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Original Article

Systolic and Diastolic Epicardial Adipose Tissue Thickness in Non-Dialysis Dependent Chronic Kidney Disease Patients: Technique, Correlates and Cardiovascular Outcomes (The EAT CKD Study)

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ABSTRACT

Background: Epicardial adipose tissue (EAT) has been related to increased cardiovascular risk in chronic kidney disease patients. However, prospective studies of EAT thickness in prediction of cardiovascular events in CKD patients are lacking. Moreover, there are inconsistencies in literature regarding cut-off of EAT thickness, standard technique and phase of measurement. **Objectives:** This study was undertaken to compare systolic and diastolic EAT thickness in prediction of CV events in non-dialysis dependent CKD patients. **Methods:** In this prospective, observational study, transthoracic echocardiography (TTE) was used to assess systolic and diastolic EAT thickness in 210 consecutive non-dialysis dependent CKD patients and followed up for at least one year for pre-defined end-points. **Results:** The mean systolic and diastolic EAT thickness in the CKD group (5.6±1.2mm and 4.2±1.1mm) was significantly higher than the non-CKD participants (4.3±1.0mm and 3.1±1.1mm), both *P*<0.001. Interclass correlation coefficient (ICC) agreement on measurements were 0.93 (95% CI: 0.79-0.98) for systolic EAT and 0.91 (95% CI: 0.74-0.97) for diastolic EAT. On multivariate linear regression analysis, only e-GFR remained as independent predictor of both systolic and diastolic EAT thickness. Receiver operating characteristics (ROC) analysis showed that diastolic EAT thickness of 5mm and systolic EAT thickness of 3.8mm had similar sensitivity (88% versus 87%, respectively) and specificity (72% versus 74%, respectively) to predict CV events in CKD patients. **Conclusion:** Both systolic and diastolic EAT thickness are significantly increased in CKD patients and can be used in CV risk stratification with similar sensitivity and specificity albeit with different cut-offs.
Key words: Coronary artery disease, CV risk, Echocardiography, Epicardial fat, Reproducibility.

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INTRODUCTION

Chronic kidney disease (CKD) is a major public health problem, defined as kidney damage (structural or functional abnormalities of the kidney) or estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 for 3 months or more, irrespective of cause.[1] The prevalence of CKD has reached up to 20% both in India[2-4] and worldwide.[5] Diabetes (DM) and hypertension (HTN) are the major risk factors and most common co-morbidities in CKD, accounting for 40–60% cases.[2-5] With the epidemic rise of DM, HTN and obesity in general population, the prevalence of CKD is expected to rise further. CKD is an independent risk factor for cardiovascular disease (CVD).[6-8] Even the early stage of CKD is associated with a worse cardiovascular prognosis.[9-10] The cardiovascular mortality in CKD is responsible for 50% of total deaths and is up to 20 times more than the general population.[11] Given the exceedingly high risk of cardiovascular disease in patients with CKD,[12] newer markers of cardiovascular risk with potential therapeutic role is being explored in CKD patients.

Epicardial adipose tissue (EAT) is emerging as a novel risk factor for cardiovascular disease development in wide variety of clinical settings. EAT is a visceral adipose tissue located within the pericardial sac with close proximity to the coronary arteries. EAT accumulation is strongly associated with the severity and development of CAD and is an effective biomarker for the prediction of CAD.[13,14] Though there is some evidence of correlation of EAT with CVD development in ESRD,[15-17] such data in non-dialysis dependent CKD patients is limited.

EAT thickness can be measured by transthoracic echocardiography (TTE), cardiac computed tomography (CT) and cardiac magnetic resonance imaging (MRI). Transthoracic echocardiography is the most commonly used modality because of easy availability, ease of use, low cost, no radiation exposure, fastness and reproducibility.[18] However, there is no consensus over the echocardiographic technique for EAT measurement.[18-20] Opinion is divided over end-systolic[21] versus end-diastolic phase of measurement.[22-25] Currently, there is no study of direct comparison between systolic and diastolic measurement. Moreover, there are inconsistencies in the literature regarding cut-off of EAT thickness.[18]

Consequently, this study was undertaken to compare systolic and diastolic EAT thickness in prediction of CV events in non-dialysis dependent CKD patients from India. To best our knowledge this is the first prospective study relating to cardiovascular risk prediction by EAT thickness in CKD patients using echocardiography. Moreover, this is the first study of direct comparison between systolic and diastolic EAT thickness in CV risk prediction.

METHODS

Patients

This was a prospective observational study done in medicine and nephrology departments of a tertiary care hospital from Aug 2016 to July 2018. Patients aged 18 years or above and diagnosed with CKD as laid down in Kidney Disease: Improving Global Outcomes (KDIGO) guidelines were included in study.[2] Patients <18 years of age, with end-stage renal disease (ESRD), on dialysis, acute renal failure or prior cardiovascular disease were excluded. Additionally, one hundred age and sex matched non-CKD individuals were taken as control. Written informed consent was obtained from all the participants. The study was performed

in accordance with good medical and laboratory practices and the ethical guidelines of the Declaration of Helsinki.

EAT measurement

EAT was measured using Esaote Mylab 50 Xvision with a 5 MHz transducer and digitally recorded. In left lateral decubitus position, optimal parasternal long-axis view (PLAX) and short-axis view (PSAX) were obtained according to the recommendations of American Society of Echocardiography. EAT is defined as a hypoechoic space anterior to the right ventricular wall and its thickness is measured between the epicardial surface and the parietal pericardium. It should be differentiated from the adjacent pericardial fat. Taking the aortic root as the reference in PLAX view and interventricular septum as reference in PSAX view, EAT was measured perpendicular to the RV free wall (Figure 1). Imaging constraints were used to ensure that the epicardial fat thickness is not measured obliquely. Enhanced depth setting and magnified views were used to assess EAT thickness more clearly (Figure 2). Additionally M-mode echocardiography was done in each patient for greater accuracy in measurements owing to significantly higher resolution than 2-D mode. M-mode was taken at the point of maximum thickness of EAT perpendicular to the right ventricular wall in both PLAX and PSAX views (Figure 3). Diastolic EAT was measured at peak of R wave in ECG and systolic EAT was measured at end of T wave (Figure 3). Maximum value of EAT obtained by above techniques were recorded each time and average value of 3 cardiac cycles was recorded. Parasternal long axis and short-axis measurements were averaged to obtain the final mean thickness. This procedure was followed in all the participants to obtain EAT thickness in end-systole and end-diastole separately.

Study protocol and end-points

Enrolled patients underwent detailed clinical evaluation, anthropometric examination and transthoracic echocardiography. Blood samples were obtained if required to ensure blood urea, serum creatinine, blood sugar and lipid profile as bare minimum. Proteinuria was directly measured by 24-hr urine samples and the glomerular filtration rate (eGFR) was estimated by the simplified Modification of Diet in Renal Disease (MDRD) equation. All the patients in CKD group were followed up for at least one year. Data from patients lost from OPD follow up were collected by telephonic enquiry. Primary endpoint was occurrence of fatal and non-fatal CV events at one year of follow up. Co-primary endpoint was all-cause mortality. Secondary end-point was CKD progression or occurrence of ERSD.

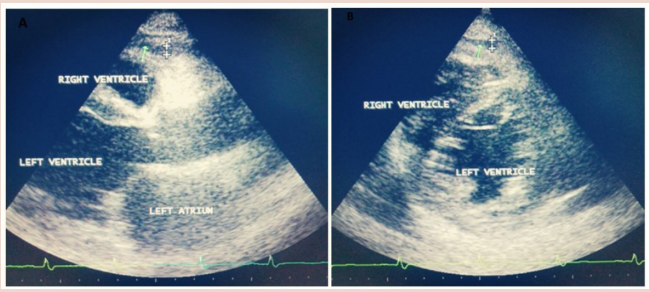

Figure 1: Transthoracic echocardiographic views for measurement of epicardial adipose tissue. Measurement is done in both parasternal long axis view and short axis view and averaged. Epicardial adipose tissue is an echo-lucent area between epicardial surface and parietal pericardium in front of the right ventricular free wall (pointed by an arrow). A) Parasternal long axis view: measurement is done over the right ventricular free wall taking aortic root as reference. Vertical length between the right ventricular free wall and parietal pericardium is measured. B) Parasternal short axis view at mid-ventricular level: measurement is done perpendicular to right ventricular free wall taking interventricular septum (IVS) as reference, usually 2cm away from IVS. Increased depth setting and zoomed views should be used to increase sensitivity of measurements.

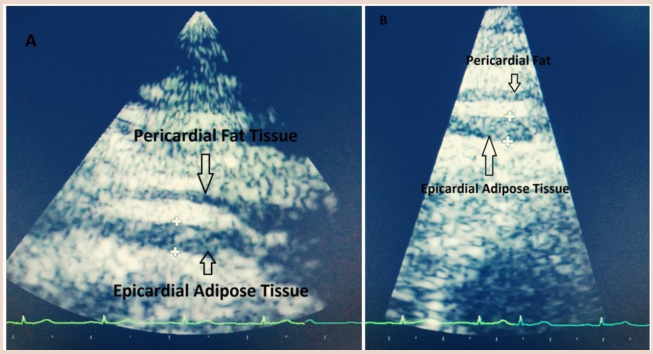

Figure 2: Epicardial adipose tissue (EAT) can be visualised more clearly by A) Enhanced depth setting; and B) Magnified view.

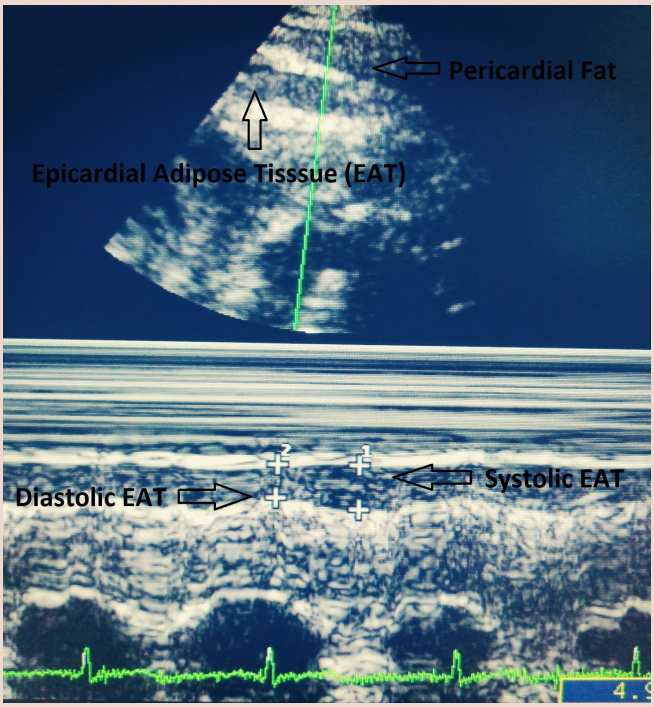

Figure 3: M-mode Echocardiogram in parasternal long axis view with increased depth setting. Epicardial adipose tissue (EAT) is identified as the hypoechoic area between the epicardial surface and parietal pericardium. It should be differentiated from the adjacent pericardial fat. M-mode is taken at the point of maximum thickness of EAT perpendicular to the right ventricular wall and parallel to the aortic annulus. Diastolic EAT is measured at peak of R wave in ECG and systolic EAT is measured at end of T wave. Diastolic EAT is inherently smaller than systolic EAT.

Definitions

CKD was defined as kidney damage (structural or functional abnormalities of the kidney) or estimated glomerular filtration rate (eGFR) <60 mL/

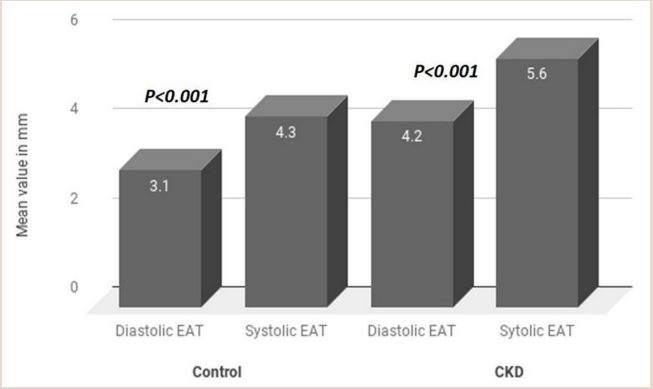

Figure 4: Mean systolic and diastolic epicardial adipose tissue (EAT) thickness in control and CKD group.

min/1.73 m^2 for 3 months or more, irrespective of cause.[2] Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg. Obesity was defined as body mass index (BMI) >25 kg/m^2 and hyperlipidemia was defined as plasma cholesterol levels ≥240 mg/dL or plasma low-density lipoprotein (LDL) levels ≥160 mg/dL or plasma triglyceride levels ≥150 mg/dL. Abnormal proteinuria was defined as protein excretion >300 mg/24 hrs. Current smoking was defined as any cigarette consumption in the last month. CKD progression was defined as 50% decline in eGFR from baseline or occurrence of ESRD (end-stage renal disease). CV events were defined as composite of stroke, myocardial infarction, angina, heart failure hospitalisation and cardiovascular death. Cardiovascular deaths included deaths due to acute myocardial infarction, congestive heart failure and stroke.

Statistical analysis

The data were analyzed using GraphPad Prism 7, version 7.04 (GraphPad Software, Inc.). Continuous variables were presented as means and SDs and categorical variables were expressed as frequencies and percentages. The *P* value for comparing two dependent continuous variables was from paired Student *t* test and two independent continuous variables were from unpaired Student *t* test. Comparison of two proportions was done by the chi-square test or Fisher exact test. Spearman and Pearson's correlation coefficient was used to calculate the correlation between EAT and patient related variables. The receiver operating characteristic (ROC) curve analysis was used to find the cut-off value of epicardial fat thickness for predicting CV events. The sensitivity and specificity were calculated. Comparison of ROC curves to test the difference between the areas under two dependent ROC curves was done with the method of DeLong *et al.*[26] Multivariate linear regression analysis was used to define independent predictors of systolic and diastolic EAT thickness among confounding variables such as age, blood pressure, BMI, fasting blood sugar, proteinuria, LDL, HDL, triglyceride and e-GFR.[27] Interclass correlation coefficient (ICC) was calculated evaluate the reliability of the systolic and diastolic EAT measurements. ICC > 0.75 was considered as excellent, 0.4 to 0.75 as good and ICC < 0.4 as poor. All tests were 2-sided and statistical significance was defined as $P < 0.05$.

RESULTS

210 consecutive non-dialysis dependent CKD patients were enrolled in the study. One hundred age and gender matched non-CKD individuals were taken as control. ICC was used to assess interobserver reproducibility of systolic and diastolic EAT measurements by echocardiography

Table 1: Patient characteristics

	CKD group (n=210)	Control (n=100)	*P*-value
Age (years)*	53.9±10	52±11	0.13
Male sex	163 (77.6)	79(79)	0.78
Obesity	19(9)	23(23)	<0.001
Diabetes	97(28.8)	21(21)	<0.001
Hypertension	130 (61.6)	29(29)	<0.001
Hyperlipidemia	81 (38.5)	21(21)	<0.001
Current smoker	94(44.7)	41(41)	0.53
Proteinuria	115(54.7)	8(8)	<0.001
eGFR (ml/min/1.73 m^2)*	46±7	74±9	<0.001
EAT thickness (mm)*			
Systolic	5.6±1.2	4.3±1.0	<0.001
Diastolic	4.2±1.1	3.1±1.1	<0.001

Table 2: Mean EAT thickness in CKD according to patient characteristics

	Systolic EAT	*P*-value	Diastolic EAT	*P*-value
Age		0.04		0.03
<60 years	5.4±1.1		4.4±1.1	
≥ 60 years	5.7 ±1.0		4.7±1.0	
Gender		0.30		0.23
Male	5.7±1.2		4.6±1.2	
Female	5.5±1.1		4.4±1.0	
Body mass index (Kg/m^2)		<0.001		<0.001
<25	5.4±0.9		4.2±1.1	
≥ 25	5.9±1.1		4.8±0.9	
Diabetes	<0.001			<0.001
Absent	5.2±0.9		4.0±1.3	
Present	6.0±1.1		4.9±0.9	
Hypertension		0.21		0.15
Absent	5.5±1.0		4.3±1.1	
Present	5.7±1.2		4.6±1.0	
Hyperlipidemia		0.001		<0.001
Absent	5.1±0.8		4.1±1.2	
Present	6.1±0.9		5.1±1.0	
Current smoker		0.001		<0.001
No	5.0±0.9		4.1±1.1	
Yes	5.9±1.0		5.0±0.9	
Proteinuria		0.01		<0.001
Absent	5.3±1.2		4.0±1.2	
Present	5.7±1.1		4.7±1.1	
eGFR (ml/min/1.73 m^2)		0.001		<0.001
≥45	4.9±0.8		3.8±0.9	
<45	6.2±1.2		5.2±0.8	

Table 3: Correlation of systolic EAT thickness with different variables

	Control (Non-CKD group)		CKD group	
	r	*P*-value	r	*P*-value
Age	0.18	0.06	0.21	0.08
Fasting blood sugar	0.14	0.19	0.22	0.11
Total cholesterol	0.23	0.20	0.25	0.30
Serum triglyceride	0.21	0.45	0.18	0.55
Serum HDL	-0.09	0.31	-0.12	0.15
Serum LDL	0.29	0.01	0.34	0.02
Systolic blood pressure	0.23	0.10	0.26	0.07
Body mass index	0.31	0.05	0.16	0.28
Proteinuria	0.18	0.12	0.23	0.10
eGFR	-0.12	0.24	-0.32	0.01

Table 4: Correlation of diastolic EAT thickness with different variables

	Control (Non-CKD group)		CKD group	
	r	*P*-value	r	*P*-value
Age	0.12	0.15	0.18	0.09
Fasting blood sugar	0.17	0.15	0.25	0.07
Total cholesterol	0.25	0.10	0.28	0.20
Serum triglyceride	0.20	0.34	0.24	0.42
Serum HDL	-0.16	0.21	-0.23	0.08
Serum LDL	0.34	0.01	0.39	0.01
Systolic blood pressure	0.21	0.10	0.24	0.09
Body mass index	0.29	0.04	0.14	0.18
Proteinuria	0.28	0.20	0.25	0.12
eGFR	-0.15	0.13	-0.35	0.01

Table 5: Multiple linear regression analysis of EAT thickness

	Systolic EAT		Diastolic EAT	
	β	*P*-value	β	*P*-value
Age	0.15	0.10	0.17	0.12
Fasting blood sugar	0.21	0.13	0.20	0.09
Total cholesterol	0.23	0.09	0.19	0.22
Serum triglyceride	0.24	0.20	0.21	0.32
Serum HDL	-0.13	0.19	-0.19	0.08
Serum LDL	0.22	0.08	0.25	0.07
Systolic blood pressure	0.18	0.12	0.22	0.11
Body mass index	0.24	0.09	0.19	0.12
Proteinuria	0.18	0.13	0.21	0.09
eGFR	-0.67	0.02	-0.63	0.04

Table 6: Clinical outcomes in CKD patients at one year follow up

	Total	EAT <5mm* (n=210)	EAT ≥5mm* (n=90)	*P*-value (n=120)
All-cause mortality	23(10.9)	9(10)	14(11.6)	0.70
Fatal CV events	12(5.7)	3(3.3)	8(6.6)	0.19
Non-fatal CV events	24(11.4)	5(5.5)	17(14.1)	0.04
Total CV events [β]	36(17.1)	8(8.8)	25(20.8)	0.01
CKD progression [§]	84(40)	23(25.5)	61(50.8)	<0.001

Table 7: ROC curve analysis of EAT thickness for prediction of CV events* in CKD

	Systolic EAT	Diastolic EAT
Area under the curve (AUC)	0.89	0.86
95% confidence interval	0.80 to 0.97	0.78 to 0.95
P value	<0.001	<0.001
Cut-off	5mm	3.8mm
Sensitivity	88%	87%
Specificity	72%	74%

in 25 CKD patients. ICC agreement on measurements were 0.93 (95% CI: 0.79-0.98) for systolic EAT and 0.91 (95% CI: 0.74-0.97) for diastolic EAT. These ICC agreement value suggest excellent reproducibility.

Patient characteristics

The patient characteristics are presented in Table 1. The participants in CKD group had significantly higher prevalence of obesity, diabetes, hypertension and hyperlipidemia, though the smoking rates were comparable. Estimated glomerular filtration rate was normal in control subject whereas markedly decreased in CKD group (74±9 versus 46±7 ml/min/1.73 m^2, P<0.001). Proteinuria in CKD group was seen in around half of the patients. According to echocardiography, the mean systolic EAT thickness in the CKD group was significantly higher than the non-CKD participants (5.6±1.2 versus 4.3±1.0, P <0.001). Similarly the diastolic EAT thickness too was significantly higher in CKD group (4.2±1.1 versus 3.1±1.1, P <0.001). However, there was significant difference between the systolic and diastolic EAT in both CKD (P<0.001) and non-CKD group (P<0.001), with the diastolic values being lower (Figure 4). Both systolic and diastolic EAT thickness were significantly higher in CKD patients with age ≥60 years, BMI ≥25 Kg/m^2, diabetes and hyperlipidemia. Additionally, EAT thickness increased with severity of CKD (lower eGFR) and in patients with proteinuria. However, there was no significant gender related difference (Table 2).

Correlation with EAT thickness

Correlation of systolic and diastolic EAT with different variables was analyzed in the control and CKD group (Table 3 and 4). Both systolic and diastolic EAT thickness had similar correlation with the analysed variables. Significant positive correlation was seen with serum LDL cholesterol in both the study groups. BMI had significant positive correlation with EAT in non-CKD group, but not in CKD group. Significant negative correlation was seen with eGFR in the CKD group but not in controls. On multivariate linear regression analysis, only e-GFR was found to be independent predictor for both systolic (β = -0.67, p = 0.02) and diastolic EAT thickness (β = -0.63, *p* = 0.04) (Table 5).

Clinical outcomes

A systolic EAT thickness of ≥5mm was considered abnormal and used as cut-off for comparison. By the end of one year, 23 patients of the CKD group were dead (10.9%, n=210). Cardiovascular mortality accounted for 52% of these (12/23). Overall, CV events were recorded in 36 patients (17%) and CKD progression was seen in 84 patients (40%). No significant difference in cardiovascular or all-cause mortality was seen between patients with EAT <5mm or ≥5mm. However, in patients with systolic EAT ≥5mm significantly greater number of CV events were observed (20.8% versus 8.8%, *P*=0.01), mainly driven by higher non-fatal CV events (Table 6). The relative risk development of CV events was 2.34 (95% CI=1.14 to 4.91). CKD progression was also significantly higher in patients systolic EAT thickness ≥5mm (50.8% versus 25.5%, *P*<0.001) with relative risk of 1.98 (95% CI=1.36 to 2.98).

ROC curve analysis

The area under the curve (AUC) for systolic EAT thickness was 0.89 (95% CI: 0.80-0.97), which was statistically significant (*P*<0.001). A systolic EAT cut-off of 5mm had a sensitivity of 88% and specificity of 72% in predicting the CV events (Table 7). ROC curve analysis for diastolic EAT thickness showed AUC of 0.86 (95% CI: 0.78-0.95, *P*<0.001). Comparison of ROC curves to test the difference between the areas under the curves, showed no significant difference (*P*=0.26). Using diastolic EAT cut-off of 3.8mm, similar sensitivity and specificity was achieved (87% and 74% respectively).

DISCUSSION

This study has demonstrated that CKD patients have significantly increased EAT thickness as compared to non-CKD patients. Both systolic and diastolic EAT thickness can be used to predict CV risk with similar sensitivity and specificity, though with different cut-offs. This simple echocardiographic variable can aid in cardiovascular risk stratification of CKD patients.

The worldwide rise in the number of CKD patients is threatening to reach epidemic proportions.[1] Similar to previous studies, hypertension (61.6%) was the most common co-morbidity in the CKD patients, followed by hyperlipidemia, diabetes and obesity.[2-5] Cardiovascular events are the most common cause of mortality and morbidity in patients with CKD, rather than renal failure itself.[6-8] Furthermore, recent studies have shown that even mild renal disease should be considered a major risk factor for cardiovascular events.[9-10] Indeed cardiovascular deaths in this study accounted for about 50% of total mortality at one year of follow up.[11]

Epicardial adipose tissue, a type of visceral fat, is emerging as a novel risk factor for cardiovascular disease.[13,14,18] EAT has endocrine, paracrine, vasocrine and inflammatory characteristics and is associated with metabolic syndrome, insulin resistance and coronary artery disease. Body mass index (BMI) is the most applied anthropometric estimate of body fat. However, it's a remote measure of visceral adiposity and thus provides an imperfect estimate of risk posed by obesity. Indeed BMI has been shown to be inverse predictor of mortality in moderate to severe CKD.[28,29] This may be due to protein malnutrition, catabolism and protein loss in CKD causing reduced muscle mass and insulin resistance, which in turn increases visceral fat including EAT.[30] Moreover, visceral fat may be high in individuals with low BMI and may cause metabolic risk similar to those with high BMI. In view of high risk of cardiovascular disease in patients with CKD and given the imperfect estimate of visceral adiposity by BMI,[31] studies focusing on epicardial fat are highly relevant. In the present study, we found that BMI was significantly lower in the CKD group. However, there was no significant correlation between BMI and EAT volume (*r*=0.16, *P*=0.28). These observations are consistent with frequent discordances between BMI and EAT volume/thickness.

Epicardial fat in CKD has been shown to be negatively correlated with eGFR independent of diabetes and obesity.[32-34] In our study eGFR was negatively correlated with both systolic and diastolic EAT thickness in the CKD group (r=-0.32, p=0.01) but not in controls. On multivariate linear regression analysis, only e-GFR remained as independent predictor of both systolic and diastolic EAT thickness, among confounding variables like age, blood pressure, BMI, fasting blood sugar, proteinuria, LDL, HDL and triglyceride. Thus, altered fat distribution seems to be key contributory factor to atherosclerosis progression in patients with CKD of any degree. Interestingly, the risk of CKD progression was significantly higher in patients with systolic EAT thickness ≥5mm (RR=1.98; 95% CI=1.36 to 2.98). This peculiar finding has never been reported in previous studies. Whether there is any significant association or this is a mere speculation would further require larger prospective studies to confirm.

Though there is some evidence of correlation of EAT with CVD development in ESRD and hemodialysis patients[15-17] such data in non-dialysis dependent CKD patients is limited.[35-37] Tsushima *et al*. using MDCT found higher EAT volume in CKD patients which was associated with high risk plaque.[36] However, all these studies were cross-sectional and relatively small. Furthermore, the outcome measure was the coronary calcium score or a weaker surrogate. Till now it remains unclear whether EAT accumulation is the cause or consequence of CKD. As of now, there has been only a single prospective study relating to CV risk prediction by EAT in CKD patients. Cordeiro *et al*. demonstrated that increased EAT volume on MDCT was a predictor for future CAD events independent of traditional cardiovascular risk factors in patients with stage 3–5 CKD.[37] However, this study was done using MDCT for EAT volume estimation, which is not feasible in regular clinical practice especially in developing countries. Transthoracic echocardiography has many advantages, such as easy availability, low cost, simplicity, no radiation exposure, fastness and reproducibility.[18] In the present study, we found significantly increased EAT thickness in CKD patients as determined by TTE, which was associated with high risk of CV events.

The underlying mechanisms of increased EAT volume and association with cardiovascular disease in CKD subjects are not fully understood. It is believed that EAT shares its microcirculation with coronaries due to proximity, thereby allowing pro-atherogenic hormones and inflammatory cytokines released from EAT to act locally and promote coronary artery disease.[38-40] Visceral adipose tissue including EAT has a reduced potential to produce adiponectin, a vasculoprotective adipokine, in obese individuals. Dietary modifications, exercises and bariatric surgery all reduce visceral and epicardial fat along with the associated metabolic risk.[41,42] Recent studies have shown that EAT is potentially modifiable in hemodialysis (HD) patients. Wilund *et al*.[43] showed EAT thickness could be reduced with exercise and Colak *et al*.[44] showed EAT thickness was significantly reduced in renal transplant patients. Experimental treat-

ment that specifically target EAT reduction are needed, with mortality and cardiovascular events as end-points.

EAT thickness can be measured by transthoracic echocardiography, cardiac computed tomography (CT) and cardiac magnetic resonance imaging (MRI). Although CT and MRI have better image quality and can perform volumetric measurements, they are limited by high costs, low accessibility and complexity of measurement. Transthoracic echocardiography has many advantages, such as easy availability, low cost, simplicity, no radiation exposure, fastness and reproducibility.[18] Echocardiography is therefore, the most preferred imaging method used to assess EAT in clinical practice with high reproducibility.[45] These measurements show good correlation with the values found on MRI ($r = 0.91$, $p = 0.001$).[18] Iacobellis was first to use echocardiography for EAT measurement and show its relation with metabolic syndrome.[21] Therefore, we used transthoracic echocardiography in our study unlike previous studies in CKD which were mostly done by MDCT. Interclass correlation coefficient (ICC) agreement on measurements were 0.93 (95% CI: 0.79-0.98) for systolic EAT and 0.91 (95% CI: 0.74-0.97) for diastolic EAT, suggesting excellent interobserver reproducibility.

There is no consensus regarding use of echocardiography in EAT measurement, but some recent reviews provide excellent guidelines to its proper use in clinical practice.[18-20,46] Maximum value of EAT obtained should used and averaged over 3 cardiac cycles. Parasternal long and short-axis measurements are averaged to obtain the mean thickness. More sensitive measurements can be made by enhancing depth setting and magnifying the view to assess EAT thickness more clearly (Figure 2).[46] We propose using M-mode echocardiography to improve accuracy of measurements owing to higher resolution of image (Figure 3). M-mode is taken at the point of maximum thickness of EAT perpendicular to the right ventricular free wall. Additionally, we used increased depth setting with the M-mode to improve sensitivity. In parasternal long axis view M-mode is aligned in the line of aortic annulus taking aortic root as reference. In parasternal short axis view M-mode is taken 2 cm away from interventricular septum at mid-ventricular level. End-diastolic EAT is measured at peak of R wave in ECG and end-systolic EAT is measured at end of T wave. Care should be taken to avoid pericardial fat and especially apical fat in parasternal long axis view. Adaptation of this technique would help in standardisation of measurement, thereby improving consistency and comparability in publications.

Opinion is further divided on which time in the cardiac cycle is most suitable for measuring EAT thickness by TTE. Proponents of measurement in systolic phase recommend it to prevent possible deformation by EAT compression during diastole.[17,21] Whereas measurement in diastole is recommended as it better coincide with other imaging modalities (CT and MRI).[22-25] It is necessary to pay attention to phase of measurement in publications because end-systolic measurements are inherently higher than the end-diastolic measurements. Unfortunately, there has been no direct study comparing systolic and diastolic EAT thickness. In our study we found that the diastolic EAT thickness in both CKD and control groups were significantly lower than systolic EAT thickness (Figure 3). However, both systolic and diastolic EAT thickness were similar in prediction of CV events. Therefore, either systolic or diastolic EAT thickness can be used in studies with due care to respective cut-off values.

Another controversial point is the normal upper-limit value for epicardial fat thickness as there are severe inconsistencies in the literature. However, few studies done in different clinical scenario suggest an EAT thickness of >5mm as abnormal and being associated with cardiovascular disease.[25,47-49] Bertaso *et al.* in a systematic review suggested that EAT measurements >5 mm might be considered abnormal.[18] However, any generalisation cannot be made because EAT thickness is influenced by age, gender, race and phase of cardiac cycle. Significant ethnic difference in EAT thickness have been noted across the studies with considerably lower values seen in Asian population. Iacobellis *et al.* in a large white population found that a cut-off of 9.5mm in males and 7.5mm in females was associated with metabolic syndrome and high abdominal fat.[46] Epicardial fat thickness values > 3.0 mm were independently associated with the presence of CAD in a Korean population of men and women.[22] In a large Indian population, EAT thickness >4.65mm predicted the presence of significant coronary stenosis.[50] In the current study, Receiver operating characteristics (ROC) analysis showed that systolic EAT thickness of 5mm and diastolic EAT thickness of 3.8mm maximize the sensitivity and specificity to predict CV events in CKD population. Systolic cut-off of 5mm and diastolic cut-off of 3.8mm had similar sensitivity (88% versus 87%) and specificity (72% versus 74%).

Study Limitations

The strengths of this study were a large sample size, longitudinal design and validation of a simple, cheap and easily available echocardiographic variable to assess cardiovascular risk in CKD patients. However, this study has several important limitations too. First, this was a single-centre study and findings are only hypothesis generating, thus further large-scale studies are necessary to confirm the present findings. Furthermore, because we enrolled only non-dialysis dependent CKD patients, the results may not be applicable to subjects with ESRD and on hemodialysis. We used transthoracic echocardiography for EAT thickness assessment which is less accurate and less reproducible than EAT volume assessment by CT and MRI. However, echocardiography has the advantage of being an easy, readily available, repeatable and low cost modality without radiation exposure. Another limitation applicable to all the current studies of EAT is lack of any threshold value either in normal population or in diseased states. EAT thickness appears to increase with age and it could be influenced with ethnicity. The study population consisted entirely of Indian patients, so the findings of this study may not be relevant to patients of other ethnic backgrounds. Finally, the findings of this study are subject to confounding and bias that are inherent to the observational studies.

CONCLUSION

Systolic and diastolic epicardial adipose tissue thickness, as determined by echocardiography, is significantly increased in CKD patients and aid in prediction of future cardiovascular events. Both systolic and diastolic EAT thickness can be used in CV risk stratification with similar sensitivity and specificity. We further propose routine use of M-mode with technical considerations as discussed to standardise the measurement and improve comparability of publications. The findings of this study are hypothesis generating, implicating the role of echocardiography based EAT assessment in cardiovascular risk stratification of CKD patients. However, larger longitudinal studies will be required to confirm or refute these findings. Finally, EAT is not just an academic curiosity, it is modifiable and appears to be an attractive target for future interventions to reduce CV risk.

CONFLICTING INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

EAT: Epicardial Adipose Tissue; **CKD:** Chronic Kidney Disease; **TTE:** transthoracic echo-cardiography; **ICC:** Interclass Correlation Coefficient; **ROC:** Receiver Operating Characteristics; **KDIGO:** Kidney Disease: Improving Global Outcomes; **ESRD:** end-stage renal disease; **PLAX** and **PSAX:** parasternal long-axis view and short-axis view ; **MDRD:**

Modification of Diet in Renal Disease; **eGFR:** estimated Glomerular Filtration Rate; **BMI:** Body Mass Index; **LDL:** low-density lipoprotein.

SUMMARY

Epicardial adipose tissue (EAT) has been related to increased cardiovascular risk in chronic kidney disease patients. However, prospective studies of EAT thickness in prediction of cardiovascular events in CKD patients are lacking. In this study we found both systolic and diastolic EAT thickness to be significantly increased in CKD patients. Moreover, they can be used for CV risk stratification with similar sensitivity and specificity albeit with different cut-offs. M-mode should be routinely used in measurement of EAT thickness to standardise the measurement and improve comparability of publications. EAT is modifiable and therefore, appears to be an attractive target for future interventions to reduce CV risk.

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