Coronary microcirculation and left ventricular diastolic function but not myocardial deformation indices are impaired early in patients with chronic kidney disease

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Abstract

Aim. To investigate abnormalities in myocardial strain and classic echocardiographic indices and coronary flow reserve (CFR), in younger vs older CKD patients. **Methods**. Sixty consecutive CKD patients (<60 years old n=30, [?]60 years old n=30) and 30 healthy controls (age- and gender-matched with younger CKD patients) were recruited. An echocardiographic assessment including myocardial strain indices was performed at baseline and following dipyridamole administration in all participants. **Results**. Younger CKD patients had higher E/e', left ventricular mass index and relative wall thickness and lower E' ($p<0.005$ for all) compared to healthy controls. Older CKD patients had lower E/A and E' ($p<0.05$ for both) compared to younger CKD patients; these differences did not remain significant after adjustment for age. CFR was higher in healthy controls compared to younger and older CKD patients ($p<0.05$ for both) without a significant difference between CKD groups. Dipyridamole-induced changes did not differ significantly among the 3 groups. **Conclusions**. Compared to healthy controls, impaired coronary microcirculation and left ventricular diastolic function, but not myocardial strain abnormalities, are found in young CKD patients and deteriorate with aging.

1. INTRODUCTION

Patients with chronic kidney disease (CKD) are at a higher risk for cardiovascular disease (CVD) compared to the general population; CVD is responsible for about 50% of deaths in CKD patients [1]. The interrelation between kidney and heart function has long been recognized and current research effort is focused in delineating the exact mechanisms behind this complex pathophysiology. Increased cardiovascular risk in individuals with CKD is due partly to the high prevalence of traditional risk factors, such as hypertension and diabetes, but also to non-traditional cardiac risk factors that are particularly relevant to patients with chronic kidney disease, including decreased glomerular filtration and albuminuria. Early atherosclerosis progression and endothelial cell dysfunction, uraemia and kidney failure, neurohormonal dysregulation, anaemia and iron disorders, mineral metabolic derangements and inflammatory pathways may all contribute to the phenotype of cardio-renal syndrome [2-6].

Non-invasive and widely available diagnostic methods that may detect preclinical functional and structural myocardial abnormalities are needed in order to identify CKD patients at higher risk for CVD [7-9]. Echocardiography is an essential tool for the assessment of cardiac structure and function in several patient groups, including CKD patients, while various echocardiographic indices have been shown to predict adverse CVD outcomes. Classic echocardiographic indices of left ventricular (LV) systolic and diastolic function may not be sensitive enough in detecting early myocardial deterioration in CKD patients [10, 11]. Two-dimensional speckle tracking echocardiography (2DSTE) is a semi-automated modality for quantification of LV systolic as

well as diastolic function in an operator-independent manner. LV myocardial deformation may be assessed in the longitudinal, radial and circumferential plane but peak global longitudinal strain (GLS) has been shown to be the most important load-independent index that gives an efficient and rather objective measurement of LV systolic and diastolic function with many prognostic implications [10, 12-17].

Dipyridamole stress echocardiography (DIPSE) is mostly used to measure coronary flow reserve (CFR) in a non-invasive way. CFR is used for evaluating both the presence of significant epicardial stenosis and the microcirculatory function in the left anterior descending artery (LAD) territory; a CFR value <2 is correlated to significant microvascular dysfunction and is proposed to be a strong predictor of epicardial coronary artery disease (CAD) [18]. Impaired CFR has been also advocated as an adverse prognosticator for CVD [19, 20].

The aim of the current study was to investigate differences in classic, 2DSTE-related indices, CFR and other DIPSE-induced changes in various echocardiographic parameters between: 1) healthy controls and age-matched younger CKD patients, 2) younger versus older CKD patients with similar clinical characteristics.

2. MATERIALS AND METHODS

2.1 Study population

We prospectively evaluated 60 consecutive CKD patients 40-75 years old who were followed-up at the CKD outpatient clinic of the University Hospital of Ioannina, Greece, between January 2013 and December 2014. The study protocol was previously approved by the local ethics committee. Exclusion criteria were: 1) history of coronary artery disease (CAD) and other established atherosclerotic disease i.e. cerebrovascular and peripheral arterial disease, 2) LV ejection fraction <50%, 3) any moderate-severevalvular heart disease (VHD), 4) atrial fibrillation, 5) advanced atrioventricular conduction disorder, 6) poor acoustic window during echocardiographic examination, 7) history of congestive heart failure, cardiomyopathy or constrictive pericarditis and 8) known allergy to dipyridamole or history of asthma/severe chronic obstructive pulmonary disease (COPD).

From a total of 106 patients attending the CKD outpatient clinic of our hospital, 46 patients were excluded at the time of study enrollment, due to poor acoustic window (16 patients), moderate-severe VHD (8 patients), presence of not previously known atrial fibrillation (6 patients), history of asthma/severe COPD (4 patients), asymptomatic (not previously known) CAD (8 patients), allergic reaction during dipyridamole infusion (1 patient) and denial to participate to the protocol (3 patients). The remaining patients (n=60) were divided and analyzed in two subgroups according to a cut-off at the age of 60 years. In the current study 30 healthy controls (40-60 years old) that were age- and gender-matched to younger CKD patients (i.e. <60 years old), were also enrolled. Finding healthy subjects in the specific age span without any known risk factor and without taking any medication was more difficult than originally thought. All patients (CKD and healthy controls) underwent a thorough physical examination and a detailed echocardiographic assessment. The study complied with the Declaration of Helsinki. All participants provided a written informed consent.

2.2 Risk factor assessment - Laboratory investigations

Clinical as well as demographic data were recorded on the day of the echocardiographic assessment. The risk factor evaluation included: 1) the cause of CKD, 2) prescribed medications at the time of the enrollment, 3) time since first diagnosis of CKD and 4) a thorough clinical examination. Hypertension was defined as systolic blood pressure (BP) >140mmHg and/or diastolic BP >90mmHg or history of anti-hypertensive drugs administration. Hypercholesterolemia in CKD patients was defined as low-density lipoprotein cholesterol (LDL) >100 mg/dl or administration of statins. Weight and height were measured and body mass index and body surface area were then calculated. Smokers were defined as those patients who were currently smoking or quitted smoking <1 year ago. Blood samples were drawn early in the morning before examination after a minimum of 12 hours overnight fast. Glomerular filtration rate (GFR) was measured by using the CKD-EPI formula [21]. Assessment of urine protein in a 24-hour urine sample was also performed.

2.3 Echocardiographic evaluation

The echocardiographic evaluation was performed by a single operator (LL). A commercially available system (Vivid 7, GE Vingmed ultrasound AS) was used for all patients. Standard parasternal and apical views were usedand acquired images were stored digitally in high analysis still images and in cine loops (in a format of three consecutive beats for analysis). A single observer blinded of the patients' identity (not the same person who performed the echocardiographic examination) performed offline analysis using EchoPac (version 113 - GE Vingmed ultrasound AS). On the day of the examination, echocardiographic assessment was performed in a two-staged approach. Initially, a basic echocardiogram was performed. All classic LV function related systolic and diastolic indices were obtained, according to the European Society of Cardiology and European Association of Cardiovascular Imaging guidelines [22]. Left atrial volume and LV mass were both indexed to patients' body surface area.

In order to obtain 2DSTE data, both parasternal and apical views (at frame rates 60-90Hz) were acquired. Thus, adequate spatial and temporal resolution and accurate frame to frame tracking (for three consecutive cardiac cycles) was ensured. The endocardial LV borders were manually traced (region of interest). When tracking was poor in more than two consecutive myocardial regions, the acquired data were declined. The timings of aortic and mitral valve opening and closure were manually defined by the use of pulsed-wave Doppler. No patient was excluded based on poor 2DSTE-related echocardiographic data. 2DSTE analysis included assessment of GLS and strain rate as also global radial and circumferential strain and strain rate. LV twist was calculated as the difference between apical and basal LV rotation as it was assessed from equivalent short-axis views. Studies with >2 consecutive segments (out of a total amount of 17) not adequately tracked were deemed as inappropriate for the measurement of LV twist and rotation. Untwist rate was measured as the peak negative time derivative of twist during diastole. The time interval from R wave peak to the maximal untwist was then calculated.

Following the baseline echocardiographic evaluation, infusion of dipyridamole for 6 minutes (0.84mg/kg) was performed. CFR was calculated as the ratio between hyperemic and basal coronary flow at the LAD area. Just before the end of dipyridamole infusion a new echocardiographic assessment (focused mainly on LV systolic and diastolic function indices) was performed. At the end of the dipyridamole infusion, 125-250mg of aminophylline was administered to the patient, to counteract any dipyridamole negative effect. The dose was dependent on the patient's status after dipyridamole infusion. Beverages containing methylxanthines such as coffee, tea, chocolate and coke were prohibited for at least 24 hours before the study. Patients with CFR values<2 were referred for another ischemia stress test and/or coronary angiographyto exclude potential significant epicardial stenosis in the LAD area. None of the enrolled patients with CFR values<2 had confirmed significant CAD. Intra-observer variability for all indices of interest included in this study has been previously reported [23].

2.4 Statistical analysis

Kolmogorov-Smirnov Z-test was used to determine the normal distribution of continuous variables. Continuous data are presented as mean±SD or median (interquartile range). The χ^2 test and unpaired Student's t-test were used to compare categorical and continuous variables respectively between two different groups of patients. Within group changes from baseline and post-dipyridamole were assessed using the paired Student's t-test. Changes in studied echocardiographic parameters following dipyridamole administration were compared using Repeated Measures ANOVA analysis. P values were always two-sided and a value of $p<0.05$ was considered significant. The SPSS statistical software package (IBM SPSS Statistics, Version 23) was used.

3. RESULTS

3.1 Baseline characteristics

The baseline demographic and metabolic characteristics of the entire CKD population (n=60) are shown in Table 1 . The median age of the CKD patients was 63 years and most of them were males (70%). Most of the CKD patients had an established history of hypertension (88%) and 28% were diagnosed with diabetes. The underlying etiology of CKD was defined in appx 75% of the patients with various diagnoses. Only 57%

of the patients were treated with a RAAS blocker. The median value of eGFR was 30.5 ml/min/1.73 m^2 and 24-hr urine protein 900 mg. There was no statistically significant difference between younger and older CKD patients in eGFR (32.9±18.2 vs 31.5±12.9ml/min/1.73 m^2, p=0.732) and 24hr urine protein [median 1134 (IQ range 498, 3000) versus 400 (IQ range 159, 2356), p=0.136].

3.2 Comparison between younger CKD patients and healthy controls *(Table 2)*

There were no significant differences in age, gender and body mass index between the two groups (p=NS). Patients with CKD had higher E/e' ratio (7.80±2.71 vs 6.06±1.37, p=0.019 Bonferroni correction), LVMI (114.7±41.1 vs 89.6±21.2, p=0.017Bonferroni correction) and relative wall thickness (0.47±0.08 vs 0.40±0.06, p=0.031Bonferroni correction) and lower E' (10.6±2.6 cm/s vs 2.3±2.9 cm/s, p=0.045Bonferroni correction) compared to healthy controls.

3.3 Comparison between CKD patient subgroups i.e. <60 versus [?]60 years old *(Table 2)*

There were no significant differences in gender or body mass index between the two groups (p=NS).Older CKD patients had higher systolic BP(143+-21mmHg vs 130+-18 mmHg, p=0.023 Bonferroni correction) and lower E/A (0.80+-0.19 vs 1.03+-0.33, p=0.007 Bonferroni correction) and E' (8.5+-1.8cm/s vs 10.6+-2.6 cm/s, p=0.003 Bonferroni correction) compared to younger CKD patients. None of the aforementioned differences remained significant after adjustment for the difference in age between the two groups (p=NS for all).

3.4 Effects of dipyridamole infusionin CKD subgroups and healthy controls *(Table 3)*

CFR was higher in healthy controls compared to both younger (3.93+-1.25 versus 3.1+-0.75, p=0.009, Bonferroni correction) and older CKD patients (3.93+-1.25versus 2.89+-0.88, p<0.001,Bonferroni correction). There was no significant difference in CFR between younger and older CKD patients (p=1.000, Bonferroni correction). Dipyridamole caused a significant increase in Sm, Sl, E'and a decrease in GLS in all groups (p<0.05 for all). LVEF was significantly increased in healthy controls and younger CKD patients while UNTWIST was significantly decreased in healthy controls and older CKD patients (p<0.05 for all) following dipyridamole administration. Dipyridamole significantly decreased E/A ratio only in younger CKD patients (p<0.05). Dipyridamole-induced changes did not differ significantly among the 3 studied groups (RM ANOVA, p=NS for all).

4. DISCUSSION

In the present study, it was shown that younger CKD patients without a previous history of CVD (i.e. <60 years old) had worse indices of diastolic function (E' and E/E'), more LV hypertrophy and signs of concentric remodeling and lower values of CFR compared to age-matched healthy controlswith similar demographic and clinical characteristics. In older CKD patients, a trend towards further worsening of these indices compared to younger CKD patients was observed but this was probably attributed to aging since all differences were lost after adjustment for differences in age between patients. This finding may be explained by the direct effect of aging on myocardial structure and function and microcirculatory function, the longer exposure to CKD and the potential relation of age to different CKD etiologies (i.e. diabetic and/or hypertensive nephropathy may be more prevalent in older CKD patients while various glomerulopathies may be more frequent in younger CKD patients). The effects of dipyridamole on classic and myocardial strain indices were similar in all studied groups.

Left ventricular diastolic dysfunction has been shown to be common in various stages of CKD and it has been suggested that the severity of CKD may be associated with the progression of asymptomatic LV diastolic dysfunction to symptomatic stages of heart failure independently of other clinical parameters [24]. In animal-based experimental CKD models, the development of LV diastolic dysfunction has been demonstrated to be independent of the presence of hypertension [25]. Various measures of LV diastolic dysfunction have been associated with increased mortality in the general population [26] while E/E' has been shown to be a very useful parameter in predicting prognosis of CKD patients [27]. Enlarged left atrium has been demonstrated early in CKD patients [28] and has emerged as a useful biomarker for risk stratification and risk monitoring

in patients with CKD [29, 30]. Currently, we showed that young asymptomatic CKD patients (mean age 49 years) had worse E' and E/E' compared to age-matched healthy controls. On the other hand, LAVI was similar between healthy controls and young CKD patients while it increased in older CKD patients suggesting that longer duration of exposure to CKD and/or aging per se are probably the main contributors of LA enlargement.

LV hypertrophy has been previously reported to be highly prevalent in patients with CKD even in early stages and related to both worsening GFR and albuminuria [31] and has been associated with cardiovascular mortality [2]. It has been demonstrated that LVH may be present up to 50-75% in patients with eGFR<60 mL/min/1.73 m^2 [32] while other forms of structural LV abnormalities including LV concentric remodeling may be also common in CKD populations (ca. 20.0%) [33]. In the present study, we showed that increased LV mass and LV concentric remodeling are prevalent in CKD patients (irrespectively of age) compared to healthy controls suggesting that these findings are more closely related to CKD per se (impaired GFR and proteinuria) and not aging. Among persons with CKD, left ventricular mass index has been associated with incident heart failure, even after adjustment for major cardiovascular risk factors [34].

CFR is a useful marker non-invasive echocardiographic index of epicardial artery stenosis of the LAD territory and coronary microcirculation; in our study in CKD patients without clinical or subclinical CAD, CFR is more likely to describe coronary microcirculation function [23, 35-39]. In agreement with our findings decreased CFR has been demonstrated in CKD patients even with mild to moderate GFR impairment [18, 40, 41]. Impaired CFR and subsequent dysfunction of coronary microcirculation has been related to LV diastolic dysfunction and other LV structural changes [23, 41-43] in various CKD populations as well as to prognosis in CKD patients [42, 44, 45].

Chronic renal disease has been proven to be associated with early and subclinical impairment of LV systolic function as assessed by 2D-myocardial strain indices and especially GLS [9, 10, 14, 46]. In a single study TWIST was shown to increase in CKD patients [46] in contrast to our findings. Currently, there were no differences in 2DSTE-related indices between young CKD patients and age-matched healthy controls as well as no differences among younger and older CKD patients with similar GFR and albuminuria values. GLS has been reported to be a predictor of adverse cardiovascular prognosis in CKD patients following adjustment for relevant clinical variables [47].

DIPSE has been previously shown to be effective for both the assessment of myocardial ischemia and CFR assessment. It has also been suggested that it could be used as a test to evaluate the systolic and diastolic myocardial reserve in various populations [23, 39, 48]. In our study, DIPSE produced similar improvement in various indices of LV systolic and diastolic function (classic and 2DSTE-related) in all three patient groups.These findings suggest that DIPSE test may not be suitable for detecting early myocardial abnormalities and risk stratification in relatively healthy asymptomatic CKD patients free of established cardiovascular disease. However, there is a need for larger longitudinal studies in order to clarify the value of DIPSE test in CKD patients besides the evaluation of CFR and assessment of myocardial ischemia.

Limitations . This was a single center study that included relatively healthy patients with CKD of various etiology without severe comorbidities and thus the results cannot be extrapolated to the general CKD population. As mentioned in the methods' section a significant portion of the CKD population was excluded due to the presence of structural heart disease or coronary artery disease. Multivariate association analysis was not performed due to the limitation of the size of the study groups. For the same reason medications' usage was not taken into account in the association analysis.

In conclusion, in this study it was demonstrated that impaired coronary microcirculation and LV diastolic function and increased LV mass with concentric remodeling are the principal findings early in the process of CKD in comparison to healthy controls. These results have implications for pathophysiological processes behind cardiorenal syndrome type 4 and targeted cardiac assessment in patients with CKD may be of value to identify the progression of subclinical myocardial abnormalities. Future studies are needed to validate our findings in terms of improvement of clinical practice but also to assess whether any therapeutic effort

aiming to delay or reverse changes in the above mentioned echocardiographic indices may have an impact on the cardiovascular prognosis in CKD patients.

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Table 1. Demographic and metabolic characteristics of CKD patients (n=60)

Age, years
Male gender, n (%)
Body mass index, kg/m^2
Hypertension, n (%)
Diabetes, n (%)
Hyperlipidemia, n (%)
Medications, n (%) Statins
Renin-Angiotensin blockers
Calcium blockers
b-blockers

Cause of CKD disease, n (%) Glomerulonephritis
Diabetic
IgA nephropathy
Vasculitis
Single kidney
Other
Unknown
Estimated GFR, ml/min/1.73 m^2
Urine protein, mg
Hemoglobin, g/dl
Fasting glucose, mg/dl
Total cholesterol, mg/dl
HDL-cholesterol, mg/dl
Triglycerides, mg/dl
Calcium, mg/dl
Phosphate, mg/dl
Sodium, mmol/l
Potassium, mmol/l
Parathormone, pg/ml
Data are presented as number of patients (%) or mean ± standard deviation or median (interquartile range). CKD, Chronic

Table 2. Comparison in study parameters among healthy controls and CKD subgroups (i.e. <60 vs [?]60 years old) using One-way ANOVA (post-hoc analysis Bonferroni).

Age, years
Male gender, n (%)
BMI, kg/m^2
Systolic BP, mmHg
Diastolic BP, mmHg
LVMI, g/m^2
LAVI, ml/m^2
LVEF, %
E/A
Sm, cm/s
Sl, cm/s
E', cm/s
E/E'
RWT
GLS, %
TWIST, degrees
UNTWIST, degrees/s
Data are presented as number of patients (%) or mean ± standard deviation. BMI, Body mass index; BP, Blood pressure; C

Table 3. Dipyridamole-induced changes in echocardiographic parameters among healthy controls and CKD patients (paired t-test within each group and RM-ANOVA to compare changes among the 3 groups)

CFR
LVEF, %
E/A
Sm, cm/s
Sl, cm/s
E', cm/s
E/E'
GLS, %
TWIST, degrees
UNTWIST, degrees/s

Data are presented as mean ± standard deviation. CFR, Coronary flow reserve; CKD, Chronic kidney disease; DIP, Dipyrid