Original Article

Assessment of subclinical atherosclerosis and endothelial dysfunction in chronic kidney disease by measurement of carotid intima media thickness and flow-mediated vasodilatation in North Indian population

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Abstract

Background: Chronic kidney disease (CKD) predisposes to accelerated atherosclerosis that is measured by carotid artery intima media thickness (CIMT) and brachial artery flow-mediated dilation (FMD). The aim of this study was to assess the noninvasive risk markers of subclinical atherosclerosis and endothelial dysfunction and their correlation with disease severity. **Methods and Results:** This was a cross-sectional study conducted in 62 patients with CKD: 38 predialysis and 24 on hemodialysis and 50 age- and gender-matched controls. In both the patients and controls, high-sensitivity C-reactive protein (CRP) levels, %FMD, and CIMT were measured. Patients with CKD had increased CRP levels {[5.8 (1.0–6.0)] mg/L vs [1.0 (0.5–2.20)] mg/L; $P < 0.001$}; %FMD was significantly lower in patients on hemodialysis (5.51%) compared with stage IV (7.62%) and stage III (15.02%) and 17.95% in control subjects ($P < 0.001$); and CIMT values in hemodialysis patients (0.88 ± 0.06 mm) were significantly higher compared with stage IV (0.67 ± 0.10) and stage III (0.61 ± 0.12) ($P < 0.001$). Increased CIMT values were seen in patients with CKD (0.82 ± 0.21 mm) than in the healthy controls (0.55 ± 0.16 mm). In patients with CKD, a significant negative correlation was found between CRP levels and FMD responses ($r = –0.315$; $P < 0.001$), while a significant positive correlation was found between CRP and CIMT values ($r = 0.327$; $P < 0.001$). Compared with predialysis, hemodialysis subjects had significantly lower FMD and higher CRP and IMT values. **Conclusion:** CKD confers a higher inflammatory status when compared with apparently healthy general population. Abnormal FMD responses and CIMT values are more commonly found in dialysis patients. Our findings suggest that CIMT and FMD can be used as noninvasive markers for early risk assessment and stratification in various stages of CKD.

Keywords: Cardiovascular risk, chronic kidney disease, noninvasive risk markers, subclinical atherosclerosis

Introduction

The incidence of cardiovascular disease (CVD), endothelial dysfunction, and oxidative risks is increased in patients with chronic kidney disease (CKD) when compared with the general population with normal renal function.[1,2] CVD is one of the leading cause of morbidity and mortality in patients with CKD.[2] Traditional risk factors of CVD such as hypertension and dyslipidemia are unable to fully explain the increased rate of cardiovascular event reported in patients with CKD. Nontraditional risk factors, such as anemia, hyperhomocystinemia, abnormal calcium and phosphate metabolism, oxidative stress, and inflammation,[3] have been postulated as potential risk

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factors in these patients. Low-grade systemic inflammation may be associated with adverse outcomes including cardiovascular events,[4] but the mechanisms relating inflammation and adverse outcomes have not been fully elucidated.[3] The relationship between endothelial dysfunction and the various stages of CKD has still not been understood. The aim of our study was to assess the noninvasive risk markers of subclinical atherosclerosis and endothelial dysfunction and to find their correlation with CKD severity.

Methods

This was a cross-sectional study conducted in the Department of Medicine, King George Medical University, Lucknow, for a period of 1 year from August 2017 to July 2018 in patients with CKD. After informed written consent and ethical clearance from institutional ethics committee were obtained, 112 subjects were enrolled for the study. Overall, 62 cases of CKD stages III–V and 50 age-matched healthy controls seeking routine health screening were enrolled. Inclusion criteria were the presence of CKD stages III–V according to the KDIGO guidelines 2012. The Modification of Diet in Renal Disease equation was used to calculate the estimated glomerular filtration rate (eGFR). The staging criteria for CKD were defined for stage II as renal damage with eGFR of 60–89 mL/min/1.73 m^2, stage III with eGFR of 30–59 mL/min/1.73 m^2, and stage IV with eGFR of 15–29 mL/min/1.73 m^2. Those with CKD stage I and II, irregular hemodialysis patients, coronary artery disease (CAD), malignancy, peripheral vascular disease, bilateral arteriovenous fistulae, and autoimmune disease were excluded from our study.

After enrollment, all patients were subjected to full clinical evaluation including medical history and clinical examination. About 5 mL of venous blood was obtained in a sterilized vial from the cases and controls for biochemical analysis. The blood sample was centrifuged at 1500 rpm for 10 min to separate the serum, and routine hematology, biochemistry, urinalysis, and urine protein measurements were performed in accordance with study protocols. An automated blood cell analyzer (BC 5380; Mindray, China) was used for routine hematology testing, and an automated clinical biochemistry analyzer (Cobas C 311; Roche Hitachi, Japan) was used for blood urea nitrogen, serum creatinine, serum uric acid, serum lipids, electrolytes, C-reactive protein (CRP), and albumin. CRP measurements were performed using CRP-Latex assay (analytical range 2–160 mg/L).

Patients and controls underwent carotid intima media thickness (CIMT) measurements, as a surrogate marker of subclinical atherosclerosis, and brachial artery flow-mediated dilation (FMD) measurements, to assess endothelial function. A standardized questionnaire was used in every subject to obtain systematic information regarding conventional cardiovascular risk factors, including hyperlipidemia, hypertension, diabetes, and family history of CVD. The subjects were investigated in our vascular laboratory, in a quiet purpose-built room maintained at a constant temperature of 22°C–24°C; after 10 min of rest in the supine position; after 12 h of overnight fasting. Patients and controls were asked not to take cardiac medications which could interfere with endothelial function, 24 h prior to the study.

Carotid Doppler ultrasonography (USG) was performed by a single operator expert ultrasonologists, and CIMT was measured in the Department of Radiology; the operator was blinded about the history and laboratory findings of patients. CIMT was defined as a hypoechogenic space between two echogenic lines containing intima media interface and media–adventitia interface on the posterior wall of the carotid artery. For performing carotid Doppler USG, the patient was asked to lie down on the examination table in the supine position. His or her neck was rotated in a superior and leftward direction. Following this, using a 7.5-MHz linear array transducer with high-resolution B-mode USG system (GE Logiq; Toshiba Xario, Japan), the length and site of bifurcation of common carotid artery were determined, posterior wall was exposed, and CIMT was measured.

Brachial artery FMD was performed according to the American College of Cardiology guidelines.[5] The brachial artery was scanned 5–15 cm above the antecubital fossa. Resting diameter was measured, and then a blood pressure cuff was inflated around the arm to at least 50 mmHg above systolic blood pressure for 4.5 min. A measurement of maximum diameter was taken 45–60 s after cuff release. Distance was measured from the anterior to the posterior M lines (media–adventitia interface). USG machine with high-resolution (B) scan 7.5 Hz linear accelerator was used to assess brachial artery diameter and its changes.

$$\text{FMD} = [(\text{postdeflation diameter} - \text{resting diameter})/\text{resting diameter}] \times 100$$

OR

$$\text{FMD} = \frac{d2 - d1}{d1} \times 100$$

where $d2$ = brachial artery diameter at 5 min postdeflation

$d1$ = baseline brachial artery diameter.

Statistical analysis

The continuous data were summarized using descriptive statistics [mean ± standard deviation (SD)]. Statistical differences between the mean values were compared using Student's *t*-test. A difference between the two values was considered to be significant if the *P* value was <0.05. The association between two or more categorical variables was tested by χ^2 statistics using appropriate correction. Prior to carrying out any test on continuous data, the normalcy of data was tested. The two-sample *t*-test was used to see the difference between the mean of two different groups if data were normally distributed. If data were not found to be normally distributed, Mann–Whitney *U*-test was used to test the level of significance between two values. One-way

analysis of variance was used to test the difference among two groups in case of normally distributed data. All calculations were done using STATA IC13 and MedCalc Version 17.5.5 software.

Results

The mean age of subjects with CKD was 40.58 ± 15.23 years and of control was 37.88 ± 11.84 years. Of the 62 subjects with CKD, the majority were stage V CKD (38.7%) followed by stage IV (32.3%) and the remaining 18 (29.0%) were stage III CKD. Among the 50 normal healthy controls, 32 (64.0%) were male and 18 (36.0%) were female. Subjects with CKD and controls were age- and gender-matched [Tables 1 and 2].

The body mass index was not different between the two groups [Table 1]. The three CKD patient cohorts had comparable baseline clinical characteristics and medical treatment at enrollment. About 85% of patients had a history of hypertension, 16% had type 2 diabetes mellitus, and 48% had hypercholesterolemia [Table 2].

In comparison to control, patients with CKD had higher levels of CRP, increased CIMT, and poorer endothelial function (FMD). Overall CRP level was found to be significantly higher [5.8 (1.0–6.0)] in comparison to control group [1.0 (0.5–2.20)] [Table 1, Figures 1 and 2].

Table 1: Baseline characteristics, brachial artery flow-mediated dilatation, and carotid intima media thickness in patients and controls

Parameter	Patients with CKD (*n*=62)	Controls (*n*=50)	*P*
Age (years)	40.58±15.23	37.88±11.84	0.012
Gender			
Male (%)	30 (48.38)	32 (64)	0.65
Female (%)	22 (35.48)	18 (36)	0.52
BMI (kg/m^2)	25.5±4.7	25.4±5.2	0.84
C-reactive protein (mg/L)	5.8 (1.0-6.0)	1.0 (0.5-2.20)	<0.001
Flow-mediated dilatation (%)	8.95±4.10	17.96±1.13	<0.001
Intima media thickness (mm)	0.82±0.21	0.55±0.16	<0.001

CKD: chronic kidney disease; BMI: body mass index

In patients with CKD, FMD and CIMT values had no relationship with total cholesterol. CIMT measurements had a negative correlation with FMD (Spearman's rho = −0.805, $P < 0.05$) [Table 3, Figure 3]. Among patients with CKD, %FMD was significantly lower in stage V CKD on hemodialysis (5.51%) when compared with stage IV (7.62%) and stage III (15.02%) and 17.95% in control subjects ($P < 0.001$) [Table 2, Figure 1].

CRP levels showed a significant negative correlation with %FMD (Spearman's rho = −0.31, $P < 0.001$) [Table 3, Figure 4] and a significant positive correlation with CIMT values (Spearman's rho = 0.327, $P < 0.001$) in patients with CKD [Table 3, Figure 5].

Discussion

Our study highlights the highly abnormal endothelial functioning and CIMT in patients with CKD and underscores the importance of early detection of subclinical atherosclerosis through noninvasive markers, thereby reducing the CV burden in CKD population.

CIMT is a noninvasive marker of generalized atherosclerosis and is a good indicator of the presence of CAD.[6,7] In addition to traditional risk factors such as age, obesity, hypertension, hyperglycemia, and hyperlipidemia, uremia-related risk factors such as hemodynamic overload, anemia, and malnutrition have been proven as the causative factors for accelerated atherosclerosis in patients with CKD.[8]

Our study also establishes the presence of low-grade systemic inflammation in patients with CKD. Patients with worse endothelial function had the highest CIMT. In our study, significantly increased CIMT values were found in all the stages of CKD, when compared with healthy controls. The mean CIMT in stage III was 0.61 ± 0.12, in stage IV was 0.67 ± 0.10, and in dialysis patient was 0.88 ± 0.06 in comparison to control. Corroborating the finding of our study are the studies by Szeto *et al.*[9] and Khandelwal,[10] which show that the mean CIMT was

Table 2: Baseline characteristics and biochemical parameter of patients with CKD

Parameter	CKD stage III (*n*=18)	CKD stage IV (*n*=24)	Hemodialysis (*n*=20)	*P*
Age (years)	39±12	42±11	40±12	*P*<0.01
Gender M (%)	12 (66.7)	20 (83.3)	16 (80%)	*P*<0.001
BMI (kg/m^2)	25.1±4.8	25.4±5.2	24.5±3.2	*P*<0.001
Hypertension (%)	14 (77.8%)	21 (87.5%)	18 (90%)	*P*<0.001
Diabetes mellitus (%)	2 (11.1)	3 (12.5%)	5 (25.0%)	*P*<0.01
Dyslipidemia	8 (44.5)	10 (41.7)	12 (60.0)	*P*<0.05
CRP (mg/L)	5.56±2.92	5.74±2.86	5.94±2.76	*P*<0.05
S. cholesterol (mg/dL)	152.11±25.71	166.8±62.19	202±47.41	*P*<0.01
S. triglyceride (mg/dL)	98.78±16.16	170.05±68.94	189.08±77.64	*P*<0.001
S. LDL (mg/dL)	86.28±14.07	90.7±22.37	130.49±50.21	*P*<0.001
S. HDL (mg/dL)	40.67±4.37	42.8±12.42	46.84±17.27	*P*<0.01
CIMT (mm)	0.61±0.12	0.67±0.10	0.88±0.06	*P*<0.001
%FMD	15.02±1.26	7.62±0.71	5.51±0.58	*P*<0.001

CKD: chronic kidney disease; BMI: body mass index; CRP: C-reactive protein; LDL: low-density lipoprotein; HDL: high-density lipoprotein; CIMT: carotid intima media thickness; FMD: flow-mediated dilation

higher in CKD. Zhang *et al.*,[11] in their study comparing stage II and III CKD patients, found significantly increased CIMT in these patients and concluded that subclinical arterial wall changes might occur earlier in course of CKD than previously thought.

It was observed that subjects with CKD had FMD values significantly lower when compared with that in controls, thereby indicating a high order of endothelial dysfunction. Our study results are in accordance to Recio-Mayoral *et al.*[12] who observed that patients with CKD had reduced FMD values when compared with controls. Similar findings were found in studies done by other authors.[10,13-16]

Increased CIMT or decreased FMD are the consequences of low-grade systemic inflammation and endothelial dysfunction. These phenomenon are seen in other chronic inflammatory disease such as rheumatoid arthritis[17] and HIV.[18]

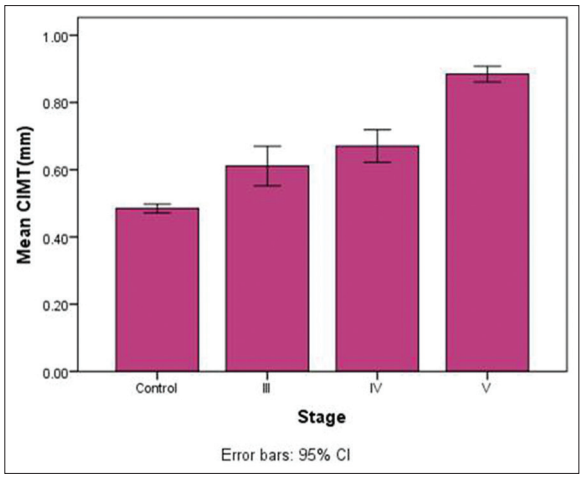

Figure 1: CIMT in patients was significantly higher when compared with controls ($P < 0.001$)

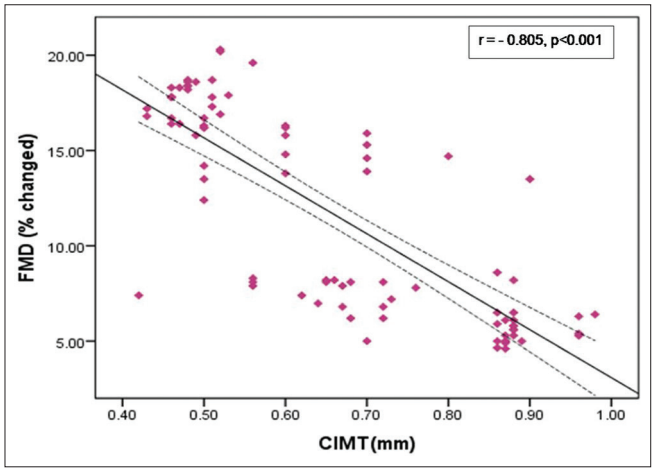

Figure 3: Scatter plot showing the correlation (i.e. linear regression) between CIMT and FMD (% changed) in patients. Significant negative correlation is observed between CIMT and FMD (% changed). The solid line represents the point-estimated value and the dotted lines represent 95% confidence intervals

CRP is a well-recognized risk factor of systemic inflammation and CVD and mortality in the general population as well as in

Table 3: Correlation of major contributing factors with carotid intima media thickness and flow-mediated dilatation (%) in patients with CKD

Variable	CIMT		FMD	
	Correlation (*r*)	*P*	Correlation (*r*)	*P*
Age	0.436	0.005	−0.479	0.002
BMI	−0.828	0.866	−0.078	0.682
CRP	0.327	0.001	−0.315	0.001
Cholesterol	−0.013	0.939	−0.256	0.110
Triglyceride	0.37	0.820	−0.253	0.115
HDL	−0.301	0.059	0.122	0.456
LDL	0.345	0.029	−0.385	0.014
Mean CIMT	1	-	−0.805	0.050
Brachial FMD %	−0.805	0.050	1	-

CKD: chronic kidney disease; CIMT: carotid intima media thickness; FMD: flow-mediated dilation; BMI: body mass index; CRP: C-reactive protein; LDL: low-density lipoprotein; HDL: high-density lipoprotein

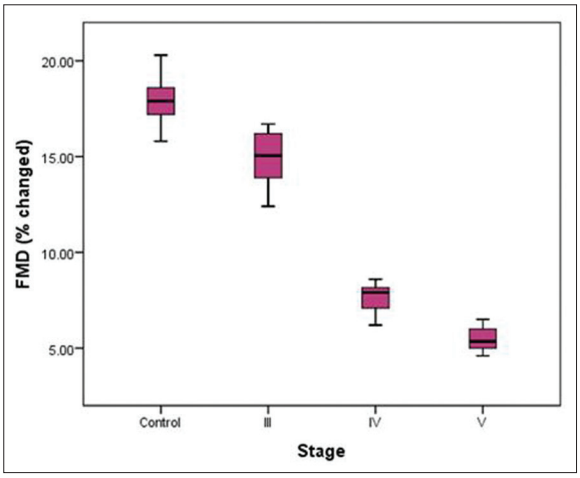

Figure 2: Showing Flow mediated dilatation (% change) in three cohorts of CKD patients and controls

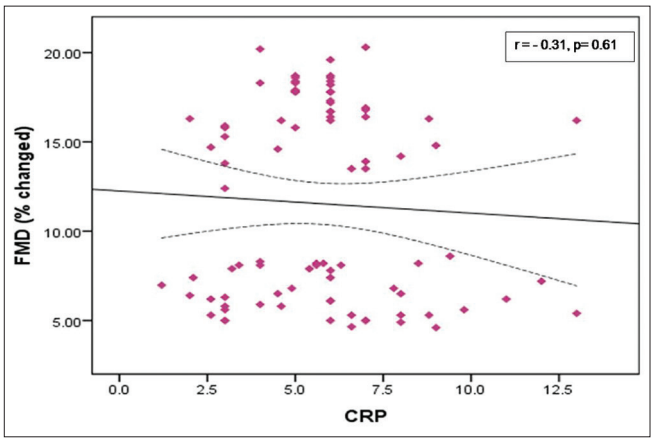

Figure 4: Scatter plot showing the correlation (i.e. linear regression) between CRP and FMD (% changed) in patients. Negative correlation is observed between CRP and FMD (% changed). The solid line represents the point-estimated value and the dotted lines represent 95% confidence intervals

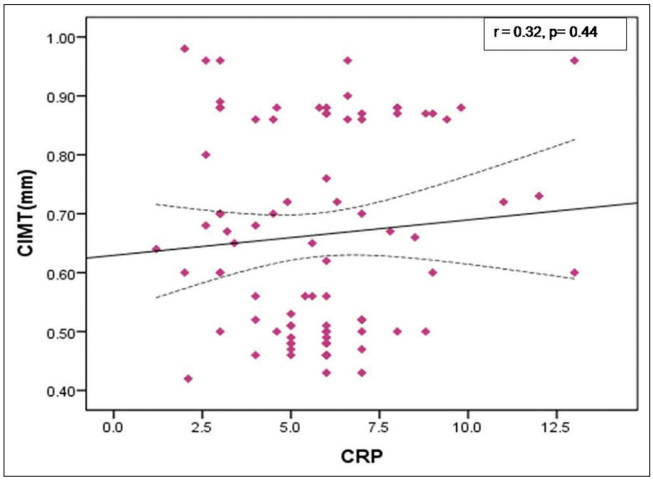

Figure 5: Scatter plot showing the correlation (i.e. linear regression) between CRP and CIMT in patients. Positive correlation is observed between CRP and CIMT. The solid line represents the point-estimated value and the dotted lines represent 95% confidence intervals

patients with end-stage renal disease. The average values of CRP were higher in this study (5.8 ± 2.76 mg/L) compared with earlier studies by Ortega *et al.*[19] (8.3 ± 14.2 mg/L) and Menon *et al.*[20] (2.2 mg/L) in predialysis patients. High CRP levels were seen in predialysis patients and the prevalence was found to be similar as reported by Owen and Lowrie in a dialysis population.[21] This highlights the fact that patients with CKD, even in predialysis stage, show signs of inflammation.

The result from our study showed that significant abnormality of the endothelial function is observed in patients with predialysis CKD subjects that the pathogenesis of cardiovascular damage starts in patients with CKD long before they had upon maintenance dialysis. This underscores the importance of early aggressive interventions targeted at preventing the devastating effects of CVD.

The limitation of our study was that we had not assessed endothelial-independent vasodilatation. Hence, we were unable to differentiate the nonendothelial vasodilatory impairments in the study subjects.

Conclusion

In patients with established CKD, prevention of CVD altogether is ideal but difficult. However, early recognition of atherosclerosis in CKD can help combat the excessive mortality due to CVD. Carotid IMT and FMD have emerged as potential noninvasive useful markers to detect early changes associated with atherosclerosis. These are easily interpreted and can help family physicians tackle the problem of CV burden in CKD at an earlier stage than before.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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