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Original Article

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Microalbuminuria, but not cystatin C, is associated with carotid atherosclerosis in middle-aged adults

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Abstract

Background. Cystatin C, a marker of renal function, has been shown to be an independent predictor of cardiovascular disease (CVD) in older adults, but few data are available in middle-aged adults. Moreover, no study has compared cystatin C and microalbuminuria as risk factors for CVD outcomes in middle-aged adults, and it is not known whether cystatin C is related to an early stage of atherosclerosis.

Methods. We evaluated the relationships between serum creatinine, estimated glomerular filtration rate (GFR), serum cystatin C (all divided into tertiles), microalbuminuria and carotid atherosclerosis in a population-based random sample of 523 adults aged 35–64 years from the Seychelles (Indian Ocean). GFR was estimated using the modification of diet in renal disease (MDRD) equation. Intima-media thickness (IMT) was assessed by B-mode ultrasound.

Results. The mean age of the study sample was 52 years, and 55% were women. Carotid IMT was higher in participants with microalbuminuria (802 *vs* 732 μm, $P < 0.001$) and was inversely associated with GFR tertiles (from 728 to 809 μm, P for trend $= 0.002$). IMT was not associated with cystatin C or creatinine (P for trend $= 0.10$ and 0.16, respectively). In multivariate analyses adjusted for cardiovascular risk factors, the association between microalbuminuria and IMT remained ($P = 0.047$), while the association between GFR and IMT disappeared (P for trend $= 0.33$).

Conclusions. Microalbuminuria, but not cystatin C, is associated with carotid atherosclerosis beyond traditional cardiovascular risk factors among middle-aged adults. Cystatin C does not have a stronger relationship with carotid atherosclerosis in middle-aged adults than creatinine.

Keywords: Africa; albuminuria; atherosclerosis; cystatin; Seychelles

Introduction

Cardiovascular diseases (CVD) are the leading causes of mortality and morbidity in western and developing countries [1,2]. Screening and treatment is one strategy for the primary prevention of CVD, but the highest-risk patients should be identified to maximize the benefit/cost ratio of treatments [3]. In recent years, several tests, ranging from serum to urinary markers, have been proposed as new cardiovascular risk factors or markers that may improve risk prediction and help to identify the highest-risk patients [4,5]. Among markers of renal function that have been associated with increased cardiovascular risk, cystatin C has recently been shown to be an independent predictor of cardiovascular events in a prospective cohort of adults aged ≥65 years [6]. Microalbuminuria has also been suggested as an independent predictor of cardiovascular events, including in subjects without diabetes [7]. The presence of chronic kidney disease, either manifested by albuminuria or reduced estimated glomerular filtration rate (GFR), appears to be an independent risk factor for CVD in prospective studies, particularly in high-risk populations [7].

It remains to be determined whether a mild decrease in GFR or other early markers of renal dysfunction are associated with increased cardiovascular risk in low-risk populations [7]. The association of microalbuminuria with cardiovascular events has, indeed, been less consistent in low-risk populations [7,8].

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The association between cystatin C and CVD has been mainly studied in prospective cohorts of older adults who have a higher prevalence of renal dysfunction [6,9]. These two markers have also not been compared in a same population, and it is not known whether cystatin C is related to an early stage of atherosclerosis. To that end, we compared the associations of serum cystatin C and microalbuminuria with the risk of carotid atherosclerosis in a population-based sample of middle-aged adults in the Seychelles Islands.

Subjects and methods

Subjects and design

Description of the population. The Republic of Seychelles is a group of islands in the Indian Ocean ~1800 km east of Kenya, and is part of the African region. A large majority of the population is of African descent. Health care, including medications, is available at no direct cost at the point of delivery to all residents through a national health system while a few private doctors also provide services on a fee for service basis. All deaths are registered in Seychelles, and vital statistics indicate a life expectancy of 69 years in men and 76 years in women. AIDS and CVD account for, respectively, ~1 and 38% of total mortality [10].

A population-based survey of cardiovascular risk factors was conducted in 2004 under the auspices of the Ministry of Health of the Republic of Seychelles (Seychelles Heart Study III), as previously described [11]. In summary, the sampling frame consisted of a sex- and age-stratified random sample of the entire population aged 25–64 years. The survey was approved by the Ministry of Health after technical and ethical reviews. Participants were free to participate, and gave written informed consent. The participation rate was 80.2%.

Study sample. Among the 1255 participants, ultrasonography was performed in all participants above 45 years seen during a 17-week period ($n = 496$), as well as in a randomly selected sample (18%, $n = 58$) of the participants aged 35–44 years. We restricted the investigation to these age classes because they were more likely to have atherosclerosis. Among these 554 participants, we excluded those with unreliable values of microalbuminuria because of menstruation or gynaecological and/or urinary tract infection ($n = 2$), similar to previous studies [12]. We also excluded those with missing data for microalbuminuria, serum cystatin or serum creatinine ($n = 19$). Participants with proteinuria (albumin/creatinine >300 mg/g) were also excluded, because of the small number ($n = 10$) and similar to previous studies using semi-quantitative measurement of microalbuminuria [13,14]. The final sample of the analyses consisted of 523 participants.

Measurements

Renal function assays. Fasting serum samples were obtained in all participants and were stored at −70°C. Serum cystatin C was measured by means of a particle-enhanced immunonephelometric assay with a nephelometer (BNII, Dade Behring) [6]. The range of detection of the assay is 0.195–7.330 mg/l, with the reference range for young, healthy persons reported as 0.53–0.95 mg/l. A previous study has shown that the assay remained stable, even after freezing [6]. Microalbuminuria was measured on a spot of second morning urine after an overnight fast using the Bayer Clinitek Status analyser that has been shown to be a reliable screening procedure for microalbuminuria [15]. It provides semi-quantitative estimation of urinary albumin excretion adjusted to creatininuria, and determines participants with microalbuminuria (30–300 mg albumin/g creatinine) and those with normal albuminuria (<30 mg/g) [16,17]. Serum creatinine was measured by means of a kinetic Jaffe method (Hitachi 917, Japan; reagents from Roche Diagnostics). We estimated GFR using the abbreviated modification of diet in renal disease (MDRD) equation [18].

Carotid ultrasound. B-mode ultrasound imaging of the carotid wall is a well-established surrogate marker for atherosclerotic disease that is increasingly used in observational and interventional studies [19]. All the scans and image analyses were carried out by the same investigator (P.Y.) who was blinded to the risk factor status of the participants. High resolution B-mode ultrasonography was conducted with an ultrasound system (General Electric LOGIQ Book) connected with a 6–10 MHz linear array transducer and equipped with a software for arterial wall analysis (M'ATH, ICN-metric, Paris, France) to perform semi-automatic measures of intima-media thickness (IMT) on frame [20]. The IMT was measured on the far wall of both common carotid arteries over a length of 1 cm on a reference site located 2 cm below the bifurcation, and the average of the left and the right maximum common carotid IMT was computed [21,22]. The far wall was used because of higher reproducibility and possible overestimation of the IMT of the near wall [22]. The recorded IMT was the mean thickness measured along the whole segment.

To examine the reproducibility, 20 randomly selected ultrasound measurements were performed twice by the same sonographer. For carotid IMT, intra-observer variability showed a coefficient of variation of 4.8% between first and second readings, similar to previous studies [23].

Covariates. Smoking was defined as current smoking of ≥1 cigarette/day. Blood pressure was defined as the average of the last two of three measurements with a mercury sphygmomanometer taken at intervals ≥2 min after the participants had been seated for ≥30 min. Hypertension was defined as use of anti-hypertensive medications or measured systolic blood pressure ≥140 and/or diastolic ≥90 mmHg. To calculate body mass index (BMI), weight was measured with electronic medical scales and height with fixed stadiometers (Seca, Germany). Blood lipids and plasma glucose were measured after fasting since midnight [11]. Diabetes mellitus was defined as fasting blood glucose ≥7.0 mmol/l, using any hypoglycaemic medication, and/or a 2 h plasma glucose ≥11.1 in an oral glucose tolerance test (performed if fasting glucose ≥5.6 mmol/l).

Statistical analysis

To examine the associations between renal markers and carotid atherosclerosis, we created tertiles of the study population according to serum cystatin C, serum creatinine and GFR levels. The upper tertile was subdivided into three groups, as levels of renal markers were relatively low in

this middle-aged population, compared to older adults [6], and to be able to assess the effect of elevated renal markers, similar to a previous study [6]. We used linear regression models to assess the association between those markers and carotid IMT. To compare the overall prediction of traditional and new risk factors on IMT, we used adjusted R^2. For each outcome, the multivariate models included the same covariates based on clinical plausibility (e.g. diabetes, lipids). We hypothesized several interactions: the relationships between renal markers and carotid atherosclerosis might differ by age, gender, hypertension and diabetes status. Results were reported as odds ratio (OR) with 95% confidence intervals (CI) or mean adjusted values for IMT. We conducted all analyses using STATA 9.0 (Stata Corporation, TX, USA).

Results

Participant characteristics

The mean age of the study sample was 52 years, and 55% were women. A majority of this population sample of Seychelles was of African descent (90%) with small minorities of European, Indian and Chinese descent. Serum cystatin C was higher in older than younger participants and in men than women (Table 1). Higher levels of cystatin C were associated with smoking and most traditional cardiovascular risk factors. Participants with higher serum cystatin had higher serum creatinine and lower GFR (P for trend < 0.001), but the prevalence of microalbuminuria did not significantly differ among tertiles of cystatin C (P for trend $= 0.10$). Cystatin C was correlated with creatinine ($r = 0.60$, $P < 0.001$) and inversely correlated with GFR ($r = -0.41$, $P < 0.001$). Participants with microalbuminuria were older and more likely to have glucose abnormalities, high blood pressure, BMI and triglycerides (Table 2). Cystatin C, but not GFR, was significantly higher in those with microalbuminuria ($P = 0.003$). Adjustment for age diminished the difference in cystatin C according to microalbuminuria ($P = 0.02$), and removed the association between cystatin C and systolic blood pressure ($P = 0.36$). All other relationships did not meaningfully change after adjusting for age. A total of 24 participants (5%) had a GFR $<60\,\text{ml/min/1.73}\,\text{m}^2$.

Traditional risk factors, serum cystatin C, microalbuminuria and carotid IMT

Carotid IMT was strongly associated with age, but not with gender (Table 3). In analyses adjusted for age and gender, IMT was also strongly associated with most traditional risk factors and microalbuminuria

Table 1. Baseline characteristics of study population according to tertiles of serum cystatin C[a]

Characteristics	Tertile 1 (<0.78 mg/l)	Tertile 2 (0.78–0.88 mg/l)	Tertile 3 (≥0.89 mg/l)	P[b]
Number of participants	178	163	182	
Age (years)	50.1 ± 7.7	52.4 ± 6.7	54.6 ± 6.8	<0.001
Woman (%)	61	54	50	0.03
Current smoker (%)	10	18	19	0.01
Glucose metabolism				0.08
Normal fasting glucose (%)	45	43	39	
Impaired FG/GT (%)	29	37	42	
Diabetes mellitus (%)	26	19	19	
BP stages [mmHg (%)[c]]				0.04
Normal BP <140/90	59	53	47	
Stage 1 BP 140–159/90–99	29	25	32	
Stage 2 BP ≥160/100	12	22	20	
Body mass index (kg/m)^2	27.7 ± 5.6	27.7 ± 5.5	29.2 ± 5.5	0.02
Systolic BP (mmHg)	132.1 ± 17.6	135.3 ± 18.8	137.8 ± 23.1	0.007
Diastolic BP (mmHg)	85.6 ± 11.1	87.4 ± 11.6	87.8 ± 13.5	0.08
LDL-cholesterol (mmol/l)	3.5 ± 1.2	3.9 ± 1.2	4.1 ± 1.3	<0.001
HDL-cholesterol (mmol/l)	1.3 ± 0.5	1.4 ± 0.5	1.3 ± 0.5	0.43
Triglycerides (mmol/l)	1.1 ± 1.0	1.2 ± 1.2	1.3 ± 0.8	0.04
Glucose (mmol/l)	6.9 ± 2.9	6.4 ± 1.9	6.5 ± 2.3	0.13
Serum creatinine (μmol/l)	73.6 ± 14.0	79.2 ± 14.6	92.0 ± 29.1	<0.001
Estimated GFR[d] (ml/min/1.73 m^2)	98.6 ± 19.1	91.4 ± 19.6	79.9 ± 19.9	<0.001
Serum cystatin C (mg/l)	0.68 ± 0.09	0.83 ± 0.03	1.03 ± 0.22	<0.001
Microalbuminuria[e] (%)	19	16	26	0.10

BP, blood pressure; impaired FG/GT, impaired fasting glucose defined as fasting glucose 5.6–6.9 mmol/l [40] or impaired glucose tolerance defined as 2 h plasma glucose 7.8–11.0 mmol/l in an oral glucose tolerance test [41].
[a]Values are mean ± SD or percentages.
[b]Chi square tests for trend for categorical variables and F-tests for trend for continuous variables across tertiles of cystatin C.
[c]Blood pressure stages according to the JNC-7 report [42]. Participants using anti-hypertensive medications with a measured blood pressure <140/90 were included in the blood pressure stage 1.
[d]GFR estimated using the abbreviated modification of diet in renal disease (MDRD) equation [18].
[e]Microalbuminuria defined as urinary albumin excretion of 30–300 mg albumin/g creatinine [17].

Table 2. Baseline characteristics of study population according to microalbuminuria[a]

Characteristics	Urinary albumin excretion (mg albumin/g creatinine)		
	Normal (<30 mg/g)	Microalbuminuria (30–300 mg/g)	P^b
No. of participants	417	106	
Age (years)	51.9 ± 7.4	54.1 ± 6.7	0.006
Woman (%)	55	55	0.94
Current smoker (%)	16	15	0.85
Glucose metabolism			<0.001
Normal fasting glucose (%)	46	31	
Impaired FG/GT (%)	37	31	
Diabetes mellitus (%)	17	38	
BP stages (mmHg %)			<0.001
Normal, BP <140/90	58	31	
Stage 1, BP 140–159/90–99	28	34	
Stage 2, BP ≥160/100	14	35	
Body mass index (kg/m^2)	27.9 ± 5.9	29.5 ± 5.4	0.01
Systolic BP (mmHg)	132.1 ± 17.9	146.9 ± 23.7	<0.001
Diastolic BP (mmHg)	85.3 ± 11.3	93.3 ± 13.4	<0.001
LDL-cholesterol (mmol/l)	3.8 ± 1.2	4.0 ± 1.5	0.21
HDL-cholesterol (mmol/l)	1.4 ± 0.5	1.3 ± 0.5	0.08
Triglycerides (mmol/l)	1.1 ± 0.9	1.5 ± 1.3	0.001
Glucose (mmol/l)	6.3 ± 2.1	7.7 ± 3.2	<0.001
Serum creatinine (μmol/l)	80.8 ± 18.0	85.3 ± 33.5	0.06
Estimated GFR (ml/min/1.73 m^2)	90.2 ± 19.9	88.6 ± 24.9	0.48
Serum cystatin C (mg/l)	0.83 ± 0.16	0.90 ± 0.31	0.003

BP, blood pressure; impaired FG/GT, impaired fasting glucose or impaired glucose tolerance.
[a]Values are mean ± SD or percentages.
[b]Chi square tests for categorical variables and *t*-tests for continuous variables.

Table 3. Multivariate analysis of traditional cardiovascular risk factors, serum cystatin C and microalbuminuria on carotid intima-media thickness (IMT)

Adjusted R^2	Age- and gender-adjusted		+Traditional risk factors[a]		+Traditional risk factors +Cystatin		+Traditional risk factors +Microalbuminuria	
	0.107[b]		0.152		0.155		0.157	
	β(SE)	*P*	β(SE)	*P*	β(SE)	*P*	β(SE)	*P*
Age (years)	8.1 (1.0)	<0.001	7.2 (1.1)	<0.001	7.6 (1.1)	<0.001	7.1 (1.1)	<0.001
Gender	13.2 (14.8)	0.37	8.0 (16.2)	0.62	5.9 (16.2)	0.72	7.3 (16.2)	0.65
Glucose metabolism		0.001		0.02		0.02		0.04
Impaired FG/GT	23.3 (16.8)		12.3 (17.0)		13.6 (17.1)		12.1 (17.0)	
Diabetes mellitus	66.6 (20.2)		49.9 (20.8)		48.1 (21.1)		43.9 (21.0)	
BP stages		0.06		0.09		0.06		0.23
Stage 1	11.6 (17.5)		6.7 (17.2)		5.1 (17.2)		3.4 (17.3)	
Stage 2	38.0 (20.5)		34.2 (20.3)		37.7 (20.3)		24.9 (20.8)	
LDL-cholesterol (mmol/l)	22.7 (5.9)	<0.001	19.0 (6.1)	0.002	20.5 (6.2)	0.001	19.0 (6.1)	0.002
HDL-cholesterol (mmol/l)	−59.4 (15.0)	<0.001	−49.9 (16.7)	0.003	−48.0 (16.8)	0.004	−49.4 (16.7)	0.003
Triglycerides (mmol/l)	8.3 (7.2)	0.25	−12.6 (8.1)	0.12	−12.1 (8.0)	0.13	−13.6 (8.0)	0.09
Current smoking	−7.0 (21.8)	0.75	6.5 (21.7)	0.76	11.6 (21.8)	0.60	6.6 (21.7)	0.76
Serum cystatin C (mg/l)		0.76				0.37		
Tertile 1 (<0.78)	0				0			
Tertile 2 (0.78–0.88)	−33.9 (18.5)				−37.9 (18.4)			
Tertile 3a (0.89–0.93)	−14.6 (25.5)				−24.3 (25.3)			
Tertile 3b (0.94–1.02)	7.3 (25.2)				−2.7 (25.4)			
Tertile 3c (≥1.02)	−29.6 (26.0)				−43.5 (25.8)			
Urinary albumin excretion (mg albumin/g creatinine)								
Normal (<30 mg/g)	0						0	
Microalbuminuria (30–300 mg/g)	52.9 (18.3)	0.004					37.5 (18.8)	0.047

[a]Adjusted for age, gender, smoking status, diabetes mellitus, blood pressure stages, LDL-cholesterol, HDL-cholesterol and triglycerides.
[b]Adjusted R^2 for the model that examined the prediction of age and gender on IMT.

($P = 0.004$), but not with cystatin C (P for trend = 0.76). In multivariate analyses adjusted for age, gender and traditional risk factors, the association between microalbuminuria and IMT remained ($P = 0.047$), while there was no association between cystatin C and IMT (P for trend = 0.37). The relationships between all traditional risk factors, microalbuminuria, cystatin C and IMT did not differ by gender (each interaction P-value >0.05). The overall prediction of traditional risk factors on IMT did not differ from models that included traditional risk factors, cystatin C and microalbuminuria (adjusted R^2 0.152 *vs* 0.157).

Measures of renal function and carotid IMT

Carotid IMT significantly increased with decreasing GFR (from 728 to 809 μm, P for trend = 0.002), but not with serum cystatin C and creatinine (Table 4). Microalbuminuria was associated with higher IMT (802 *vs* 732 μm, $P < 0.001$). In multivariate analysis adjusted for age, gender and traditional risk factors, the association between GFR and IMT disappeared (P for trend = 0.33). The associations between cystatin C, creatinine and IMT were further reduced (P for trend = 0.37 and 0.24, respectively). The association between microalbuminuria and IMT was reduced (regression coefficient reduced by about a third), but remained borderline significant ($P = 0.047$). The relationships between microalbuminuria and carotid IMT did not differ by age, gender, presence of hypertension or diabetes (each interaction P value >0.20). Using creatinine, GFR and cystatin C as continuous variables yielded similar results, with no statistically significant associations with IMT. In multivariate analyses, each SD increase of 22 μmol/l in creatinine was associated with an increase in IMT of 5 μm ($P = 0.53$), each SD decrease of GFR with an increase in IMT of 3 μm ($P = 0.71$) and each SD increase of 0.20 mg/l of cystatin C with a decrease in IMT of 5 μm ($P = 0.50$). In comparison, as shown in Table 4, presence *vs* absence of microalbuminuria was associated with an increase in IMT of 37 μm ($P = 0.047$).

Discussion

In this population-based study of middle-aged adults, microalbuminuria, but not cystatin C, was associated with carotid atherosclerosis independently of age and traditional cardiovascular risk factors. In contrast, the relationship between cystatin C and carotid atherosclerosis appeared to be largely confounded by age and other cardiovascular risk factors. We found no consistent evidence that serum cystatin C had a stronger relationship with carotid atherosclerosis in middle-aged adults than serum creatinine or GFR in this population that mostly included participants with mild or no renal dysfunction.

Previous prospective studies in the elderly have found that serum cystatin C was a stronger predictor of cardiovascular events and cardiovascular mortality than either serum creatinine or GFR [6,9], including in those with a GFR ≥ 60 ml/min/1.73 m^2 [24]. Our results on cystatin C and IMT could only be directly compared with one small cross-sectional study of 60 hypertensive Japanese patients (mean age: 58 years) that found a correlation between serum cystatin C levels and IMT ($r = 0.54$, $P < 0.001$) [25], but did not adjust for potential confounders and only presented Pearson correlation coefficients that have been shown to be very sensitive to outliers, yielding to potentially biased results [26]. Moreover, a prospective study in middle-aged adults did not confirm the strong association found in older adults [27]. In this study, the association between cystatin C and coronary event was borderline significant after adjustment for age and traditional risk factors (RR per SD: 1.19, 95%CI: 1.01–1.40) and no longer statistically significant after further adjustment for C-reactive protein (CRP) (RR: 1.13, 95%CI: 0.95–1.34). Several potential explanations could explain why the association between serum cystatin C and CVD might differ between middle-aged adults in our cross-sectional data and in the prospective study described above [27] and older adults in prospective studies [6,9]. First, most participants in our study population had relatively low levels of cystatin C, as compared with studies that included older adults [6] or large proportions of patients with renal dysfunction [28,29]. Those in the first two tertiles of cystatin had lower cystatin than participants aged ≥65 in the second quintile of cystatin in the Cardiovascular Health Study, and the risk of myocardial infarction did not significantly differ between the 1st and 2nd quintiles (adjusted hazard ratio: 0.97, 95%CI: 067–1.41) in this previous study [6]. Only 10 (2%) of our participants would belong to the 5th quintile of cystatin of this study. Similarly, serum creatinine levels in our sample were lower and GFR higher than in older adults, and only 24 participants (5%) had chronic kidney disease of at least stage 3 (GFR < 60 ml/min/1.73 m^2) [30]. Thus, our population-based results are, expectedly, not representative of adults with advanced renal disease or patients with a GFR < 60 ml/min/1.73 m^2 [30] who have an increased risk of CVD [7]. The fact that most participants of our study had a GFR ≥ 60 ml/min/1.73m^2 (95%) might explain why markers of glomerular filtration (serum creatinine and cystatin C) were not associated with carotid IMT. However, even those in the highest subtertile of tertile 3 of cystatin in our study population did not have an increased risk of carotid atherosclerosis in multivariate analyses. The levels of cystatin C in our population in the African region were also similar to levels found in an European prospective study that included men aged 50–59 years of the general population [27]. Secondly, in our population, cystatin C was strongly associated with some traditional risk factors, such as low density lipoprotein (LDL)-cholesterol and smoking status, that were not associated with cystatin C in an older population [6]. Moreover, a previous study has found that cystatin C

Table 4. Carotid intima-media thickness (IMT) in μm according to measures of renal function

	Unadjusted		Age-adjusted		Multivariate-adjusted[a]	
	Mean (95% CI), μm	P^b	Mean (95% CI), μm	P^b	Mean (95% CI), μm	P^b
Urinary albumin excretion (mg albumin/g creatinine)						
Normal (<30 mg/g)	732 (715–749)	<0.001	679 (657–700)	0.004	678 (656–699)	0.047
Microalbuminuria (30–300 mg/g)	802 (768–836)		731 (695–768)		715 (678–753)	
Serum cystatin C (mg/l)		0.10[c]		0.70[c]		0.37[c]
Tertile 1 (<0.78)	743 (716–769)		702 (675–728)		702 (675–728)	
Tertile 2 (0.78–0.88)	728 (700–755)		667 (637–697)		664 (634–693)	
Tertile 3a (0.89–0.93)	754 (709–800)		686 (640–732)		678 (632–723)	
Tertile 3b (0.94–1.02)	783 (739–828)		679 (658–700)		699 (653–745)	
Tertile 3c (≥1.02)	763 (718-808)		670 (622–718)		658 (611–706)	
Serum creatinine, μmol/l		0.16[c]		0.29[c]		0.24[c]
Tertile 1 (<72)	740 (714–767)		687 (659–715)		680 (651–710)	
Tertile 2 (73–85)	738 (711–765)		673 (644–703)		672 (643–701)	
Tertile 3a (86–92)	732 (684–780)		674 (626–721)		668 (621–716)	
Tertile 3b (93–101)	791 (747–835)		740 (697–784)		741 (697–785)	
Tertile 3c (≥102)	756 (710–801)		683 (637–729)		683 (635–732)	
Estimated GFR, ml/min/1.73 m^2		0.002[c]		0.13[c]		0.33[c]
Tertile 1 (≥97)	728 (702–754)		680 (652–708)		683 (656–711)	
Tertile 2 (79–96)	736 (710–762)		690 (663–718)		684 (656–712)	
Tertile 3a (73–78)	758 (715–802)		690 (645–736)		681 (635–726)	
Tertile 3b (67–72)	766 (718–814)		692 (643–742)		687 (638–737)	
Tertile 3c (<67)	809 (761–857)		725 (675–776)		711 (661–761)	

[a]Adjusted for age, gender, smoking status, diabetes mellitus, blood pressure stages, LDL-cholesterol, HDL-cholesterol and triglycerides.
[b]Chi-square tests for trend for multilevel categorical variables and statistical test from the linear regression model for other variables.
[c]Chi-square tests for trend.

was influenced by several factors other than renal function alone, such as BMI and smoking [31]. It should be noted that cystatin C was not independently associated with IMT in our study, although the participants, despite being truly representative of the general population, were at relatively high cardiovascular risk with a high prevalence of cardiovascular risk factors. Another explanation for these age differences might be differences in study designs (cross-sectional *vs* prospective data).

Despite some conflicting results [32], several cross-sectional studies found that microalbuminuria was associated with higher IMT [14,33,34], similar to our data. However, previous studies were limited by the focus on high-risk adults in selected settings, such as hypertensive [33] or diabetic subjects [34], or by the lack of adjustment for diabetes [14]. One study in the MESA (the Multi-Ethnic Study of Atherosclerosis) cohort found an association between microalbuminuria and carotid IMT in unadjusted analyses (mean ± SE: 900 ± 10 μm in those with microalbuminuria *vs* 850 ± 3 μm in those without, $P < 0.001$), but this association was no longer significant in multivariate analyses [32]. However, microalbuminuria was positively associated with other markers of subclinical cardiovascular disease, such as coronary artery calcification and left ventricular mass. One potential explanation for the differences between our results and the results in the MESA cohort might be the older age of the population in MESA (mean age 63 years, range 45–85 years). Another study found an association between microalbuminuria and carotid IMT in unadjusted analyses, which remained significant in multivariate analyses in diabetics, but not in non-diabetic participants [35], while the relationship between microalbuminuria and carotid IMT did not differ by presence of diabetes in our study population. Potential explanations for the differences with our data might be the unusual way to measure IMT (only left carotid artery measured) and the likely inclusion of mostly White subjects, while our study includes predominantly persons of African descent.

The mechanisms by which microalbuminuria increases cardiovascular risk are still uncertain. Traditional risk factors [36], inflammatory markers [36] and endothelial dysfunction [37] may contribute to increased cardiovascular risk in subjects with microalbuminuria.

Our study has several limitations. As the study was cross-sectional, we cannot determine the direction of the association between renal markers and carotid atherosclerosis. For example, it has been suggested that microalbuminuria might just be a marker of generalized vascular dysfunction [38], and the substantial attenuation of the magnitude of the association between microalbuminuria and carotid IMT in our study when adjusting for risk factors, such as hypertension and diabetes, may be consistent with such a mechanism. Although microalbuminuria was independently associated with carotid IMT, the magnitude of the associated risks for future CVD would likely be low. In the Cardiovascular Health Study, a difference of 200 μm in IMT was associated with a 27% increased risk of myocardial infarction [39]. In our study, the IMT difference associated with microalbuminuria was 70 μm, which would

correspond to a 9% increase in cardiovascular risk. As our measurement of microalbuminuria was semi-quantitative and dichotomized, we could not examine whether higher levels of microalbuminuria would be linearly associated with higher IMT. We also had limited power to perform subgroup analyses, because of the relatively small sample size. Strengths of our study sample were its population-based design with a high participation rate, which allows inferring findings to the general population of middle-aged adults, and the availability of all traditional cardiovascular risk factors, which allowed adjusting for several potential confounding variables in our analysis. Also, few studies had previously assessed the relationship between cystatin C and microalbuminuria and carotid IMT in persons of African descent, less so in populations in the African continent.

In summary, microalbuminuria is associated with carotid atherosclerosis, as assessed by IMT, beyond traditional cardiovascular risk factors among middle-aged adults, but not cystatin C. The absence of an association in this population-based cross-sectional study of middle-aged adults and an association found in prospective studies among older adults suggests that the relationship between markers of renal function and cardiovascular disease might differ between middle-aged and older adults and/or depending of the target population. Before using cystatin C for cardiovascular risk stratification in clinical practice, the relationships between cystatin C, microalbuminuria and CVD should be further examined in prospective studies that include middle-aged adults.

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