Microalbuminuria, but not reduced eGFR, is associated with cardiovascular subclinical organ damage in type 2 diabetes

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Abstract

Aim. – This study explored the association between reduced estimated glomerular filtration rate (eGFR) and microalbuminuria vs. subclinical organ damage in patients with type 2 diabetes.

Methods. – Data from middle-aged patients with type 2 diabetes (n = 706) treated in primary care were analyzed for microalbuminuria, defined as a urinary albumin/creatinine (uACR) ≥ 3.0 mmol/mol, and reduced eGFR, defined as < 60 mL/min/1.73 m², in relation to blood pressure, pulse wave velocity (PWV), left ventricular mass index (LVMI), and carotid intima–media thickness (IMT) and lumen diameter (LD).

Results. – Patients with microalbuminuria had significantly higher 24-h ambulatory systolic blood pressure (ASBP) compared with subjects with a uACR < 3 mg/mmol: 137 vs. 128 mmHg (P < 0.001). There were no differences in ASBP in patients with eGFR < 60 mL/min/1.73 m². However, patients with vs. without microalbuminuria had increased PWV (11.4 vs. 10.1 m/s; P < 0.001), LVMI (134.4 vs. 118.6 g/m²; P < 0.001), LD (7.01 ± 0.93 vs. 6.46 ± 0.74 mm; P < 0.001) and IMT (0.78 vs. 0.74 mm; P = 0.047), respectively. The associations between uACR vs. PWV and LVMI were more robust after adjusting for age, diabetes duration, ASBP, HbA1c, LDL-cholesterol, and antihypertensive and lipid-lowering therapy compared with uACR vs. IMT. There were no statistically significant differences in PWV, LVMI or IMT between patients with reduced (< 60 mL/min/1.73 m²) vs. normal eGFR.

Conclusion. – Levels of urinary albumin excretion, but not reduced eGFR, were associated with increased arterial stiffness, left ventricular mass and atherosclerosis in patients with type 2 diabetes.

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Keywords: Albuminuria; GFR; Cardiovascular disease markers; Atherosclerosis; Type 2 diabetes

1. Introduction

Microalbuminuria is associated with atherosclerosis risk factors as well as cardiovascular disease (CVD) [1–3], which may be explained in part by endothelial damage or dysfunction [4]. However, it is less clear whether modestly impaired renal function per se, as reflected by a decreased glomerular filtration rate (GFR), represents a risk factor for the development of atherosclerosis.

According to the 2002 clinical practice guidelines, renal impairment can also refer to chronic kidney disease (CKD) and, in adults, is defined as either kidney damage (including persistent proteinuria) or GFR < 60 mL/min/1.73 m² for ≥ 3 months [5,6]. A reduced GFR has been proposed as a risk factor for CVD in type 2 diabetes (T2D), and some studies have suggested that a decreased GFR is associated with an increased CVD risk in T2D.
independently of microalbuminuria [7,8]. The aim of the present study was to explore the relationships between estimated GFR (eGFR) and microalbuminuria and subclinical vascular organ damage in T2D patients.

2. Patients and methods

Data from 706 patients who participated in the Cardiovascular Risk Factors in Patients with Diabetes: a Prospective Study in Primary Care (CARDIPP) was analyzed. The study was launched in 2005 and baseline data collection was completed in November 2008. The general aim of CARDIPP was to investigate cardiovascular risk factors in middle-aged patients with T2D to facilitate early and individually adjusted risk intervention. Patients aged 55–65 years were consecutively recruited during their usual annual follow-ups at 22 primary healthcare centres in the counties of Östergötland and Jönköping, Sweden, irrespective of their previous blood pressure and CVD status. The centres varied in size and were located in different geographical areas, but all followed the national guidelines for diabetes care. Patients with severe concomitant diseases such as cognitive impairment and cancer were not included.

Altogether, CARDIPP enrolled 761 patients, but because of missing data for microalbuminuria in 32 patients and no information on creatinine levels in 25 cases, 55 patients were excluded, leaving a total study sample of 706 subjects with a mean age of 61 ± 3 years and mean duration of T2D of 7 ± 6 years. The present study was approved by the regional ethics review board based in Linköping, and all participants gave their written informed consent.

A questionnaire also investigated the participants’ lifestyle habits, including alcohol consumption and smoking status, while a standard medical history provided data on diabetes duration and ongoing medication.

As for diabetes treatments, 203 patients (28.8%) were treated by lifestyle recommendations only, 288 patients (40.8%) were treated by oral antidiabetic drugs (OADs) and 215 (30.5%) patients by insulin, either alone or in combination with OADs. Of the OADs, metformin was the most frequently used agent (n = 372, 52.7%), followed by sulphonylureas (n = 107, 15.2%). Among antihypertensive drugs, the use of renin–angiotensin–aldosterone system (RAAS) blockers such as angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) predominated (n = 311, 44.1%), followed by beta-blockers (n = 250, 35.4%), calcium antagonists (n = 112, 15.9%), thiazides (n = 74, 10.5%) and loop diuretics (n = 53, 7.5%). Treatment with lipid-lowering agents (statins) was found in 396 subjects (56.1%) and low-dose acetylsalicylic acid in 204 patients (28.9%).

Urine and blood samples for laboratory analyses were taken in the morning following at least 10 h of fasting. Routine blood tests were done and analyzed at the healthcare centres. The Swedish standard high-performance liquid chromatography (HPCL) Mono-S method was used to measure HbA1c, although the data were then converted to Diabetes Control and Complications Trial (DCCT) standards (%) and International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) units (mmol/mol). Other clinical data such as body weight, height and waist circumference were also recorded. Blood pressure was calculated as the average of three sitting measurements taken 1-min apart by specially trained nurses at each primary healthcare centre, and 24-h ambulatory blood pressure monitoring was performed with a Spacelabs Medical 90217 ABPM device (Spacelabs Healthcare, Issaquah, WA, USA).

Blood and urine samples were frozen for later analyses of plasma cystatin C, and urinary albumin excretion (UAE) rate was measured using the urinary albumin/creatinine ratio (uACR). Microalbuminuria was defined as uACR ≥ 3.0 mg/mmol for both men and women in accordance with the American Diabetes Association (ADA) definition [9]. However, as the larger muscle volume in men can affect uACR, this calculation was further supplemented by the UK National Institute for Health and Care Excellence (NICE) recommendations of uACR > 2.5 mg/mmol for men and > 3.5 mg/mmol for women [6]. Renal glomerular function was defined by eGFR, calculated by the Modification of Diet in Renal Disease (MDRD) study equation [10], as defined in Table 48 in the Kidney Disease Outcomes Quality Initiative (KDOQI) chronic kidney disease (CKD) guidelines [5]. Although patients’ race was not noted, Caucasians are generally still strongly predominant in the Swedish population. As far as the present authors are aware, there were no black African participants in the study that generated the MDRD equation.

Reduced GFR was defined as < 60 mL/min/1.73 m², as in stage 3 of the CKD classification system, representing a loss of at least half the normal glomerular kidney function [5]. Also calculated were the eGFR based on cystatin C levels and absolute eGFR, using the Cockcroft–Gault equation.

Clinical physiological examinations were performed at the Department of Physiology of Linköping University Hospital and at County Hospital Ryhov in Jönköping. Common carotid intima–media thickness (IMT) was determined during diastole from still images of 10-mm sections taken in close proximity to the carotid bulb, using B-mode ultrasound to obtain mean carotid lumen diameter (LD) and far-wall IMT. Mean IMT and LD values calculated from two registrations on each side were used for all analyses. Arterial stiffness was evaluated in supine position by pulse wave velocity (PWV), measured as the transit time between carotid and femoral arterial pulse waves, and calculated by measurement of the surface distance at jugulum–carotid and jugulum–femoral locations, and by electrocardiogram (ECG)-gated recordings of the carotid and femoral arterial pulse waves using the foot-to-foot method, with the foot of the wave defined by the end of diastole. Echocardiography was performed with the patient in a left semilateral position, while left ventricular mass (LVM) was determined using the method described by Devereux and Reichek [11]; body surface area was calculated and expressed as g/m², as is most commonly recommended [12]. The LVM index (LVMi) based on height at an allometric power of 2.7 adapted for overweight patients to avoid underdiagnosis of left ventricular (LVH) [13] was also calculated.
2.1. Statistical analysis

SPSS version 18.0 software for Windows was used for all statistical analyses. Data are given as means ± SD, and Table 1 presents the results of independent two-sample Student’s t test, with \( P < 0.05 \) considered statistically significant, while chi-square tests were used to compare categorical variables. Multiple linear-regression analyses were used to further analyze the associations between uACR and clinical physiological examination findings. Bivariate correlations between uACR, eGFR, ambulatory systolic blood pressure (ASBP) and other variables are shown with Pearson’s correlation coefficients.

3. Results

3.1. Kidney markers

The mean uACR value was 3.0 ± 12.4 mg/mmol, and 85.5 ± 16.4 μmol/L for plasma creatinine. Mean eGFR was 75 ± 16 mL/min/1.73 m² according to the MDRD equation, 101 ± 26 mL/min by the Cockcroft–Gault formula, and 92 ± 20 mL/min/1.73 m² using cystatin C to calculate eGFR. Table 1 shows the patients’ baseline characteristics according to the presence of microalbuminuria and/or reduced eGFR (<60 mL/min/1.73 m²).

Microalbuminuria was prevalent in 116 cases, or 16.4% of the study population. The same number, 116 patients (16.4%), had an eGFR <60 mL/min/1.73 m² and 112 participants (15.9%) were in the range of 45 to <60 mL/min/1.73 m², with the lowest eGFR found in four patients with values between 30 and <45 mL/min/1.73 m². Fig. 1 shows the distribution of uACR in the entire study population. Altogether, 23 patients (3.3%) had concomitant microalbuminuria and eGFR <60 mL/min/1.73 m². In addition, 19.8% of patients with reduced eGFR had microalbuminuria.

3.2. Ambulatory blood pressure

The 24-h ASBP was higher at 137 ± 16 vs. 128 ± 13 mmHg (\( P < 0.001 \)) in patients with uACR ≥ 3 mg/mmol vs. those with uACR < 3 mg/mmol, respectively, and the same pattern was observed both during the day (ASBP: 143 ± 16 vs. 134 ± 13 mmHg, respectively; \( P < 0.001 \)) and at night (ASBP: 129 ± 18 vs. 116 ± 15 mmHg, respectively; \( P < 0.001 \)). On comparing ASBP according to the eGFR classification cutoff of 60 mL/min/1.73 m², there were no statistically significant differences in ASBP between the two categories.

3.3. Subclinical organ damage

Patients with vs. without microalbuminuria had increased IMT (0.78 ± 0.19 vs. 0.74 ± 0.22 mm, respectively; \( P = 0.047 \)), LD (7.01 ± 0.93 vs. 6.46 ± 0.74 mm, respectively; \( P < 0.001 \)), PWV (11.4 ± 2.4 vs. 10.1 ± 2.0 m/s, respectively; \( P < 0.001 \)) and LVMI (134.5 ± 34.1 vs. 118.6 ± 28.1 g/m², respectively; \( P < 0.001 \)). However, there were no statistically significant differences in IMT, PWV or LVMI between patients with reduced renal function according to eGFR compared with eGFR >60 mL/min/1.73 m². There was a significant difference (\( P = 0.015 \)) for LD dichotomized by eGFR, but the proportions were reversed, with values representing more reduced renal function having a more reduced lumen diameter (6.39 ± 0.64 vs. 6.58 ± 0.82, respectively). ASBP was significantly higher in patients with microalbuminuria irrespective of whether or not eGFR was reduced. Fig. 2 shows the correlation coefficients between eGFR, uACR and ASBP vs. baseline values. The relationships between uACR vs. PWV, LVMI and IMT were further

![Fig. 1. Histogram of the distribution of urinary albumin/creatinine ratio (uACR; mg/mmol) in 706 male and female patients aged 55–65 years with type 2 diabetes.](image)

![Fig. 2. Pearson’s correlation coefficients of estimated glomerular filtration rate (eGFR), urinary albumin/creatinine ratio (uACR) and mean 24-h systolic blood pressure for various studied variables. * \( P < 0.05 \); ** \( P < 0.01 \).](image)
explored via multiple linear-regression analyses in variously adjusted models (Table 2). The associations between uACR vs. PWV and LVMI were more robust after adjustments compared with those between uACR and IMT. The prevalence of variables above the suggested cutoff values for elevated cardiovascular risk [6,14] are shown on Fig. 3 by gender.

4. Discussion

The present cross-sectional study found associations between elevated urinary albumin excretion (UAEx) and subclinical cardiovascular organ damage, defined as increased LVMI, PWV, and carotid IMT and LD in patients with T2D. However, there were no significant associations between reduced eGFR (defined as stage 3 CKD) and subclinical organ damage.

Another cross-sectional study showed eGFR < 60 mL/min/1.73 m² to be associated with increased prevalence of CVD in Japanese patients with T2D [8]. Furthermore, eGFR < 60 mL/min/1.73 m² was a predictor of mortality in the general population [15], and was also associated with increased risks of death, cardiovascular events and hospitalization [16]. However, in yet another study, normoalbuminuric diabetics patients with reduced eGFR did not present with CKD progression or die during the 38 ± 11 months of follow-up, as did patients with microalbuminuria and especially macroalbuminuria, thereby highlighting albuminuria as the more important risk factor than eGFR [17]. Nevertheless, the present study failed to confirm an association between eGFR < 60 mL/min/1.73 m² and subclinical organ damage in a cohort of middle-aged Swedish patients with T2D in primary care. This may be explained
in part by the fact that most (97%) of our subjects with eGFR < 60 mL/min/1.73 m² had only modest renal impairment within an eGFR range of 45–60 mL/min/1.73 m².

In previous studies showing an association between eGFR < 60 mL/min/1.73 m² and increased risk for CVD or mortality, the risk for adverse events rose sharply for those with an eGFR < 45 mL/min/1.73 m² for every outcome examined in the overall cohort [15,16] as well as in subgroup analyses [16]. In addition, one study reported a U-shaped relationship of eGFR, as assessed by the MDRD study equation, with mortality [15]. However, as pointed out by those authors, the finding has to be interpreted with caution. The MDRD equation is known to underestimate eGFR at levels ≥ 60 mL/min/1.73 m² in healthy individuals and to overestimate eGFR in those with reduced muscle mass due to ill health. Such overestimation could potentially contribute to the reported U-shaped association of eGFR with mortality [15].

In the present study, eGFR was estimated by the Cockcroft–Gault formula, MDRD study equation and cystatin C levels. However, the US National Kidney Foundation has reported conflicting data with the latter when calculating eGFR, and thus questions whether cystatin C provides a sufficient improvement compared with creatinine to justify its widespread clinical use [5]; indeed, the prevailing attitude towards the method is to use it with caution [18]. Also, a major problem with the Cockcroft–Gault formula is overestimation of eGFR when GFR is low, which can delay the diagnosis of severe renal failure. Although imperfect, the MDRD equation appears to be more accurate in diabetic patients [19], which is why the present study data were primarily analyzed by this equation; it is also still referred to by the National Kidney Foundation and ADA [5,9].

Measurement of PWV is accepted as the most simple, robust and reproducible method for describing arterial stiffness and, of the various methods, carotid–femoral PWV is considered the most clinically relevant, ‘gold-standard’ technique [20]. There is considerable evidence to support the predictive value of PWV for cardiovascular events and all-cause mortality [20–22]. In our present study, the association between uACR and arterial stiffness was very robust, and remained so even after adjusting for ASBP.

The association between PWV and transition from normo- to microalbuminuria or micro- to macroalbuminuria in T2D patients has been reported elsewhere [23] and is in agreement with our present results. In fact, microalbuminuria has been reported as a determinant of increases in both PWV and IMT independently of conventional cardiovascular risk factors in patients with T2D [24]. Yet, in a cross-sectional Japanese study, the stage of diabetic nephropathy as graded by UAE was not associated with carotid IMT in T2D patients [25]. However, the study’s subjects, who had a mean age of 65 years, did not resemble the usual primary healthcare patients, as 50% of them were classified as CKD stages 3–5 and only 4% were on diet-only treatment for glycaemic control. Also, 16% of our present primary healthcare T2D population had a uACR > 3.0 mg/mmol compared with 53% in the Japanese study, which probably reflects a more diseased population. Overall, IMT was the clinical physiological method resulting in the weakest significances and correlations in our study. Although this raises the question of the applicability of IMT in this category of patients, our study is not able to answer the question.

There was an association between microalbuminuria and IMT after adjusting for age and diabetes duration, although it was no longer statistically significant after further adjustments in a model including ASBP, HbA1c, low-density lipoprotein (LDL) cholesterol, and antihypertensive and lipid-lowering therapy. In contrast, the associations between microalbuminuria and PWV and LVMI remained significant after the same adjustments. Furthermore, our finding of microalbuminuria as a determinant of increased LVH has been reported previously in a large hypertensive population [26], and there was a linear association between albuminuria and mortality [15,27].

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**Fig. 3.** Prevalence (%) of risk factors with values above cutoff for elevated risk of cardiovascular disease by gender.

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**Table 2**

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uACR model 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.163</td>
<td>(0.733–1.592)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>uACR model 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.934</td>
<td>(0.470–1.398)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>uACR model 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.850</td>
<td>(0.374–1.326)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uACR model 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.979</td>
<td>(11.189–24.769)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>uACR model 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.993</td>
<td>(8.515–23.471)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>uACR model 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.667</td>
<td>(6.287–21.046)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IMT</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>uACR model 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.046</td>
<td>(0.000–0.092)</td>
<td>0.049</td>
</tr>
<tr>
<td>uACR model 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.034</td>
<td>(–0.019–0.088)</td>
<td>0.206</td>
</tr>
<tr>
<td>uACR model 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.041</td>
<td>(–0.013–0.096)</td>
<td>0.139</td>
</tr>
</tbody>
</table>

<sup>a</sup> Multiple linear-regression analysis with PWV, LVMI and IMT as dependent variables adjusted for uACR (± 3.0 mg/mmol), age and diabetes duration.

<sup>b</sup> Model 1 plus adjustments for systolic blood pressure (SBP), HbA1c, and LDL-cholesterol.

<sup>c</sup> Model 2 plus adjustments for antihypertensive and lipid-lowering therapy.
The pathogenesis of LVH is multifactorial, although hypertension is important and can lead to concentric hypertrophy. Hypertension can also increase arterial stiffness, which is itself associated with LVH as well as activation of the hormone and endocrine systems. The significant differences found in triglycerides, high-density lipoprotein (HDL) cholesterol and waist circumference in our microalbuminuric patients compared with those with uACR < 3.0 mg/mmole were also consistent with the typical characteristics of the metabolic syndrome, whereas there were no significant differences in either total or LDL-cholesterol. Patients with the metabolic syndrome have an increased risk of developing higher urinary protein excretion [28]. Both microalbuminuria and the metabolic syndrome could reflect RAAS activation, oxidative stress and inflammation, leading to endothelial injury, smooth muscle cell migration and proliferation and, later, to renal and cardiac fibrosis [29].

Nevertheless, the use of LVMI may be controversial. LVM indexed to height at an allometric power of 2.7 was applied to patients in our study, characterized by obesity, although this may overestimate LVH in smaller individuals while underestimating LVH in taller ones; indexation to height at an allometric power of 1.7 is an alternative [14].

Some studies have shown that LVH is common among diabetics who also have CKD [30,31]. However, the lack of association between CKD and LVH in our study suggests that it most likely represents middle-aged patients with T2D within a narrow distribution of GFR due to a low prevalence of CKD.

Regarding the prevalence of hypertension at baseline in our study, there were more patients taking antihypertensive therapy than patients with hypertension based on their medical history. Other than a diagnosis that is not on record, this might be explained by the fact that some patients taking antihypertensive drugs have other diagnoses such as angina pectoris and heart failure.

The gender perspective includes the influence of sex hormones. Being female appears to be protective in terms of loss of kidney function until the onset of menopause. Although few studies have examined the effects of hormone therapy, it appears that oral oestrogen use is associated in a dose-dependent manner with an increased risk of kidney dysfunction [32]. In a study comparing overweight postmenopausal women aged 52–62 years who were former or current hormone therapy users, the former users had poorer cardiovascular profiles, including a larger arterial diameter statistic, which may best be explained by their use of hormone therapy and heavier weight [33].

Measuring the common carotid artery by LD or interventitial diameter may be an underused method for evaluating vascular status. A small diameter better balances shear and tensile stresses whereas an enlarged diameter may be a sign of attenuated vasoregulation [34]. In our present study, patients with microalbuminuria had significantly increased LD (P < 0.001) whereas the significance of IMT was weaker, with reversed proportions in those with reduced renal function, whose values showed decreases in LD.

Clinical and laboratory guidelines recommend uACR as the measure of albuminuria for defining and managing CKD [5,6,14,35]. However, our study was limited by having only a single measurement of renal function and only one spot urine sample; the cross-sectional design of our study was a further limitation. Thus, it was not possible to draw any conclusions of causality between renal impairment and subclinical vascular damage.

On the other hand, the strength of our study lies in the fact that cardiovascular subclinical organ damage was assessed by three different well-established clinical physiological methods, along with measurements of PWV, LVMI, and carotid IMT and LD. To our knowledge, this is the first study to explore the relationship between kidney function and cardiovascular subclinical organ damage in T2D patients to this extent.

In conclusion, microalbuminuria, defined as uACR ≥ 3 mg/mmole, and not slightly impaired renal function according to eGFR, is a marker of the prevalence of subclinical organ damage in terms of arterial stiffness, atherosclerosis and increased LVM in middle-aged patients with T2D.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.diabet.2013.09.008.

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