Coronary artery calcification and coronary flow velocity in HD patients

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Abstract

Background. Decreased coronary flow reserve (CFR) is a marker of endothelial dysfunction, coronary artery calcification and inflammation, well-known cardiovascular risk factors in haemodialysis (HD) patients. In this study, we aimed to investigate the correlation of coronary artery calcification scores (CACS) with CFR in HD patients.

Methods. Sixty-four end-stage renal failure patients were enrolled in this study (38 males, 26 females). Thirty-nine healthy subjects (22 males, 17 females) were included in the control group. Biochemical parameters and acute-phase inflammation marker [high-sensitivity C-reactive protein (hs-CRP)] of patients were recorded before dialysis. The CACS were measured by electron beam computerized tomography method. CFR recordings were performed by trans-thoracic Doppler echocardiography. The relationship between CACS and CFR was evaluated.

Results. The mean CACS was 281 ± 589 and 29 patients had CACS < 10. Patients with CACS > 10 had significantly lower CFR values compared to patients with CACS < 10 (1.56 ± 0.38 vs 1.84 ± 0.53, P = 0.024). However, there was no difference in hs-CRP values between the groups. CFR was negatively correlated with CACS (r = −0.276, P = 0.030). In multiple stepwise regression analysis, CACS was found to be an independent variable for predicting CFR (P = 0.048). During a follow-up of 18 months, 10 patients had experience of cardiovascular events. Patients with CACS > 10 had significantly higher event rate [34.5% (10/29) vs 0% (0/24)] compared to those with CACS < 10 (P = 0.001). Patients who developed cardiovascular events had significantly higher mean CACS and lower CFR values than the remaining group (P = 0.019 and P = 0.039). All of four patients who died during follow-up were in the CFR < 2 and CACS > 10 groups.

Conclusions. CACS was associated with CFR in HD patients. However, we did not find any association of inflammation with CACS and CFR. This association between CFR and CACS might indicate two different (anatomical and functional) aspects of the common pathophysiology of the arterial system in HD patients.

Keywords: coronary artery disease; coronary flow reserve; electron beam computed tomography; haemodialysis; vascular calcification

Introduction

Cardiovascular mortality is a major cause of death in chronic kidney disease (CKD) [1,2]. In haemodialysis (HD) patients, cardiovascular complications such as endothelial dysfunction, atherosclerosis, valvular disease and left ventricular hypertrophy (LVH) are the most commonly encountered clinical challenges and the most prevalent rea-
sons for morbidity and mortality [1–5]. Therefore, the identification of coronary artery disease (CAD) in CKD is an important task for nephrologists. The assessment of coronary artery stenosis severity depends on either determination of the anatomic dimensions of the stenosis by angiographic and non-invasive techniques or assessment of the functional significance of the stenosis by measurement of its effect on blood flow. The coronary artery calcification score (CAC) in uraemic patients undergoing HD reflects the severity of atherosclerotic vascular disease and predicts the cardiovascular events [6–9]. Electron beam computerized tomography (EBCT) is non-invasive and quantitatively measures the CACS which is associated with the presence of atherosclerotic plaques [6,7]. Although, in CKD patients, coronary artery calcification (CAC) can occur in the absence of occlusive coronary atherosclerosis, recent studies of CKD patients have shown the association of CAC with obstructive atherosclerosis [7]. Haydar et al. reported that severe CAC predicted the presence of CAD with 92.8% sensitivity and 44% specificity in HD patients [7]. However, on the basis of anatomic studies, one cannot determine the presence and extent of ischaemia, and functional testing will remain necessary to decide whether revascularization or medical therapy is indicated. Another non-invasive test, coronary flow reserve (CFR) detected by trans-thoracic Doppler echocardiography (TTDE), represents the capacity of the coronary circulation to dilate following an increase in myocardial metabolic demands and provides information for functional significance of CAD [10]. By using this method, impairment of CFR can be assessed before the development of angiographically detectable stenosis in epicardial coronary arteries and early coronary microvasculature pathology can be investigated [11]. CFR impairment was an early marker of subclinical coronary atherosclerosis and associated with endothelial dysfunction [12]. CFR value < 2.0 had a sensitivity of 92% and a specificity of 82% for the presence of significant CAD [13]. Impairment of coronary microvascular functions and decreased CFR has recently been reported in HD patients [14]. Although coronary EBCT is a promising diagnostic tool to evaluate CAD in HD patients, its correlation with CFR has not yet been investigated. The aim of the present study is to investigate the correlation of CACS with CFR in HD patients without known CAD.

Materials and methods

Study groups

In this study, 64 (38 males, 26 females) chronic HD patients and 39 (22 males, 17 females) normotensive healthy controls were enrolled for the assessment of CAC by EBCT and CFR by TTDE between April 2008 and June 2008 (Table 1). A review of medical records including information on age, sex, weight, duration of HD treatment, the aetiology of CKD, CVD risk factors and cardiac functions was undertaken. Patients younger than 70 years of age and treated for more than 6 months with HD were included in the study. Time spent on dialysis was 88 ± 42 months (range, 24–218 months). In the HD study group, patients were receiving thrice weekly dialysis for a 4-h period with a standard bicarbonate-containing dialysate bath, using biocompatible HD membrane (Polysulfone, FX-80 series, Fresenius, Germany). Blood flow rates ranged from 250 to 300 ml/min, while dialysate flow rate was kept constant at 500 ml/min. All HD patients were maintained at their target dry body weight and received an adequate dose of dialysis (double pool Kt/V ≥ 1.4). Exclusion criteria were: valvular heart disease, any prior coronary intervention, congestive heart failure, cardiac arrhythmia and active infection or non-infectious overt inflammation. Patients whose left anterior descending (LAD) coronary artery could not be visualized adequately by Doppler echocardiography were also excluded. A total of 88 HD patients were evaluated and 24 (27.2%) patients were not enrolled in the study cohort. Of these 24 patients, 12 (13.6%) patients had previously documented CAD and coronary intervention, four (5.4%) patients had valvular heart disease, four (5.4%) patients had cardiac arrhythmia and three (3.4%) patients had congestive heart failure without CAD. One (1.1%) patient was excluded because the LAD coronary artery could not be visualized adequately by Doppler echocardiography. For all enrolled patients, the patients' charts were reviewed and the data for cardiovascular events including new-onset angina, nonfatal myocardial infarction, angioplasty or coronary artery bypass surgery or cardiac death between April 2008 and November 2009 were collected. Our examinations of the patients conformed to good medical and laboratory practices and the recommendations of the Declaration of Helsinki on Biomedical Research Involving Human Subjects. This study was approved by the Ethical Committee of Istanbul School of Medicine. This study is registered with ClinicalTrials.gov, number NCT00921089.

Laboratory data

Fasting serum samples were obtained in the early morning for biochemical analyses. All biochemical blood samples were collected before the mid-week HD session in the study group. Most laboratory values including complete blood cell counts and serum levels of urea nitrogen, creatinine, electrolytes, calcium, phosphorus, total protein, albumin, total cholesterol, triglycerides and intact parathyroid hormone were measured. As a marker of inflammation, serum high-sensitivity C-reactive protein (hs-CRP) levels were measured.

Coronary flow measurement

The coronary flow velocity recordings were performed to all 105 subjects in the study or control group by a single investigator who was experienced in this data acquisition. One subject in each group was not suitable for measurement of CFR due to poor acoustic windows. CFR recordings were performed with the Vivid 7 echocardiography device (General Electric, USA) using a middle-range-frequency (3–8 MHz) broadband transducer in the LAD coronary artery by TTDE, as has been previously described [10]. The acoustic window was around the mid-clavicular line in the fourth and fifth intercostal spaces in the left lateral decubitus position. The left ventricle (LV) was imaged in the long-axis cross-section and the ultrasound beam was inclined laterally. The coronary blood in the mid-to-distal LAD coronary artery was examined by colour Doppler flow mapping guidance with the optimal velocity range (+12 to +15 cm/s). Then, the sample volume (1.5 or 2.0 mm wide) was positioned on the colour signal in the LAD coronary artery: Variables of the LAD coronary artery velocity were measured by fast Fourier transformation analysis. After baseline recordings of flows, dipyridamole (0.56 mg/kg; Persantin, Boehringer Ingelheim) was infused over a 4-min period. An additional infusion of dipyridamole (0.28 mg/kg over a 2-min period) was used if the heart rate did not exceed a 10% increase from the baseline. Two minutes after the end of the infusion, hyperaemic spectral profiles in the LAD coronary artery were recorded. All images were recorded for playback analysis and were later measured off-line. Average diastolic peak flow velocity (APDv) and average mean diastolic flow velocity were measured at baseline and under hyperaemic conditions. CFR was defined as the ratio of ADPV at hyperaemia to ADPV at baseline. The intra-observer variability of CFR measurement was 3.6% in the current study. CFR ≥ 2.0 was considered normal in the present study [15,16]. LV mass was calculated from M-mode records taken on parasternal long-axis images according to Devereux’s formula [17].

All of the measurements were performed between 8 and 9 AM and all of the subjects abstained from caffeine-containing drinks for at least 12 h before testing. TTDE examinations were performed on the day after dialysis in all HD patients.

Electron beam computed tomography examination

Eligible patients for the study group were scheduled to have an EBCT scan within 15 days after their CFR recordings for research purposes only. EBCT
Clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>HD group</th>
<th>Control group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>41 ± 13</td>
<td>37 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.4 ± 3.7</td>
<td>23.4 ± 4.1</td>
<td>NS</td>
</tr>
<tr>
<td>Time spent on dialysis (months)</td>
<td>87 ± 42</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Aetiologies of ESRD [n [%]]

- Hypertensive nephrosclerosis: 16 (25)
- Diabetic nephropathy: 8 (12.5)
- Chronic pyelonephritis: 7 (11)
- Chronic glomerulonephritis: 7 (11)
- Polycystic kidney disease: 2 (3)
- Unknown: 24 (37.5)

Laboratory data

<table>
<thead>
<tr>
<th>Laboratory parameters</th>
<th>HD group</th>
<th>Control group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (mmHg)</td>
<td>113 ± 19</td>
<td>113 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>71 ± 16</td>
<td>72 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>11.40 ± 1.65</td>
<td>14.18 ± 1.53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>8.73 ± 0.80</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>4.93 ± 1.30</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>iPTH (pg/dl)</td>
<td>364 ± 388</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.99 ± 0.29</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>185 ± 105</td>
<td>129 ± 42</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>165 ± 36</td>
<td>188 ± 31</td>
<td>NS</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>3.99 ± 0.29</td>
<td>1.45 ± 2.30</td>
<td>NS</td>
</tr>
<tr>
<td>CFR</td>
<td>1.69 ± 0.28</td>
<td>2.65 ± 0.66</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Statistical analysis

The statistical analysis was carried out by the Statistical Package for Social Sciences for Windows ver. 15.0 (SPSS Inc., Chicago, IL, USA). Data are expressed as the mean ± SD, with a significance level of P < 0.05. For dichotomous variables, the frequency of positive occurrences are given along with their corresponding percentages. Statistical comparisons of individual groups were based on Student’s t-test for continuous variables and on Fisher’s exact test for discrete variables. The Kruskal–Wallis with post-hoc test and one-way analysis of variance with Scheffe post hoc test evaluated unrelated observations between groups, whereas repeated-measures analysis of variance with Scheffe post hoc analysis determined group differences between repeatedly measured variables. Relationships were determined with Pearson’s correlation coefficient.

Results

Patient baseline characteristics

The baseline characteristics of the 64 patients (mean age, 41 ± 13 years) and 39 controls (mean age, 37 ± 9 years) were given in Table 1. The number of former or current smokers in the HD and control groups were 16 (25%) and 13 (33%), respectively. In the HD study group, hypertension and diabetes were noted in 15 (23.4%) and eight (12.5%) patients, respectively. There were no differences in age, gender, body mass index (BMI),
smoking status and pre-dialysis systolic and diastolic blood pressure levels between the two groups (Table 1). Among laboratory parameters, there were no significant differences in levels of serum cholesterol, triglyceride and hs-CRP between the study groups. Haemoglobin levels were significantly lower in the HD group than the control group, as expected ($P < 0.001$) (Table 1).

Electron beam computed tomography examination

The mean CACS of 64 patients was $281 \pm 589$ with a median of 20.8 (interquartile range, 0.40–193.55). According to the guidelines on interpretation of calcium scores proposed by Rumberger et al. [19], lesions were minimal (score, $\leq 10$) in 29 (45.3%) subjects, mild (score, 11 to 100) in 14 (21.8%), moderate (score, 101 to 400) in eight (12.5%) and severe with a high probability for stenosis (score, $>400$) in 13 (20.3%) subjects. Eight of our patients (12.5%) had no CACS.

Age, time on dialysis, haemoglobin levels and serum total cholesterol levels were significantly higher in patients with CACS $>10$ compared to patients with CACS $\leq 10$. However, serum albumin levels in patients with CACS $>10$ were lower (Table 2). In addition, patients with CACS $>10$ had significantly lower CFR values compared to patients with CACS $\leq 10$ (Table 2) (Figure 1). However, there were no differences in hs-CRP levels between the study groups. Patients with CACS $>10$ had significantly lower ADPV values at hyperaemia ($r = -0.258$, $P = 0.046$) compared to patients with CACS $\leq 10$ ($59.72 \pm 20.78$) ($P = 0.04$), although there were no differences regarding ADPV values at baseline between the two groups.

In the univariate correlation analysis, CACS was positively correlated with age, time on dialysis, haemoglobin levels, serum cholesterol levels and triglyceride levels. CACS was negatively correlated with albumin ($r = -0.308$, $P = 0.015$) and CFR ($r = -0.276$, $P = 0.030$) (Figure 2). When the CACS of coronary arteries were separately evaluated, CACS of the LAD coronary artery was also negatively correlated with CFR ($r = -0.252$, $P = 0.048$). The correlation of CACS with ADPV at baseline was also examined, but no correlation was found ($r = -0.087$, $P = 0.50$). However, the total CACS and CACS of the LAD coronary artery were significantly correlated with the ADPV at hyperaemia ($r = -0.25$, $P = 0.048$ and $r = -0.27$, $P = 0.032$, respectively).

Coronary flow measurements

On TTDE examination, CFR values were significantly lower in the HD group ($1.69 \pm 0.48$) than in the control group ($2.65 \pm 0.66$) ($P < 0.001$) (Table 1). In 49 of 64 (76.6%) HD patients, CFR was $<2.0$; however, in one of 39 (2.6%) healthy controls, it was $\approx 2.0$ ($P < 0.001$). There were no correlations between CFR and BMI, time on HD, systolic and diastolic blood pressures, triglyceride levels and hs-CRP levels. Age ($r = -0.269$, $P = 0.036$) and serum total cholesterol levels ($r = -0.279$, $P = 0.017$) were correlated with CFR values in the HD group. There was no correlation between hs-CRP levels and CFR values. Multiple stepwise regression analysis was performed to predict CFR. Age, serum cholesterol levels and CACS were taken as independent variables. CACS was found to be an independent variable for predicting CFR ($r = 0.26$, standardized $\beta = -0.259$, $P = 0.048$). However, age and serum cholesterol levels were not predictors of CFR.

When the HD study group was divided into two subgroups, according to CFR measurements ($CFR < 2$ and $CFR \geq 2$), age, serum cholesterol levels and triglyceride levels were significantly higher in the CFR $<2$ group than the CFR $\geq 2$ group (Table 3). The CACS of the CFR $<2$ group ($358 \pm 653$) was significantly higher than the patients in the CFR $\geq 2$ group ($101 \pm 258$) ($P = 0.046$) (Figure 3).

Longitudinal follow-up results for cardiovascular events

During a follow-up of 18 months, six patients died [cardiovascular disease ($n = 4$), malignancy ($n = 2$)] and nine of them have undergone transplantation. Ten patients had experience of cardiac events: four of them died of myocardial infarction, six underwent coronary angiography
because of new-onset angina and three of them required coronary artery bypass surgery. In the group of patients with low CFR (<2), cardiovascular event rate was 21.4% (n = 9/42) compared with 9% (n = 1/11) in patients with normal CFR (≥2) (P = 0.352). Patients with CACS > 10 had significantly higher event rate [34.5% (10/29) vs 0% (0/24)] compared to those with CACS ≤ 10 (P = 0.001). On the other hand, patients who developed cardiovascular events within the 18-month period had significantly higher mean CACS compared to others (1017 ± 975 vs 129 ± 336, P = 0.019). In addition, the mean CFR values of the patients who developed cardiovascular events [1.40 ± 0.43 (n = 10)] were also significantly lower than the remaining group [1.70 ± 0.40 (n = 43)] (P = 0.039). All of the four patients who died during the follow-up were in the CFR < 2 and CACS > 10 groups.

**Table 3.** Demographic and clinical characteristics of the subgroups of HD patients according to CFR values (mean ± SD)

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>CFR &lt; 2 group (n = 49)</th>
<th>CFR ≥ 2 group (n = 15)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>29/20</td>
<td>9/6</td>
<td>NS</td>
</tr>
<tr>
<td>Age</td>
<td>44 ± 13</td>
<td>35 ± 13</td>
<td>0.026</td>
</tr>
<tr>
<td>Time spent on dialysis (months)</td>
<td>91 ± 43</td>
<td>78 ± 39</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.9 ± 4.4</td>
<td>22.0 ± 3.4</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>112 ± 21</td>
<td>121 ± 13</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>71 ± 15</td>
<td>71 ± 21</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline heart rate (bpm)</td>
<td>85 ± 12</td>
<td>84 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>Laboratory data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>11.5 ± 1.5</td>
<td>11.0 ± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Serum albumin (g/dl)</td>
<td>3.97 ± 0.28</td>
<td>4.08 ± 0.32</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>172 ± 32</td>
<td>139 ± 36</td>
<td>0.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>200 ± 114</td>
<td>134 ± 40</td>
<td>0.001</td>
</tr>
<tr>
<td>hs-CRP (mg/dl)</td>
<td>1.57 ± 2.43</td>
<td>1.10 ± 1.89</td>
<td>NS</td>
</tr>
<tr>
<td>CACS</td>
<td>358 ± 653</td>
<td>101 ± 258</td>
<td>0.046</td>
</tr>
</tbody>
</table>

M, male; F, female; CFR, coronary flow reserve; NS, not significant; hs-CRP, high-sensitivity C-reactive protein; CACS, coronary artery calcification score.

In the present study, CFR measured by TTDE during pharmacological coronary vasodilation corresponded closely to quantitative CACS measurements of the coronary artery, especially the LAD coronary artery. To our knowledge, this is the first study to show that the assessment of CAC by EBCT correlates with the functional significance of CAD measured by CFR.

CACS is regarded as an index of the severity of atherosclerotic vascular disease, helps to anatomically assess the CAD and may also predict future adverse cardiovascular events, especially in patients on dialysis [18–22]. The CFR measurement which reflects coronary microvascular function and endothelial functions of epicardial coronary arteries by TTDE, as a cheaper and easy screening test, may be used as a detection method in the assessment of major epicardial coronary arteries [10–13]. Measurement of CFR during maximal pharmacological vasodilation (vasodilator reserve) has been used to examine the functional consequences of a stenosis on perfusion in the LAD coronary artery. A CFR of <2 may be evidence of severe CAD [10–13]. Although the accuracy of EBCT in evaluating coronary anatomy is improving, the relation between anatomical and functional assessment of coronary artery remains unclear. Therefore, we would like to examine the correlation between CAC severity and flow reserve in the LAD coronary artery by simultaneously performing these two tests in a cross-sectional cohort of HD patients. The main result of our investigation is that there is an association between CFR and CACS. The association between CFR and CAC may suggest that CFR is a marker for the development of atherosclerotic CAD and could be a powerful non-invasive tool to assess cardiovascular risk in patients with CKD.

Arterial calcification might occur in two different locations of the artery: the tunica intima and the tunica media. Intimal calcification is a marker of atherosclerotic disease and is associated with severity of stenotic lesions. However, medial calcification might also influence outcome by promoting arterial stiffening whose principal consequences are development of LVH and altered coronary perfusion. Recent studies suggested the association of occlusive coronary intimal atherosclerosis with CACS in CKD patients [6,7]; however, this is not confirmed by other studies [23]. In an autopsy study, it was reported that HD patients had heavy calcification involving the intima and media layers with severe atherosclerosis [22]. Association of CACS with CFR may represent an inappropriate vasodilatory response to pharmacological stimulus due to the accumulation of calcium in the media layer. In addition, calcium deposition in the intima, which may impair endothelial function, could be another possible mechanism for decreased CFR. Therefore, it is not clear whether CFR impairment (failure of coronary artery vasodilation to pharmacological stimulus) is due to medial calcification or endothelial dysfunction itself [6,7]. Although EBCT provides a sensitive method for detecting coronary calcification, it cannot differentiate between intimal plaque calcification and media calcification, of which the latter occurs mainly in patients with uraemia [24].
vascular ultrasonography may be helpful in the identification of coronary calcification site.

Some inflammation markers, i.e. hs-CRP, fibrinogen and interleukin-6, have been found to be associated with atherosclerosis, which was also reported for uremic population [25–27]. However, in this study, no correlation was found between hs-CRP levels and CACS or CFR. Possibly, the lower level of inflammation and CACS in the present study patients as a result of strict exclusion criteria to eliminate the other factors that may affect CFR measurements might play a role in this finding.

Many well-defined cardiovascular risk factors may be present in individuals who require dialysis. In our study, mean age, serum cholesterol levels and triglyceride levels, which are known cardiovascular risk factors, were significantly higher in patients with impaired CFR. Serum total cholesterol levels were also negatively correlated with CFR values, which is a finding that is previously reported also in the normal population.

Conclusion

This study shows that anatomical assessment of coronary artery lesion by EBCT correlated with the functional severity of lesions measured by CFR. HD patients had lower CFR values and higher CACS, which may be regarded as an early finding of an affected cardiovascular system. This association might indicate two different (anatomical and functional) aspects of the common pathophysiology of the arterial system in HD patients. Further studies to determine the prognostic value of these EBCT-derived scores and CFR values in daily clinical practice are required.

Conflict of interest statement. None declared.

References


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