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Blood pressure and not uraemia is the major determinant of arterial stiffness and endothelial dysfunction in patients with chronic kidney disease and minimal co-morbidity

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ABSTRACT

Introduction: Patients with chronic kidney disease (CKD) have increased risk of cardiovascular disease to which co-morbidity and associated conventional risk factors contribute. We hypothesised that arterial stiffness (AS) and endothelial dysfunction (ED), as surrogates of cardiovascular risk, would worsen as renal function declined even in patients without co-morbidity and that this would relate to emerging cardiovascular risk factors.
Methods: Carotid–femoral pulse wave velocity (PWV), as a measure of AS, and flow-mediated dilatation (FMD) of the brachial artery, as a measure of ED, were assessed in CKD patients without established cardiovascular disease or diabetes mellitus.
Results: PWV increased linearly as renal function declined ($r^2 = 0.08$, $p < 0.01$) whereas FMD was reduced only in patients with advanced kidney disease. In multivariable analysis, blood pressure was the major determinant of PWV and FMD. High-sensitivity C-reactive protein and asymmetric dimethylarginine, and isoprostanes and endothelin-1, were independent predictors of PWV and FMD, respectively. However, renal function did not independently predict either AS or ED.
Conclusions: These findings suggest that declining renal function, in the absence of significant co-morbidity, is associated with progressive arterial stiffness, but only patients close to dialysis exhibit endothelial dysfunction. Whilst blood pressure remains the major determinant of PWV and FMD, inflammation, oxidative stress and endothelin–nitric oxide balance contribute to cardiovascular risk, in this non-comorbid cohort.

1. Introduction

Patients with chronic kidney disease (CKD) are at increased risk of cardiovascular disease, with cardiovascular mortality at least 10–20-fold higher in dialysis patients than in the general population [1]. Indeed, CKD patients have a substantially higher chance of dying from cardiovascular disease than of progressing to end-stage renal disease [2]. Conventional (Framingham) cardiovascular risk factors, including hypertension, hypercholesterolaemia, diabetes mellitus and smoking, are common in CKD patients. However, these only partly explain the high cardiovascular risk. Thus, the study of emerging cardiovascular risk factors has been an area of intense investigation [3].

Inflammation, oxidative stress, and a shift in the balance of the vasodilator nitric oxide and vasoconstrictor endothelin systems have all been identified as potential contributors to increased cardiovascular risk in CKD patients [4]. These are all common in a typical CKD population [3,5], but are also increased by conventional cardiovascular risk factors and by established cardiovascular disease [5,6]. Hence, the contribution of renal dysfunction to these emerging risk factors remains unclear, or indeed whether renal dysfunction has an independent effect on CVD risk.

Increased arterial stiffness, as measured by pulse wave velocity, is a commonly recognised feature of CKD [7], a marker of cardiovascular risk [7,8], and an independent predictor of mortality and

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survival in dialysis patients [8,9]. The endothelium is an important regulator of arterial stiffness [10], and endothelial dysfunction is also a common feature of CKD [11–13] and a predictor of cardiovascular disease [14]. However, most studies of arterial stiffness and endothelial dysfunction in CKD involve dialysis patients, or those with complex co-morbidity, including diabetes mellitus and established cardiovascular disease, which are themselves associated with arterial stiffening and endothelial dysfunction [8,9,12,13]. Recent evidence suggests that an increase in arterial stiffness and endothelial dysfunction occurs in the early stages of CKD, with progression as glomerular filtration rate (GFR) falls [15,16]. However, these and other such studies again include patients with conventional cardiovascular risk factors as well as major cardiovascular co-morbidity [15–21].

In this study, therefore, we measured arterial stiffness and endothelial dysfunction in a group of CKD patients with minimal co-morbidity across a wide range of renal function from early stage CKD to pre-dialysis. We also assessed the prevalence of inflammation, oxidative stress, and markers of nitric oxide/endothelin activity in this group. Finally, we examined the relationships of arterial stiffness and endothelial dysfunction with both conventional and emerging cardiovascular risk factors. We aimed to establish at what CKD stage arterial stiffness and endothelial dysfunction become apparent in renal patients without established cardiovascular risk factors, how emerging risk factors would accumulate as renal function declined in this non-comorbid group and the role these emerging risk factors play in cardiovascular risk in these patients.

2. Methods

This study was prospective and cross-sectional in design. It was approved by the Multi-centre Research Ethics Committee for Scotland, and performed in accordance with the Declaration of Helsinki. All subjects provided written informed consent.

2.1. Subjects

Subjects were recruited from the renal outpatient clinic at the Royal Infirmary of Edinburgh. Subjects were categorised into 5 stages of CKD on the basis of the Kidney Disease Outcome Quality Initiative (K/DOQI) classification [22]. We aimed to recruit around 30 subjects in each of CKD stages 1–3, and 30 subjects in stages 4 and 5 combined. We also aimed to enroll around 30 age-matched healthy subjects from the community as a control group.

The inclusion criteria were: male or female CKD patients, 18–65 years old, clinic blood pressure (BP) $\leq$ 160/100 mmHg, whether or not on anti-hypertensive medication. We excluded patients with a renal transplant or on dialysis, patients with systemic vasculitis or connective tissue disease, those with a history of established cardiovascular disease, peripheral vascular disease, diabetes mellitus, respiratory disease, or neurological disease, those with current alcohol abuse or pregnancy, and patients treated with an organic nitrate or β-agonist. Patients' medical records were reviewed, and where necessary clarification sought with their nephrologist, prior to an enrolment to the study. Patients continued their usual medications. Smokers and hypercholesterolaemic patients were not excluded but the latter had to be established on statin medication, with good cholesterol control, for at least 3 months before taking part in the study.

2.2. Measurements

Systolic BP (SBP) and diastolic BP (DBP) were recorded in duplicate, with an appropriately sized cuff, using a validated oscillometric sphygmomanometer, the Omron HEM-705CP [23], and values were presented as an average of two recordings. Mean arterial pressure (MAP) was calculated as MAP = DBP + [(SBP − DBP)/3]. Pulse pressure (PP) was calculated as PP = SBP − DBP. Body mass index (BMI) was calculated as BMI = weight (kg)/height $(m)^2$.

Arterial stiffness was assessed by measuring carotid–femoral pulse wave velocity (CF-PWV) as previously described [24], using the SphygmoCor apparatus (SphygmoCor BPAS-1; AtCor Medical, Sydney, Australia). Peripheral arterial waveforms were measured at the radial artery using the same tonometer. The corresponding central waveforms, and aortic SBP, DBP, MAP and PP estimated, were generated via the SphygmoCor's transfer function. Central augmentation index (cAIx) and cAIx corrected to a heart rate of 75 (cAIx@HR75) were estimated as previously described [24].

Brachial artery flow-mediated dilatation (FMD) was used to assess endothelium-dependent vasomotor function, as previously described [25]. FMD was quantified as a percentage change from baseline in brachial artery diameter after 5 min of forearm ischaemia. Endothelium-independent vasomotor function was assessed using 25 μg of nitroglycerine (NTG) by sublingual administration.

2.3. Laboratory investigations

On the study day, venous blood samples were obtained from subjects after 12 h of overnight fasting. Plasma glucose, total cholesterol and erythrocyte sedimentation rate (ESR) were quantified in the hospital biochemistry laboratory. For measurements of serum high-sensitivity C-reactive protein (CRP) and oxidised low density lipoprotein, and interleukin-6 (IL-6), isoprostanes, asymmetric dimethylarginine (ADMA), and endothelin-1, venous blood samples were collected in EDTA tubes that were immediately centrifuged at 2500 × *g* for 20 min at 4 °C. Samples were stored at −80 °C until analysis.

Serum high sensitivity CRP concentrations were quantified in the hospital biochemistry laboratory using a validated latex particle enhanced immunoturbidimetry technique (Vitros® 5, 1 FS Chemistry Systems, Ortho-Clinical Diagnostics, Inc., New York, USA) (intra- and inter-assay variations 2.3% and 5%, respectively). Serum oxidised low density lipoprotein concentrations were measured using a commercially available kit of sandwich enzyme-linked immunosorbent assay (ELISA) (Mercodia AB, Uppsala, Sweden; assay variations 7.3% and 6.2%). Plasma IL-6 concentrations were quantified by an ELISA (Cayman Chemical, Ann Arbor, MI; assay variations 5% and 15%). Plasma isoprostane concentrations were quantified by an ELISA (Cayman Chemical, Ann Arbor, MI; assay variations 9% and 13%). Plasma ADMA concentrations were measured using an optimised and fully validated high performance liquid chromatography method, as previously described [26] (assay variations 1.9% and 2.3%). Plasma endothelin-1 concentrations were determined by standard radioimmunoassay (Peninsular Laboratories Europe, St. Helens, UK), as previously described [27] (assay variations 6.3% and 7.2%).

2.4. Renal function assessment

Creatinine clearance, as an estimate of glomerular filtration rate, was calculated according to the Cockcroft and Gault equation [28]: [(140 − age (years) × weight (kg) × 1.23 for male or 1.05 for female]/serum creatinine (μmol/L). To obtain serum creatinine in mg/dl, the value in μmol/L was divided by a correction factor of 88.4. The Cockcroft and Gault equation was selected to assess renal function in this study because it is more accurate than the modification of diet in renal disease (MDRD) equation when used to assess mild renal insufficiency [28,29]. It was further corrected by body surface area.