The Relationship between Epicardial Adipose Tissue and Malnutrition, Inflammation, Atherosclerosis/Calcification Syndrome in ESRD Patients

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Summary
Background and objectives Malnutrition, inflammation, atherosclerosis/calcification (MIAC) and endothelial dysfunction are the most commonly encountered risk factors in the pathogenesis of cardiovascular disease in ESRD patients. Epicardial adipose tissue (EAT) is the true visceral fat depot of the heart. The relationship between CAD and EAT was shown in patients with high risk of coronary artery disease. In this study, we aimed to investigate the relationship between EAT and MIAC syndrome in ESRD patients.

Design, setting, participants, & measurements Eighty ESRD patients and 27 healthy subjects enrolled in this cross-sectional study. EAT and coronary artery calcification score were measured by a multidetector computed tomography (MDCT) scanner. Patients with serum albumin <3.5 mg/dl were defined as patients with malnutrition; those with serum C-reactive protein level >10 ng/dl (normal range, 0–5 ng/dl) had inflammation; and those with CACS >10 had atherosclerosis/calcification.

Results Total CACS and EAT measurements were significantly higher in ESRD patients when compared with healthy subjects. There was a statistically significant relationship between EAT and CACS in ESRD patients (r = 0.48). EAT measurements were higher in PD patients than HD patients. Twenty-four of the patients had no component, 31 had one component, 17 had two components, and nine had all of the MIAC components. EAT was found to be significantly increased when the presence of MIAC components increased. EAT was positively correlated with age, body mass index, and presence of MIAC. These parameters were also found as independent predictors of increased EAT.

Conclusions We found a relationship between EAT and components of MIAC syndrome in ESRD patients.

Introduction Cardiovascular diseases (CVD) are the most common cause of mortality and morbidity in patients with ESRD receiving hemodialysis (HD) and peritoneal dialysis (PD) (1). Malnutrition, inflammation, atherosclerosis, endothelial dysfunction, coronary artery calcification (CAC), and left ventricular hypertrophy are the most commonly encountered risk factors in the pathogenesis of CVD in ESRD patients (2–4). Malnutrition, inflammation, atherosclerosis/calcification (MIAC) syndrome has been defined as the interaction between increased levels of proinflammatory cytokines, malnutrition, and atherosclerosis/calcification in ESRD patients (5,6). The presence of MIA components was found to be associated with increased mortality and morbidity in ESRD patients receiving PD (7) or HD (8). The coronary artery calcification score (CACS) in patients with ESRD reflects the severity of atherosclerotic vascular disease and predicts cardiovascular events (9,10). Epicardial adipose tissue (EAT) is the true visceral fat depot of the heart that accounts for approximately 20% of total heart weight, covers 80% of the cardiac surfaces, and is mostly in the grooved segments along the paths of coronary arteries (11–13). Recent studies showed a close relationship between coronary artery disease (CAD) and EAT using multidetector computed tomography (MDCT) and echocardiography in healthy subjects and patients at a high risk of CAD (14–17). In a recent study, the authors concluded that EAT acts as an extremely active organ that produces several bioactive adipokines as well as proinflammatory and proatherogenic cytokines such as tumor necrosis factor–α, monocyte chemotactic protein-1, IL-6, and resistin (16,18–21). Levels of most of these cytokines are also increased in ESRD patients (22–24). It is therefore reasonable to postulate that EAT is a source of inflammatory signals in patients with ESRD. Studies focusing on the association between the MIAC syndrome and EAT in ESRD patients are lacking. In this study, we investi-
gated the relationship between EAT and MIAC components in ESRD patients.

**Study Population and Methods**

The study protocol was approved by the Medical Ethics Committee of Selcuk University (Meram School of Medicine, Konya, Turkey). Written informed consent was obtained from all of the subjects included in the study.

This was a cross-sectional study involving 80 ESRD patients (31 women, 49 men; mean age, 49 ± 14 years) receiving PD or HD for ≥6 months in the dialysis unit of Selcuk University and 27 healthy control subjects (14 women, 13 men; mean age, 54 ± 12 years) between February and June 2009. The Minitab 16 statistical program (Minitab, State College, PA) was used to determine sample size. The minimal sample volume was used to determine a difference of 20 cm³ in EAT with 80% power, and the 95% confidence interval was calculated to be 79.

Patients aged 18 to 70 years willing to participate in the assessment of CAC and EAT by MDCT were screened. A review of medical records (including information on age, gender, weight, duration of renal replacement treatment, medications, and primary disease of ESRD) was undertaken. Exclusion criteria were: (1) congestive heart failure; (2) active infection; (3) autoimmune disease; (4) secondary hyperparathyroidism; and (5) nephrotic-range proteinuria. Ninety-five patients were evaluated, and 15 patients were excluded from the study. Of these 15 patients, five patients had congestive heart failure (New York Heart Association class III–IV); four patients had active infection; three patients had secondary hyperparathyroidism; and three patients had autoimmune disease (including systemic lupus erythematosus and microscopic polyangiitis). None of the patients included in the study had nephrotic-range proteinuria and arrhythmia on the basis of electrocardiography. The remaining 80 ESRD patients fulfilled the above criteria and were enrolled in the study. Twenty-seven age-matched and gender-matched healthy individuals referred from outpatient clinics of the Internal Medicine Department of Selcuk University were also enrolled as control subjects. They were subject to the same inclusion and exclusion criteria as the patients. HD patients were receiving thrice-weekly dialysis for a 4-hour period with a standard bicarbonate-containing dialysate bath using a biocompatible HD membrane (Polysulfone, FX-80 series, Fresenius, Germany). Dialysate flow rates were 500 ml/min, and blood-flow rates were 250 to 300 ml/min. The systolic BP (SBP) and diastolic BP (DBP) of patients and healthy subjects were measured in the upright sitting position after ≥5 minutes of rest using an Erka sphygmomanometer (PMS Instruments Limited, Berkshire, UK) with an appropriate cuff size. Two readings were recorded for each individual. The mean value of two readings was defined as the BP. Patients with SBP and DBP >140 and <90 mmHg, respectively, or who were already on antihypertensive treatment were assumed to be hypertensive.

Twenty-three patients were taking antihypertensive drugs (14 of them on angiotensin-converting enzyme inhibitors; eight receiving an angiotensin-II receptor blockers; and one receiving a calcium channel blocker and an angiotensin-converting enzyme inhibitor). Thirty-two patients were taking calcium containing phosphate binders.

**Biochemical Analyses**

Venous blood samples for biochemical analyses were drawn after an overnight fast before first exchange in PD patients and before the midweek session in patients receiving HD. All of the biochemical analyses, including those for total cholesterol, LDL cholesterol, HDL cholesterol, and plasma triglyceride concentrations, were undertaken using an oxidase-based technique by the Roche/Hitachi Modular System (Mannheim, Germany) in the Central Biochemistry Laboratory of the Meram School of Medicine.

**MIAC Components**

In this study, the serum levels of albumin and C-reactive protein (CRP), as well as CACS, were used to assess malnutrition, inflammation and atherosclerosis/calcification, respectively, just as in studies evaluating high cardiovascular mortality and morbidity in ESRD patients (25). Therefore, patients with a serum albumin of <3.5 mg/dl were defined as patients with malnutrition; those with a serum CRP level of >10 ng/dl (normal range, 0 to 5 ng/dl) had inflammation; and those with a CACS of >10 had atherosclerosis/calcification. The patients were classified according to the presence of MIAC components. Hence, patients without any component are classified as MIAC 0; those with one of the components are classified as MIAC 1; those with two of the components are classified as MIAC 2; and those with all of the components are classified as MIAC 3.

**Evaluation of CACS and EAT**

Unenhanced coronary computed tomography (CT) was quantified on retrospectively electrocardiography-gated cardiac CT using 64-slice MDCT (Sensation 64 Siemens Medical Solutions, Erlangen, Germany). The coronary CT protocol was: slice collimation, 64 × 0.6 mm; gantry rotation time, 0.33 seconds; pitch, 0.2; tube voltage, 120 kV; and tube current, 600 mA. If the heart rate was >65 beats per minute, heart rate control was achieved with a β-blocker. Multiplanar data reconstructions were obtained in standardized ventricular short-axis planes at the basal, mid-cavity, apical, and the horizontal long-axis plane with 3-mm slice thickness and 2-mm slice interval (26). To quantify CAC, all of the reconstructions were transferred to a personal computer-based workstation (Synog CaScoring Wizard, Siemens Medical Solutions). CACS was defined as >2 contiguous pixels with Hounsfield units >130 as designed by Agatston et al. (27). All of the values of the left anterior descending coronary artery, circumflex coronary artery, and right coronary artery were added to calculate the CACS. In this study, CACS <10 was defined as the absence of atherosclerosis/calcification, and CACS >10 was defined as the presence of atherosclerosis/calcification. The rationale of using >10 as a cut off for CAC was that the functional deterioration of coronary arteries starts from low levels of CACS (e.g., >10) (38). To quantify EAT volume, all of the reconstructions were transferred to the personal computer-based workstation. A CT attenuation threshold between -200 and -20 Hounsfield units was used to isolate epicardial fat. Measurements of EAT and CACS were evaluated by two radiologists blinded to the study protocol. The interobserver variability was <10%.
Statistical Analyses
Statistical analyses were carried out using the Statistical Package for Social Sciences for Windows version 15.0 (SPSS, Chicago, IL). The parametric data are the means ± SDs, and the nonparametric data are the median values (interquartile range). The normal distribution of all variables was tested using the Kolmogorov–Smirnov test. Dichotomous variables were compared using the chi-squared test. Statistical differences between parametric data of two groups were analyzed using the Kolmogorov–Smirnov test. Differences between subgroups was tested using the Mann–Whitney U test. The Mann–Whitney U test was used to determine differences between nonparametric data. Statistical differences among parametric data of three groups were analyzed using the one-way ANOVA test, and the difference between subgroups was assessed with the post hoc Tukey test. Linear associations between continuous variables were assessed using the Spearman correlation test. Multivariate linear regression analyses were undertaken to determine independent associations among EAT and other variables. Age, body mass index (BMI), dialysis vintage, presence of MIAC, SBP, and diabetes mellitus (DM) were entered into the regression model as independent variables, and EAT was entered as a dependent variable. The backward elimination method was preferred in the stepwise regression analysis, and 0.1 was used as a criterion for elimination in this model. At the end of the fourth step, three variables including age, BMI, and the presence of MIA remained statistically significant in the model. P < 0.05 was considered significant for all tests.

Results
Baseline Characteristics of Patients
The baseline characteristics of 80 ESRD patients and 27 healthy subjects are shown in Table 1. The etiology of ESRD patients was diabetic nephropathy (n = 18), chronic glomerulonephritis (n = 12), hypertensive nephropathy (n = 23), polycystic kidney disease (n = 7), nephrolithiasis (n = 5), and unknown (n = 15). There were no differences with respect to the following variables between ESRD patients and healthy subjects: age, gender, BMI, predialysis levels of SBP and DBP, serum levels of LDL cholesterol, HDL cholesterol, triglyceride, and CRP.

Evaluation of CACS and EAT
Total CACS and EAT measurements were significantly higher in ESRD patients compared with healthy subjects (Table 1; P = 0.01 and P = 0.02, respectively). There was a statistically significant relationship between EAT and CACS in ESRD patients (P < 0.0001, r = 0.48). When patients were classified according to their renal replacement therapy (HD and PD), EAT measurements were higher in PD patients than in HD patients (155.9 ± 69.0 versus 139.4 ± 62.7 cm³), but this difference was NS (P = 0.30) (Figure 1).

MIAC Components
Patients were classified according to MIAC components. Twenty-four patients had none, 30 had one component, 17 had two components, and nine had all MIAC components. EAT measurements of the patients according to MIAC components are shown in Table 2. EAT was significantly increased if the number of MIAC components increased (Figure 2).

Linear Correlations
In the bivariate correlation analysis, EAT was positively correlated with age and BMI (r = 0.44, P = 0.001 and r = 0.44, P = 0.001, respectively). The multivariate linear regression analysis revealed that age, as well as the presence

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ESRD Patients (n = 80)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Mean ± SD) or Median (IQR)</td>
<td></td>
</tr>
<tr>
<td>Healthy Subjects (n = 27)</td>
<td>(Mean ± SD) or Median (IQR)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>54 ± 12</td>
<td>49 ± 14</td>
</tr>
<tr>
<td>Men/women</td>
<td>14/13</td>
<td>49/31</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>26.2 ± 5.5</td>
<td>26.7 ± 5.1</td>
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<tr>
<td>Dialysis vintage (months)</td>
<td></td>
<td>56 ± 4</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>132 ± 22</td>
<td>138 ± 28</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>80 ± 11</td>
<td>86 ± 16</td>
</tr>
<tr>
<td>Hemoglobin (mg/dl)</td>
<td>12.5 ± 2.1</td>
<td>11.9 ± 1.7</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.8 (3.5–4.0)</td>
<td>3.7 (3.4–3.9)</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>21 ± 4</td>
<td>18 ± 6</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>16 ± 5</td>
<td>17 ± 7</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>94.3 ± 6.0</td>
<td>119.8 ± 35</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>37.3 ± 11.6</td>
<td>37.4 ± 14</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>122.1 ± 86</td>
<td>147 ± 94</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>8.9 ± 0.8</td>
<td>8.5 ± 0.8</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>4.3 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>8.25 (3.75–14.5)</td>
<td>9.6 (6.1–17.1)</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>353 ± 309</td>
<td></td>
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<tr>
<td>Total CACS</td>
<td>41 ± 112</td>
<td>144 ± 278</td>
</tr>
<tr>
<td>Epicardial adipose tissue (cm³)</td>
<td>121.5 ± 37.5</td>
<td>160 ± 76</td>
</tr>
</tbody>
</table>

AST, aspartate aminotransferase; ALT, alanine aminotransferase; PTH, parathyroid hormone; CACS, coronary artery calcification score; BMI, body mass index; SBP, systolic BP; DBP, diastolic BP; CRP, C-reactive protein; IQR, interquartile range.
of MIAC and BMI, were independent predictors of increased EAT. Regression results are shown in Table 3.

Table 2. The relationship between EAT and MIAC components in ESRD patients

<table>
<thead>
<tr>
<th>Number of MIAC Components</th>
<th>Number of ESRD Patients (n = 80)</th>
<th>EAT (cm³) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>24 (30%)</td>
<td>121 ± 51</td>
</tr>
<tr>
<td>One component</td>
<td>30 (38%)</td>
<td>145 ± 61</td>
</tr>
<tr>
<td>Two components</td>
<td>17 (21%)</td>
<td>152 ± 75</td>
</tr>
<tr>
<td>All components</td>
<td>9 (11%)</td>
<td>221 ± 101a</td>
</tr>
</tbody>
</table>

One-way ANOVA test result was showed in the table. The P value of ANOVA test was 0.003. MIAC, malnutrition, inflammation, atherosclerosis/calcification; EAT, epicardial adipose tissue.

EAT difference between three components versus no components of MIAC, P = 0.01; EAT difference between three components versus one component of MIAC, P = 0.02; EAT difference between three components versus two components of MIAC, P = 0.06.

Discussion

There were five main findings of this study. First, EAT measured by MDCT was increased in ESRD patients when compared with healthy subjects. Second, EAT measurements were significantly correlated with the MIAC syndrome in ESRD patients. Third, there was a statistically significant relationship between EAT and CACS in ESRD patients. Fourth, EAT was significantly increased if the number of MIAC components was increased. Lastly, EAT was positively correlated with age, BMI, and MIAC in the stepwise analysis. This was the first study that evaluated the relationship between EAT and the MIAC syndrome in ESRD patients.

Several factors contribute to the high mortality seen in ESRD patients, but CVD remains the main cause of morbidity and mortality despite recent developments in renal replacement therapies (3,28). This can be attributed to many factors: advanced age, atherosclerosis, endothelial dysfunction, hypertension, anemia, hyperparathyroidism, chronic inflammation, DM (and its macrovascular and microvascular complications), left ventricular hypertrophy, malnutrition, and vascular calcification.

The MIAC syndrome was first defined in ESRD patients by Stenvinkel et al. (5,29). They concluded that malnutrition, inflammation, and atherosclerosis cause a vicious cycle and that proinflammatory cytokines play a central part in this process (7). CAC is part of the extended state of vascular calcification that can be detected even in the early decades of patients with ESRD (30). Wang et al. (6) showed an important association between malnutrition, inflammation, and atherosclerosis and valvular and vascular calcification in PD patients. In this study, CACS <10 was defined as the absence of atherosclerosis/calcification, and CACS >10 was defined as the presence of atherosclerosis/calcification. Caliskan et al. (38) investigated the correlation of CACS with coronary flow reserve (CFR) in HD patients. They concluded that patients with CACS of >10 had a significantly lower CFR. Hence, the functional deterioration of coronary arteries (decreased CFR) starts from low levels of CACS (e.g., >10). Taken together, these factors may contribute to premature CVD and the markedly increased mortality in patients with ESRD.

EAT and intra-abdominal visceral fat depots originate from the splanchnopleuric mesoderm (31). Mazurek et al. (16) concluded that, like abdominal visceral adipose tissue, EAT is also metabolically active because it can secrete proinflammatory cytokines and utilize free fatty acids (FFAs). Despite the smaller adipocyte size, EAT has a higher rate of uptake and secretion of fatty acids than other visceral fat depots. In health, EAT may act as a “buffering system” by scavenging excess FFAs that are toxic to the myocardium. However, under ischemic conditions, EAT may serve as a local energy source by providing FFAs for the increased metabolism of the myocardium (32,33). In a recent study, the authors concluded that EAT acts as an extremely active organ that produces several bioactive adipokines, as well as proinflammatory and proatherogenic cytokines (tumor necrosis factor–α, monocyte chemotactic protein-1, IL-6, resistin, visfatin, omentin, leptin, plasminogen activator inhibitor-1, and angiotensinogen) (16,18–21). The levels of most of these proinflammatory cytokines were, in general, increased, and these cytokines were
found to be associated with atherosclerosis and CAC in ESRD patients (22–24). In this study, increased EAT as measured by MDCT was related to CAC. We also found a close relationship between the components of MIAC and EAT. EAT measurements showed a significant increase with increasing components of the MIAC syndrome. EAT was highest among patients having all three components (221 ± 101 cm$^3$) and lowest among those who do not have the MIAC syndrome (121 ± 51 cm$^3$). This association might be attributed to increased levels of proinflammatory cytokines secreted by EAT.

When we classified ESRD patients according to renal replacement therapy, PD patients had similar EAT measurements when compared with HD patients. When comparing PD with HD, hypertriglyceridemia and obesity are commonly seen in PD patients (especially secondary to the use of high glucose ingredients of peritoneal dialysates). In contrast to the similar EAT values in the two groups, EAT was affected by more variables beyond BMI and hypertriglyceridemia.

Ix and Sharma (34) concluded that there is a link between obesity, CKD, and nonalcoholic fatty liver disease (NAFLD). Targher et al. (35) showed an association between NAFLD and CKD in patients with type 2 DM. However, in this study, there was no difference between ESRD patients and healthy subjects in terms of BMI and serum levels of aspartate aminotransferase and alanine transaminase. We therefore assumed that our patients did not have NAFLD.

EAT was found to be positively correlated with age, BMI, and the presence of MIAC in the stepwise analysis. Studies have shown an important association between increased age, obesity, and the presence of the MIAC syndrome and cardiovascular mortality and morbidity in CKD populations (2–3,6). Our findings were consistent with those of other studies. Therefore, defining EAT could be valuable for further studies such as the determination of cardiovascular risk stratification in ESRD patients.

Despite the simplicity of evaluating EAT with echocardiography (36), EAT should be measured in three dimensions by MDCT: regional thickness, cross-sectional area, and total volume (33,37). Therefore, MDCT can be used to assess CAC and EAT in ESRD patients at a high risk of CVD. Echocardiography has been used for the measurement of EAT with a higher reproducibility and reliability in the general population, but there are no data about echocardiography for the assessment of EAT in ESRD patients (36). The lower cost, simplicity, and rapidity of echocardiography could make it the preferred measurement method for EAT.

Our study had two main limitations. First, this was a cross-sectional analysis of ESRD patients focusing on CAC and EAT. Second, the sample size was relatively small. This was not a prospective controlled study, so we cannot draw cause-and-effect relationships from our findings.

In conclusion, we found a relationship between EAT as defined by MDCT and components of the MIAC syndrome in ESRD patients. Further clinical and experimental studies are needed to determine the relationship between EAT and the MIAC syndrome.

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Disclosures

None.

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