33, 0.73
Diabetes, %	<0.0001	2.86	1.82, 4.63
Former smoker and TG T1 versus ref.	0.29	1.42	0.74, 2.76
Current smoker and TG T1 versus ref.	0.12	1.99	0.85, 4.80
Non-smoker and TG T2 versus ref.	0.11	1.58	0.90, 2.82
Former smoker and TG T2 versus ref.	0.17	1.59	0.83, 3.12
Current smoker and TG T2 versus ref.	0.0008	4.47	1.92, 11.10
Non-smoker and TG T3 versus ref.	0.61	0.85	0.47, 1.54
Former smoker and TG T3 versus ref.	0.0002	4.61	2.14, 10.67
Current smoker and TG T3 versus ref.	0.0002	5.49	2.32, 14.08
P T2 versus P T1	0.29	1.27	0.82, 1.96
P T3 versus P T1	0.07	1.53	0.97, 2.42
Hosmer-Lemeshow, P		0.2496	
AUC		0.821	

TG T1, triglycerides tertile 1 (<103 mg/dL); TG T2, triglycerides tertile 2 (103–155 mg/dL); TG T3, triglycerides tertile 3 (>155 mg/dL); ref, non-smoker and TG T1; P T1, phosphorus tertile 1 (<3.11 mg/dL); P T2, phosphorus tertile 2 (3.11–3.62 mg/dL); P T3, phosphorus tertile 3 (>3.62 mg/dL); AUC, area under curve.

Moreover, the prevalence is higher the more severe the CKD is, with the highest prevalence among dialysis patients, for all ages and both sexes. Furthermore, as in the general population, atheromatous disease was more prevalent among diabetics and male patients.

Table 5. Odds ratio, 95% confidence interval and P-values for the multivariate logistic regression to model presence of plaque at any territory: patients in CKD stage 4–5

	P	OR	IC 95%
Age, years	<0.0001	1.12	1.10, 1.14
Sex, women versus men	0.003	0.53	0.35, 0.80
Diabetes, %	0.07	1.51	0.97, 2.34
Former smoker versus non-smoker	0.0016	2.12	1.33, 3.39
Current smoker versus non-smoker	0.0001	3.10	1.79, 5.49
P T2 versus P T1	0.17	1.38	0.87, 2.21
P T3 versus P T1	0.01	1.75	1.11, 2.77
T-Chol T2 versus T-Chol T1	0.05	1.59	0.99, 2.56
T-Chol T3 versus T-Chol T1	0.06	1.57	0.98, 2.54
hsCRP T2 versus hsCRP T1	0.86	1.04	0.66, 1.63
hsCRP T3 versus hsCRP T1	0.035	1.66	1.04, 2.67
Hosmer-Lemeshow, P		0.5154	
AUC		0.834	

P T1, phosphorus tertile 1 (<3.71 mg/dL); P T2, phosphorus tertile 2 (3.71–4.32 mg/dL); P T3, phosphorus tertile 3 (>4.32 mg/dL); T-Chol T1, total cholesterol tertile 1 (<163 mg/dL); T-Chol T2, total cholesterol tertile 2 (163–192 mg/dL); T-Chol T3, total cholesterol tertile 3 (>192 mg/dL); hsCRP T1, high-sensitivity C-reactive protein tertile 1 (<1.19 mg/L); hsCRP T2, high-sensitivity C-reactive protein tertile 2 (1.19–3.38 mg/L); hsCRP T3, high-sensitivity C-reactive protein tertile 3 (>1.38 mg/L); AUC, area under curve.

Table 6. Odds ratio, 95% confidence interval and P-values for the multivariate logistic regression to model presence of plaque at any territory: patients in CKD 5D

	P	OR	IC 95%
Age, years	<0.0001	1.12	1.10, 1.14
Sex, women versus men	<0.0001	0.38	0.24, 0.60
Diabetes, %	0.02	2.24	1.16, 4.57
Former smoker versus non-smoker	0.0027	2.16	1.31, 3.60
Current smoker versus non-smoker	0.12	1.54	0.90, 2.65
P T1 versus P T2	0.01	1.90	1.15, 3.17
P T3 versus P T2	0.008	2.03	1.21, 3.43
25,OH D3 T2 versus 25,OH D3 T1	0.52	0.84	0.49, 1.44
25,OH D3 T3 versus 25,OH D3 T1	0.0057	0.47	0.28, 0.80
Hosmer-Lemeshow, P		0.1733	
AUC		0.863	

P T1, phosphorus tertile 1 (<4.3 mg/dL); P T2, phosphorus tertile 2 (4.3–5.32 mg/dL); P T3, phosphorus tertile 3 (>5.32 mg/dL); 25-OH D3 T1, 25(OH)-vitamin D_3 tertile 1 (<11.25 ng/mL); 25-OH D3 T2, 25(OH)-vitamin D_3 tertile 2 (11.25–16.6 ng/mL); 25-OH D3 T3, 25(OH)-vitamin D_3 tertile 3 (>16.6 ng/mL).

To evaluate the adjusted effect of the severity of CKD on the prevalence of subclinical atheromatosis, we calculated the estimated prevalence of plaques using a logistic regression model including the most common atherogenic factors (age, sex and diabetes). The results of this analysis were interesting. First, the adjusted contribution of CKD to the logistic model increases with the severity of CKD, being the highest in dialysis. Second, the contribution of age showed a quadratic profile, although with weaker association for females. Third, diabetes was associated with higher prevalence of plaque at all stages of CKD, with similar magnitude for both sexes and all age groups. Thus, this simple model, without biochemical parameters, by setting the age, sex and medical history of diabetes, the estimation of the risk of having an ahteromatous plaque.

The results showed that diabetes was associated with a higher prevalence of atheromatous plaques among both sexes and at any stage of CKD. Notably, the well-known protective effect of female sex was still present in CKD patients. In addition, the higher prevalence of atherosclerosis among dialysis patients compared with a population with normal renal function supports the existence of dialysis-specific risk factors that could increase the risk of having an atheromatous plaque.

In order to better understand the factors associated with the presence of plaque in every stage of CKD, we performed stratified multivariate logistic analysis. Thus, we introduced in the analysis of 17 variables known to have a relationship with the presence of atherosclerosis in the normal population, or with CV complications in CKD. Some of the continuous variables were introduced in tertiles because it has been previously reported to have a non-linear relationship with atherosclerosis in CKD. Furthermore, in some of these variables, the reverse epidemiology phenomenon has also been described [29].

Age and sex maintain a significant association in every stage of CKD. The presence of diabetes also shows a strong effect, although in CKD 4–5 does not reach statistical significance.

In CKD 3, there is also an important quadratic effect of age and a strong interaction of triglycerides and smoking habits. This interaction has been previously reported in the general population [30] and it is still present at the early stages of CKD. However, in advanced stages of CKD, the interaction is lost and there only the association of smoking with the presence of plaques remains. The effect of higher triglycerides in the CKD population has been previously described [31]. In our population, former or current smokers with triglycerides over 155 mg/dL showed an increase in plaque presence, independently of other factors. These results suggest the need for more studies to determine the role of triglycerides in CV disease in early stage CKD.

In CKD 4–5, the association between triglycerides and plaque presence is lost and only total cholesterol shows a weak association. These results are in agreement with results from Tonelli *et al.* [32], which showed that the association between higher LDL-cholesterol and risk of myocardial infarction is weaker for people with lower baseline eGFR. In dialysis patients, none of the lipid profile parameters showed an association with the presence of plaque. This could be explained by the rising influence of non-traditional risk factors in the advanced stages of CKD (like inflammation, malnutrition, etc.).

The alterations in mineral metabolism have been reported to be associated with CV disease in CKD patients. In our study Ca and PTH was not significantly associated with the presence of plaque. Although P levels showed a non-significant tendency in stage 3 ($P = 0.07$), in stage 4–5, having P levels in the highest tertile was associated with a higher presence of plaque. In dialysis patients both the highest and the lowest tertile were associated with the presence of plaque, showing the characteristic U shaped curve described in other studies [33]. This effect of low P levels could be related to the existence of malnutrition [34]. The levels of hsCRP were also associated with the presence of plaque in CKD 4–5, as previously reported by other studies [35].

Finally, the multivariate analysis detected an independent association between the presence of plaque and levels of 25(OH)-vitamin D_3 in the lowest tertile in dialysis patients. There is a high amount of information regarding the role of vitamin D in vascular health [36]. However, to date, the protective effect of restoring 25(OH)-vitamin D_3 levels to normal values has not been

proven. Thus, the association of lower levels of 25(OH)-vitamin D_3 with higher plaque presence could be related to a worse general health in the patients with atheromatosis.

Our study suffers from a number of limitations. First, its cross-sectional nature does not allow us to make causal associations. Second, we could not perform the same multivariate logistic analysis in the non-CKD group because of the lack of data in mineral metabolism parameters. Third, we lack data on proteinuria in many CKD patients, a parameter which has been associated with CV risk in CKD patients [37].

Our study also has some strengths. First, the high number of patients allows us to perform stratified multivariate logistic analysis, minimizing the survival bias inexorably associated with this kind of studies. Furthermore, the vascular exploration was performed by the same itinerant team and evaluated by a unique reader, minimizing the variability associated with the technique.

In summary, the data presented here support the high prevalence of atheromatous disease in the CKD population, which is higher in advanced stages of CKD, and suggest the existence of specific risk factors that favour the atheromatous process. Furthermore, we have provided a prevalence table that could be used as a reference for future studies. We also provide with a simple tool (non-requiring of any analytical parameters) to visualize the higher prevalence of atheroma plaque in asymptomatic CKD patients associated to renal function decrease, taking into account age, sex and diabetes. Indeed, this method should be validated in different populations. The fact that patients in the early CKD stages had a higher prevalence of atheromatous plaques than the population without CKD demonstrates the need for the early detection of atheromatous disease in this population. The different risk factors associated in every stage of CKD suggest the need of specific prevention measures depending on the severity of CKD. The use of carotid and femoral ultrasound can aid the detection of the disease, particularly in this population, in whom the classical risk scoring is inefficacious.

CONFLICT OF INTEREST STATEMENT

None declared.

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