ABSTRACT

Aim To evaluate the relationship between cardiovascular remodelling and glomerular filtration rate (eGFR) in pre-dialysis chronic kidney disease (CKD) patients without cardiovascular diseases (CVD) and in renal transplant recipients (RTR).

Methods The cross-sectional study included 83 patients with eGFR<60 mL/min/1.73m² (45 with CKD 3 stage and 38 with CKD 4 stage). Thirty six RTR had eGFR 67.8 (57.3-73.7) mL/min/1.73m² and control group consisted of 44 patients with eGFR>60 mL/min/1.73m². All patients were evaluated by echocardiography and X-ray.

Results Left ventricular hypertrophy (LVH) was present in 74.7% CKD patients, most frequently in CKD 4 stage and in RTR. Calcifications of abdominal aorta (CAA) were present in 87% CKD 4, 60% RTR and in 44% CKD 3 patients. Calcifications of the mitral valve were found in 34.2% CKD 4, 25.0% RTR and in 6.7% CKD 3 stage patients. Aortic valve calcifications were most frequently present in CKD 4 stage (26.3%). The LV mass index negatively correlated with eGFR (p<0.001), and positively with parathyroid hormone (p<0.001), phosphorus (p=0.043), age (p<0.001) and diabetes (p=0.043). In multivariate regression analysis the risk factor for calcifications of the mitral and aortic valve, as well as for CAA was the decline in eGFR (p<0.001).

Conclusion Renal transplant recipients have a higher incidence of CV remodelling than patients with CKD 3 and less than patients with CKD 4 stage, indicating incomplete regression of CV calcifications and LVH after kidney transplantation. A decrease of renal function represents a significant risk factor for valvular and vascular calcifications occurrence in CKD patients.

Key words: chronic kidney disease, echocardiography, remodelling, transplantation, vascular calcification
INTRODUCTION

The prevalence of chronic kidney disease (CKD) in the world is increasing. Currently, about 11% of the world’s population is suffering from CKD (1,2). It is a complex disease with a high prevalence of cardiovascular diseases (CVD). Patients with CKD are exposed to severe hemodynamic changes, which ultimately lead to the development of CVD. It has been proven that CKD is an independent risk factor for cardiovascular (CV) complications such as arterial hypertension (3), left ventricular hypertrophy (LVH) (4), heart failure (5) and accelerated atherosclerosis (3). However, it is still scarce knowledge about the association between different stages of CKD and changes in cardiac structure and function in patients without a history of heart disease (6). Left ventricular hypertrophy and impaired left ventricular geometry are predictors of various adverse outcomes in general population, but knowledge about it in CKD is still insufficient (7).

Vascular calcifications (VC) are an independent risk factor associated with CVD and CV mortality in general population and in patients with CKD too. Several previous studies have demonstrated that pre-dialysis CKD patients have a high prevalence of VC (8, 9). Vascular calcifications occur in the intima and in the media of the arterial wall (10) and their occurrence in CKD is associated with an impaired homeostasis of calcium, phosphorus and parathyroid hormone (PTH), as well as with increased oxidative stress and loss of calcification inhibitors (11).

Results of a large number of studies conducted in the last decade have confirmed an increased prevalence of aortic valve calcifications among patients on hemodialysis in the range of 28-85%, which is significantly higher than in the general population, where aortic valve sclerosis was found in about 25% of subjects over 65 years. Only a few studies examined the prevalence of valvular calcifications in pre-dialysis patients with moderately impaired renal function (12,13).

Calcifications of the abdominal aorta are independently associated with CV events in patients on hemodialysis and in general population, but data on pre-dialysis CKD patients and in particular in RTR are still insufficient (14).

The aim of this study was to evaluate the relationship and connection between cardiovascular remodelling and estimated glomerular filtration rate (eGFR) in pre-dialysis CKD patients and in RTR without known heart disease.

PATIENTS AND METHODS

Patients and study design

One hundred nineteen CKD patients were included in a cross-sectional study conducted from November 2016 to June 2018 at the Clinical Centre of the University of Sarajevo (CCUS). All patients were recruited from the Nephrological Counselling Centre of the Nephrology Clinic. Forty five (27.6%) patients had a mean eGFR 46.6 (41.4-51.6) mL/min/1.73m² (CKD 3 stage) and 38 patients (27.6%) had an average value of eGFR 27.1 (19.8-29.7) mL/min/1.73m² (CKD 4 stage). Average value of eGFR in the RTR group (36, 22.1%) was 67.8 (57.3-73.7) mL/min/1.73m², and the average period after transplantation in this group was 5.7±2.0 years (range 4.0 - 8.5 years). A control group consisted of 44 patients with eGFR>60 mL/min/1.73m² and matching age and sex as in the test group.

The average age of patients was 55.9±9.3 years (range 40-65 years). All patients were without signs of heart failure and peripheral vascular disease. The study did not include CKD patients on dialysis treatment, patients with acute kidney injury, heart failure, primary hyperparathyroidism, mitral and aortic valve diseases, connective tissue disease, acute infectious diseases, and patients with a malignant disease.

Demographic and medical data, including age, smoking habits and comorbidities, had been taken from medical records or through interviews. The body mass index (BMI) measurement and measurement of systolic and diastolic blood pressure were performed in all patients. Routine laboratory findings, serum vitamin D, plasma parathormone, proteinuria, estimation of glomerular filtration rate, lateral lumbar X-ray and echocardiography were performed for each patient.

All patients gave informed consents to participate in the study. The study protocol was approved by the Ethic Committee of the Clinical Centre, University of Sarajevo and was conducted in accordance with all ethical standards of medical research and the Declaration of Helsinki.
Methods

All biochemical blood tests were performed with standard laboratory procedures at the Clinic for Biochemistry and Immunology of the CCUS. The level of vitamin D in the serum was determined at the Clinic for Nuclear Medicine using the standard radioimmunoassay method (Vitamin D 30-50 ng/mL) and the intact parathormone concentration (iPTH) in plasma by chemiluminescence method (PTH 10-65 pg/mL). Glomerular filtration rate was estimated using the Modification of Diet and Renal Disease Study Equation (MDRD) (15). For the classification of CKD the Kidney Disease Improving Global Outcomes (KDIGO) guideline was used (16).

At the Institute for Occupational Medicine of Canton Sarajevo an X-ray in lumbar lateral position with a soft exposure was performed to each patient to examine the presence of VC on the anterior and posterior wall of the abdominal aorta. The semi-quantitative scoring system developed by Kauppila et al. was used for estimating the total score of VC (17). Calcifications at the level of the first four lumbar vertebrae were analyzed on each segment separately for the anterior and posterior aortic wall. Calcific deposits were graded on a scale of 0-3 at each segment: grade 0 - no calcite deposits, grade 1 - calcite deposits less than 1/3 of the aorta wall, grade 2 - 1/3 to 2/3 wall calcified, and grade 3 - 2/3 or more of the aortic wall calcified. Summing up the grades of each segment the Kauppila score (A+P score) ranging from 0-24 points was obtained. Morphological changes on the anterior and posterior wall of aorta were graduated, so that the A+P≥6 marked moderate and severe changes.

Echocardiographic examination was performed on the Toshiba PowerVision 7000 ultrasound scanner, equipped with a 2.5 MHz transducer by a cardiologist who did not have access to clinical data of patients. The wall thickness and left ventricular diameter at the end-diastole, left ventricular mass, ejection fraction (EF), diastolic function based on the E/A ratio (ratio of early and late diastolic flow rates over the mitral valve orifice) and the diameter of the initial part of the abdominal aorta in the systole and diastole were examined. Left ventricular mass and left ventricular mass index (LVMI) were estimated by LV cavity dimension and wall thickness at end-diastole. Left ventricular mass index was calculated according to the formula: LV Mass (g) = 0.8 \{1.04 \{[LVEDD + IVSd + PWd] 3 - LVEDD3]\} + 0.6 (18).

LV hypertrophy (LVH) was defined as LV mass index > 115 g/m² in males and > 95 g/m² in females. Relative wall thickness (RWT) for the assessment of LV geometry was calculated using the formula: (thickness of interventricular septal wall + LV posterior wall in diastole)/LV diameter in diastole. According LV geometry patients were classified into: concentric hypertrophy, eccentric hypertrophy and normal (19). Relative wall thickness (RWT > 0.42) indicates concentric hypertrophy and RWT < 0.42 eccentric hypertrophy (18).

Statistical analysis

Descriptive statistics was used for data processing. The significance of differences in continuous independent variables that follow a normal distribution was tested by Student’s t test if two groups were investigated, or ANOVA test if three or more groups were followed. The significance of the difference in continuous variables that do not follow a normal distribution was tested by Mann Whitney or by the Kruskall Wallis test depending on how many groups were examined. Spearman and Pearson correlation coefficients were used to analyse the relationship between the observed variables. The multivariate linear regression analysis was used to analyse the association between monitored variables and vascular calcifications in observed patients. The level of significance was set at p<0.05.

RESULTS

Average values of erythrocytes and hemoglobin were higher in controls and RTR group in the comparison to the patients with CKD 3 and CKD 4 stage (p<0.001). The highest values of parathormone were found in CKD 4 stage, 127.5 (82.2-241.8) pg/mL, followed by RTR, 110.0 (91.5-221.5) pg/mL, CKD 3 stage, 65.7 (40.5-91.7) pg/mL, and controls, 43.3 (32.8-55.4) pg/mL (p<0.001). Patients with CKD 3 and CKD 4 stages had higher serum phosphorus levels than RTR: 1.1 (0.9-1.2) mmol/L, 1.1 (0.9-1.4) mmol/L and 0.9 (0.8-1.1) mmol/L, respectively (p<0.001). Vitamin D had the lowest value in the CKD 4 stage group (p<0.001) (Table 1).
The left ventricular mass as well as the LVMI were significantly higher in patients with CKD 4 and CKD 3 stage compared to RTR (p<0.001) and control group (p<0.001). Left ventricular hypertrophy was present in 74.7% CKD patients, most frequently in CKD 4 stage, while 50% of RTR and 15.9% control patients had LVH. Systolic function of LV presented as EF was significantly lower in patients with CKD 4 stage (p<0.001), as well as average values of the E/A ratio (parameter of diastolic function of LV), which were also lower in patients with CKD 4 stage compared to other observed groups (p=0.004) (Table 1).

Mitrail valve fibrosis was most common in patients with CKD 3 stage, 37 (82.2%), followed by RTR, 26 (72.2%), while calcifications were most commonly recorded in CKD 4 stage and RTR patients, 13 (34.2%) and nine (25.0%) patients, respectively. Fibrotic aortic valve was also the most common in patients with CKD 3 stage, 35 (77.8%). Calcifications of this valve were more frequently presented in patients with CKD 4 stage, 10 (26.3%) (Table 1).

Significant negative correlation between the LVMI and eGFR was determined (rho=-0.519; p<0.001) and a positive one between LVMI and PTH plasma levels (rho=0.379; p<0.001), serum phosphorus (rho=0.158; p=0.043), age (rho=0.389; p<0.001) and diabetes (rho=0.159; p=0.043) (Figure 1).
A significant negative correlation between calcifications of the heart valves and the eGFR (p<0.001) and a positive correlation with plasma levels of PTH, serum phosphorus, age and diabetes was confirmed (Table 2). The results of this study have confirmed the presence of LV hypertrophy in 74.7% CKD patients, predominantly concentric type, and in 50% RTR. This research suggests that LVH is very early present in the course of chronic renal disease since 62.2% of our patients with CKD 3 stage had LVH. Significantly larger numbers of patients with CKD 4 stage had LVH, indicating the association of renal function loss with cardiovascular remodelling.

Pluta et al. (3) found the presence of concentric LVH in 22.2% cases and eccentric hypertrophy in 18.9% patients with CKD, with significantly higher prevalence of LVH in patients with CKD 4 stage compared to CKD 3 stage and RTR (p<0.001) (Figure 2). Significant negative correlation between the abdominal aorta calcifications (A+P score) and the eGFR was confirmed (rho=-0.424; p<0.001).

In the multiple linear regression analysis, investigating independent risk factors as predictors of valve and vascular calcifications in chronic kidney disease, the level of eGFR was determined to be an independent negative predictor and a significant risk factor for calcifications on the mitral valve, 95% CI -0.007 (-0.011-0.003) (p=0.001) on the aortic valve, 95% CI -0.007 (-0.011-0.003) (p=0.001), as well as on the abdominal aorta walls, 95% CI -0.084 (-0.134-0.033) (p=0.001). It was also found that the size of left ventricular end-diastolic diameter represented a significant independent positive predictor of the occurrence of vascular (p<0.002), mitral valve (p<0.001) and aortic valve calcifications (p=0.017) in CKD (Table 3).

**DISCUSSION**

The results of this study have confirmed the presence of LV hypertrophy in 74.7% CKD patients, predominantly concentric type, and in 50% RTR. This research suggests that LVH is very early present in the course of chronic renal disease since 62.2% of our patients with CKD 3 stage had LVH. Significantly larger numbers of patients with CKD 4 stage had LVH, indicating the association of renal function loss with cardiovascular remodelling.

Table 2. The relationship between valvular calcifications and tested parameters

<table>
<thead>
<tr>
<th></th>
<th>Mitral valve calcifications</th>
<th>Aortic valve calcifications</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rho</td>
<td>p</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>0.421</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ca (mmol/L)</td>
<td>0.044</td>
<td>0.576</td>
</tr>
<tr>
<td>P (mmol/L)</td>
<td>0.152</td>
<td>0.043</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m2)</td>
<td>-0.459</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>age (years)</td>
<td>0.301</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.110</td>
<td>0.164</td>
</tr>
<tr>
<td>HTN (%)</td>
<td>0.093</td>
<td>0.236</td>
</tr>
<tr>
<td>DM (%)</td>
<td>0.149</td>
<td>0.037</td>
</tr>
<tr>
<td>smokers (%)</td>
<td>-0.018</td>
<td>0.822</td>
</tr>
</tbody>
</table>

**Table 3. Multivariate multiple regression model of independent risk factors for predicting valvular and vascular calcification in chronic kidney disease**

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Calculations on mitral valve</th>
<th>Calculations on aortic valve</th>
<th>A+P score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>p</td>
<td>β (95% CI)</td>
</tr>
<tr>
<td>PTH pg/mL</td>
<td>1.298E-005 (-0.001-0.001)</td>
<td>0.966</td>
<td>-9.205E-005 (-0.001-0.001)</td>
</tr>
<tr>
<td>Vit. D ng/mL</td>
<td>-0.001 (-0.010-0.009)</td>
<td>0.867</td>
<td>0.002 (-0.008-0.012)</td>
</tr>
<tr>
<td>LVIDd cm</td>
<td>0.230 (0.115-0.345)</td>
<td>&lt;0.001</td>
<td>0.148 (0.027-0.270)</td>
</tr>
<tr>
<td>IVSd cm</td>
<td>0.527 (-0.043-0.106)</td>
<td>0.069</td>
<td>10.010 (10.405-1.614)</td>
</tr>
<tr>
<td>LVMI g/m²</td>
<td>0.001 (-0.003-0.004)</td>
<td>0.752</td>
<td>0.000 (-0.004-0.003)</td>
</tr>
<tr>
<td>eGFR mL/min/1.73m2</td>
<td>-0.007 (-0.011-0.003)</td>
<td>0.001</td>
<td>-0.007 (-0.011-0.003)</td>
</tr>
</tbody>
</table>

**Note:** PTH, parathyroid hormone; Vit. D, vitamin D; LVIDd, left ventricular end-diastolic diameter; IVSd, interventricular septal end-diastolic dimension; LVMI, LV mass index; EF, ejection fraction; eGFR, estimated glomerular filtration rate
higher LVMI values in patients in the third stage of CKD compared to those in the first stage. In a three-year follow up study on 4,175 subjects Matsumita et al. (20) found that LV mass was significantly higher in patients with a lower glomerular filtration. The large CRIS study among 3,487 patients verified LVH in 32% of patients with eGFR ≥60 mL/min/1.73 m², in 48% with eGFR 45-59 mL/min/1.73 m², and in 57% with eGFR 30-44 mL/min/1.73 m² (6). Ali et al. found LVH in 76/135 (56.3%) outpatients with CKD; LVH was significantly higher in patients with CKD 4 stage (21). The LVH is associated with reduced myocardial capillary supply, interstitial fibrosis and thickening of the intramyocardial arteries, which increases the risk of ischemic damage and favor the development of arrhythmias, myocardial infarction and sudden cardiac death (22,23).

The results of our study suggest that an early occurrence of phosphorus and parathormon metabolism disorder in CKD patients, when the glomerular filtration rate is reduced below 60 mL/min, could affect the disposal of calcification deposits in soft tissues, cardiac arteries and blood vessel walls. In this process, phosphorus serves as a substrate deposited inside a media or intima of blood vessels, and also plays an important role in mediating the activation of transcription of certain genes in smooth muscle cells, resulting in their transformation into osteoblast-like cells (24). A disbalance of phosphorus in secondary hyperparathyroidism and advanced CKD leads to an increase in the mortality rate from CV events (25). On the other hand, parathormone acts as cardiotoxin in CKD and is a risk factor for increased cardiovascular morbidity in relation to the general population (26, 27). The analysis of the functional status of the left ventricle showed that systolic and diastolic parameters of LV function were significantly lower in subjects with CKD 3 and 4 stages compared to RTR.

In addition to the cardiac alterations, specific structural changes of the abdominal aorta are present and consist of importantly marked calcification of the aortic walls (28). Vascular calcifications affect arterial system and/or heart valves. Deposits of hydroxyapatite in the arterial wall occur either in the intima or in the media of blood vessels. Local vascular upregulation of pro-inflammatory and pro-osteogenic molecules are thought to be present in early CKD stages and they could be responsible for vascular calcifications (29).

Moderate to severe changes on the abdominal aorta determined by the Kauppila method (A+P score, KS≥6) were found in 87% CKD 4, 60% RTR and in 44% CKD 3 stage patients in our study. Gorriz et al. investigating the presence of VC using the Kauppila and Adragao method in a prospective observational study conducted in Spain in 39 centres among 742 non-dialysis patients (stages CKD 3-5) found vascular calcifications in 79% patients and strongly indicated in 47% (AS≥3 or KS> 6) (8). Peeters et al. (14) did not find calcifications in 28% patients; in 22% small (<4), and in 50% patients moderate and severe calcifications (≥4) were found (CAA score ≥4 was independently associated with cardiovascular events). Vascular calcifications are associated with a more stiffened arterial system contributing to increased cardiovascular disease in patients with chronic kidney disease (30).

Death with still functional graft is one of the main reasons for graft loss in patients with renal transplant. Cardiovascular events are the main cause of mortality in this population (36-55%). The influence of VC on the morbidity and mortality of patients with RTR is still not taken seriously enough (3.5-5.0% of RTR have a cardiovascular event per year). The prevalence of coronary artery calcifications is very high in these patients (61-75%) and higher than in patients with CKD 3 stage, but lower than in patients on hemodialysis (28).

Our research is one of the few that monitored the frequency and occurrence of VC in RTR and showed that VC was significantly less presented in this group of patients than in patients with CKD 4 stage, but it was still high. This can increase CV morbidity and mortality in this category of patients. The question of influence of the renal graft function on the presence of vascular and valvular calcification in relation to time remains still open, which could be a subject of future research on a large number of patients in different post-transplant time.

Mitral valve calcifications in our study were observed in 34.2% patients with CKD 4 stage, 25.0% RTR and in 6.7% patients with CKD 3 stage. Aortic valve calcifications were most often presented in CKD 4 (26.3%) and CKD 3 stage
(13.3%) patients. In the Framingham Offspring study valvular calcifications were more common in patients with advanced CKD, and the significance was established only for the mitral, but not the aortic valve (31), which is comparable with our results. Opposite of that, Rong et al. found calcifications in high percent on both valves, at the aortic (22.9%) and at the mitral (21.2%) valve in CKD patients (32).

Leskien et al. in a cross-sectional study on 135 patients with CKD (58 predialysis CKD patients, 36 hemodialysis patients and 41 RTR) found the combination of mitral and aortic valve calcifications in 31% pre-dialysis patients and in 29% RTR, but in 50% hemodialysis patients (33). In our study we confirmed the existence of valvular calcification in 38.5% of CKD patients and in 36.1% of RTR indicating that the occurrence of valve calcifications in CKD patients are related to the loss of renal function and type of renal replacement therapy.

The lack of our study is a relatively small number of patients, and larger studies are needed to evaluate the occurrence of calcifications and cardiovascular remodelling in pre-dialysis CKD patients and in renal transplant recipients.

In conclusion, the findings of this study suggest that local vascular pro-osteogenic molecules are present in pre-dialysis CKD patients and significantly contribute to the formation of CV calcifications and vascular remodelling. Impaired renal function represents a significant risk factor for the emergence and progression of valvular and vascular calcification in pre-dialysis CKD patients. However, RTR has a higher incidence of CV remodelling compared to patients with CKD 3, indicating incomplete regression of CV calcifications and LVH after successful kidney transplantation. Examination of ACC and valvular calcification in CKD patients could help identify patients at increased cardiovascular risk.

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**TRANSPARENCY DECLARATION**

Competing interests: None to declare.

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