Fetuin-A is an independent determinant of change of aortic stiffness over 1 year in non-diabetic patients with CKD stages 3 and 4

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Abstract

Background. Vascular calcification is highly prevalent in chronic kidney disease (CKD) patients. This calcification leads to arterial stiffening. Fetuin-A is an endogenous inhibitor of vascular calcification and has been associated with arterial stiffness and mortality in dialysis patients. We tested the relationship between fetuin-A and change in arterial stiffness in CKD stages 3 and 4.

Methods. We measured fetuin-A concentrations in 92 patients with CKD stages 3 and 4 and studied the association with clinical, biochemical and vascular parameters including arterial stiffness measured by carotid–femoral pulse wave velocity (PWV) at 0 and 12 months.

Results. Fetuin-A was significantly lower in the non-diabetic group (n = 73) compared to the diabetic group (n = 19, P = 0.018). There was a significant interaction between diabetic status and fetuin-A concentration. Univariate analysis of the non-diabetic group showed association between change in aortic stiffness over 1 year with fetuin-A (r = −0.481, P < 0.0001) and systolic blood pressure (r = 0.389, P = 0.001) and baseline PWV (r = 0.240, P = 0.041). In multivariate analysis, fetuin-A, systolic blood pressure and baseline PWV independently predicted change in carotid–femoral PWV at 1 year (β = −0.355, P < 0.001; β = 0.426, P < 0.001; and β = −0.383, P < 0.001, respectively; model R² = 0.455).

Conclusions. In patients with non-diabetic CKD stages 3 and 4, fetuin-A is an independent risk factor for progressive arterial stiffness.

Keywords: aortic stiffness; blood pressure; calcification inhibitor; chronic kidney disease; fetuin-A

Introduction

Mortality and cardiovascular event rates progressively increase as renal function declines, and cardiovascular dis-
ease is the most common cause of death in chronic kidney disease (CKD) [1,2]. Patients with CKD have a higher prevalence of traditional cardiovascular risk factors such as hypertension and diabetes [3,4]. However, the contribution of non-traditional risk factors including inflammation, oxidant stress and arterial stiffness to this excess cardiovascular mortality is increasingly recognized.

Arterial stiffness is increased in CKD [5]. In cross-sectional studies, arterial stiffness is associated with increasing severity of renal impairment [6], age [7], hypertension [8] and diabetes mellitus [9]. Age, heart rate and renal function are determinants of change of arterial stiffness in hypertensives [10], whilst baseline vascular calcification is associated with progressive calcification, itself a determinant of progressive arterial stiffening, in the CKD population [11]. In a prospective study of a haemodialysis population, aortic stiffness, measured by the gold standard [12] carotid–femoral pulse wave velocity (C–F PWV), was shown to be an independent predictor of all-cause and cardiovascular mortality [13].

Stiffness of the artery is partially determined by composition of the arterial wall. In renal failure, there is excess calcification in the media [14] and also in atherosclerotic plaques [15]. The process of calcification in the vessel wall is, however, not merely the passive result of alteration in the serum calcium and phosphate concentrations seen in renal failure, but is actively controlled by a complex variety of cellular processes and circulating proteins such as fetuin-A [16].

Fetuin-A is a heaptically synthesized 62-kDa glycoprotein and a negative acute-phase reactant. Fetuin-A interacts with small mineral nuclei to form soluble colloidal particles thereby preventing precipitation of basic calcium phosphate (BCP) and acting as a calcification inhibitor in the supersaturated extracellular environment [17]. In addition, at the cellular level, fetuin-A inhibits apoptosis of vascular smooth muscle cells (VSMC) and is released in matrix vesicles from VSMC to prevent mineral nucleation [18]. Fetuin-A also aids binding of apoptotic bodies to neighbouring cells leading to their clearance and thereby reducing the potential for these substances to nucleate BCP [19].

Fetuin-A is also known to inhibit the insulin-stimulated tyrosine kinase receptor [20] and has recently been shown to be associated with the metabolic syndrome [21] and to be an independent risk factor for the development of type 2 diabetes mellitus [22,23].

In adult dialysis patients, serum fetuin-A is reduced compared to healthy controls [13]. This reduction is independently associated with significantly increased cardiovascular and all-cause mortality [13]. A study of CKD stage 5 patients showed an inverse relationship between serum fetuin-A and coronary calcification scores [24].

We hypothesized that reduction of fetuin-A may have a causative role in the increased aortic stiffness of patients with CKD stages 3 and 4. We, therefore, examined the relationship between fetuin-A concentration and change in arterial stiffness over 1 year in patients with CKD stages 3 and 4.

Materials and methods

Patients

All participants were enrolled in a prospective cohort study of cardiovascular risk in patients with CKD stages 3 and 4 between March 2006 and August 2007. Exclusion criteria included previous diagnosis of left ventricular failure with left ventricular ejection fraction <35%, aortic stenosis with gradient >30 mmHg, atrial fibrillation with ventricular rate >100 b.p.m. and age <40 years or >90 years. All participants were treated with the aim of achieving United Kingdom Renal Association targets for management of blood pressure (BP) in CKD [25]; a target BP of 135/80 mmHg in patients with urine protein/creatinine ratio <100 mg/mmol and 125/75 mmHg for those with protein/creatinine ratio >100 mg/mmol. The choice of antihypertensive medication was left to the discretion of the patient’s clinician but followed British Hypertension Society guidelines [26].

The study was approved by the West Sussex Research Ethics Committee and patients gave written informed consent. The study was conducted in accordance with the Declaration of Helsinki.

Clinical data and laboratory investigations

All participants were outpatients. A full history covering renal disease, cardiovascular disease and risk factors was obtained at entry to the study. All measurements were undertaken in a quiet, temperature-controlled room. Oscillometric BP was measured twice using an appropriate cuff size with the patient supine after 5 and 10 min of rest (Omron 705 CP, Tokyo, Japan). The mean of the two recordings of systolic BP (SBP) and diastolic BP (DBP) was recorded. Mean arterial pressure (MAP) was calculated as follows: MAP = DBP + (SBP – DBP)/3.

Estimated glomerular filtration rate (eGFR) was calculated using the four-variable equation derived from the modification of diet in renal disease (MDRD) study [27]. Urine protein/creatinine ratio (uPCR) was measured on a random urine sample obtained during the clinic visit. Standard biochemical analysis was performed using a routine automated analyser (Roche Modular, Haywards Heath, UK). Intact parathyroid hormone (PTH) was measured using Elecsys sandwich electrochemoluminescence immunoassay method. This has no cross-reactivity to the 1–37 PTH fragment.

Statistical analysis was performed using biochemical results at time of study entry.

Plasma and serum samples were taken from patients at their baseline study visit and frozen at −70°C. Plasma fetuin-A concentration was measured using a commercially available enzyme-linked immunosorbent assay kit (Biomedex, Brno, Czech Republic). Between- and within-batch coefficients of variability were 5.6% and 6.2%, respectively, across the concentration range measured. Paired serum and plasma samples showed excellent correlation in the same assay with no significant difference between the means.

C–F PWV measurement was performed using Complior® (Colson, Les Lilas, France) [28] at baseline and 12 months. Dedicated mechano-sensors were directly applied to the skin overlying the carotid and femoral arteries. The transit time was determined by means of a correlation algorithm between each simultaneous recorded wave. The validation and reproducibility of this method have been previously published [28].

Measurement of C–F PWV was performed by three observers throughout the study. A repeatability study was performed, which demonstrated no significant inter-observer variability. Intra-observer variability analysis demonstrated 94.1% of readings were within the limits of agreement.

Cardiovascular disease was defined as a history of myocardial infarction, angina, coronary artery bypass grafting, stroke, transient ischaemic attack or peripheral vascular disease given by the patient and confirmed from the medical notes.

Statistical analysis

MAP is a recognized determinant of PWV [29]. C–F PWV values were, therefore, adjusted for MAP and values throughout relate to adjusted C–F PWV.

After examination of distribution and skew, correlations were assessed using Pearson’s correlation for parametric or Spearman’s rank correlation
for non-parametric data. All categorical variables are reported as percentages. Quantitative variables that were normally distributed are reported as mean ± standard deviation (SD). Skewed quantitative variables were log-transformed and the geometric mean (with 95% confidence interval, CI) reported. Comparisons between diabetic and non-diabetic groups were made using unpaired Student’s t-test and chi-squared test for categorical variables.

Analysis of the results demonstrated a significant interaction between the effect of diabetes and fetuin-A on change in C-F PWV. Therefore, further analysis was performed separately on diabetics and non-diabetics.

Univariate regression analysis was performed for selected biochemical, clinical and anthropometric variables. Variables with P < 0.05 were entered into a multivariate model.

Statistical analyses were performed using Stata software (version 10.1; StataCorp LP, College Station, TX, USA).

Results

Baseline characteristics

Of the 133 patients recruited into the study, 92 had C-F PWV measurement at baseline and 12 months. Of the 41 patients for whom recordings were unavailable, 16 were no longer active in the study and 25 could not be recorded for technical reasons. There were absent femoral pulses or previous aorto-femoral vascular grafting in eight, absent carotid pulses in two and the waveform could not be detected by the software for a further 15. Of the 16 patients no longer in the study, 11 had withdrawn and five had either started renal replacement therapy or had died. The baseline characteristics of 92 active patients are summarized in Table 1.

Fetuin-A was correlated with serum phosphate and MAP but not with log C-reactive protein (CRP) or log uPCR (Table 2).

Mean fetuin-A was significantly lower in the non-diabetic group compared to the diabetic group (0.28 ± 0.07 vs 0.23 ± 0.06 g/l; P = 0.018).

Table 1. Baseline demographic and clinical data of patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-diabetics</th>
<th>Diabetics</th>
<th>All</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 73)</td>
<td>(n = 19)</td>
<td>(n = 92)</td>
<td></td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>60:13</td>
<td>15:4</td>
<td>75:17</td>
<td>0.746</td>
</tr>
<tr>
<td>Age (years)</td>
<td>67.4 ± 12.1</td>
<td>68.4 ± 9.3</td>
<td>67.7 ± 11.5</td>
<td>0.639</td>
</tr>
<tr>
<td>Smoking history</td>
<td>47 (64.4%)</td>
<td>10 (52.6%)</td>
<td>57 (62.0%)</td>
<td>0.439</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28.4 ± 5.7</td>
<td>30.6 ± 4.1</td>
<td>28.9 ± 5.5</td>
<td>0.117</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>33.6 ± 11.6</td>
<td>32.1 ± 8.9</td>
<td>33.3 ± 11.1</td>
<td>0.583</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>154.5 ± 19.5</td>
<td>150.3 ± 21.3</td>
<td>153.6 ± 19.8</td>
<td>0.407</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>84.8 ± 11.1</td>
<td>78.4 ± 10.8</td>
<td>83.9 ± 11.3</td>
<td>0.025*</td>
</tr>
<tr>
<td>Heart rate (b.p.m.)</td>
<td>70.9 ± 11.3</td>
<td>70.7 ± 14.0</td>
<td>70.9 ± 11.8</td>
<td>0.953</td>
</tr>
<tr>
<td>Mean no. of antihypertensives</td>
<td>2.1 ± 1.2</td>
<td>2.4 ± 1.7</td>
<td>2.2 ± 1.3</td>
<td>0.456</td>
</tr>
<tr>
<td>Phosphate binder treatment</td>
<td>2 (2.7%)</td>
<td>1 (5.3%)</td>
<td>3 (3.3%)</td>
<td>0.505</td>
</tr>
<tr>
<td>Vitamin D₃ treatment</td>
<td>2 (2.7%)</td>
<td>1 (5.3%)</td>
<td>3 (3.3%)</td>
<td>0.505</td>
</tr>
<tr>
<td>1 Hydroxyvitamin D₃ treatment</td>
<td>7 (9.6%)</td>
<td>2 (10.5%)</td>
<td>9 (9.8%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Statin treatment</td>
<td>45 (61.6%)</td>
<td>11 (57.9%)</td>
<td>56 (60.9%)</td>
<td>0.796</td>
</tr>
<tr>
<td>Phosphate (mmol/l)</td>
<td>1.08 ± 0.20</td>
<td>1.10 ± 0.17</td>
<td>1.08 ± 0.20</td>
<td>0.602</td>
</tr>
<tr>
<td>Corrected calcium (mmol/l)</td>
<td>2.28 ± 0.12</td>
<td>2.29 ± 0.10</td>
<td>2.28 ± 0.11</td>
<td>0.748</td>
</tr>
<tr>
<td>Fetuin-A (g/l)</td>
<td>0.23 ± 0.06</td>
<td>0.28 ± 0.07</td>
<td>0.24 ± 0.06</td>
<td>0.018*</td>
</tr>
<tr>
<td>CRP ≥ 5 mg/l (%)</td>
<td>18 (24.7%)</td>
<td>7 (36.8%)</td>
<td>25 (27.2%)</td>
<td>0.157</td>
</tr>
<tr>
<td>uPCR (mg/mmol)</td>
<td>31.6 (24.3, 41.0)</td>
<td>44.4 (24.1, 82.0)</td>
<td>33.9 (26.7, 43.0)</td>
<td>0.254</td>
</tr>
<tr>
<td>iPTH (ng/l)</td>
<td>76.9 (66.9, 88.4)</td>
<td>70.8 (51.0, 92.3)</td>
<td>75.6 (66.5, 85.8)</td>
<td>0.598</td>
</tr>
</tbody>
</table>

P-values relate to comparison between non-diabetic and diabetic cohorts.

*P < 0.05, uPCR and iPTH are expressed as geometric mean ± 95% CI. Other continuous variables are expressed as mean ± SD.

Table 2. Correlations of baseline fetuin-A with clinical parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>Entire cohort</th>
<th>Non-diabetic cohort</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.009</td>
<td>0.035</td>
<td>0.766</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>−0.172</td>
<td>−0.189</td>
<td>0.108</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>−0.219</td>
<td>−0.224</td>
<td>0.056</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>0.124</td>
<td>0.041</td>
<td>0.729</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>−0.012</td>
<td>0.055</td>
<td>0.645</td>
</tr>
<tr>
<td>Corrected calcium (mmol/l)</td>
<td>−0.222</td>
<td>−0.259</td>
<td>0.027*</td>
</tr>
<tr>
<td>Log iPTH (ng/l)</td>
<td>−0.022</td>
<td>−0.141</td>
<td>0.240</td>
</tr>
<tr>
<td>Log CRP (mg/l)</td>
<td>0.079</td>
<td>0.036</td>
<td>0.759</td>
</tr>
<tr>
<td>Log uPCR (mg/mmol)</td>
<td>−0.004</td>
<td>−0.022</td>
<td>0.854</td>
</tr>
</tbody>
</table>

*P < 0.05.

Mean C-F PWV was not significantly different between diabetics and non-diabetics at baseline (Table 3).

Determinants of change in carotid–femoral pulse wave velocity

Univariate analysis of the non-diabetic group showed change in C-F PWV at 1 year was related to SBP (r = 0.389, P = 0.001), baseline fetuin-A concentration (r = −0.481, P < 0.001) and baseline C-F PWV (r = −0.240, P = 0.041; Table 4 and Figure 1), but not to other considered variables. There was no significant correlation between change in C-F PWV and change in eGFR at 1 year (r = 0.086, P = 0.468).

Univariate analysis of determinants of change in C-F PWV in the non-diabetic group using CKD stage as a categorical variable was also performed. Results were similar across all groups (data not shown).
SBP, fetuin-A and baseline C–F PWV were entered into a multivariate model where all variables remained significant (Table 5). The model predicted 45.5% of variation in C–F PWV over 1 year.

In the numerically small diabetic group (n = 19), none of the variables including fetuin-A reached statistical significance.

The non-diabetic patients were divided into those who had an increase in C–F PWV over 1 year and those who had a decrease. Those who had an increase had a significantly lower fetuin-A concentration at baseline (0.214 ± 0.051 vs 0.265 ± 0.522 g/l, P < 0.001).

**Discussion**

This is the first study to describe the relationship between baseline fetuin-A and change in arterial stiffness in the predialysis CKD population. The findings of this study are consistent with the recognized role of fetuin-A as an inhibitor of calcification, i.e. the reduced concentration of the inhibitor of calcification leads to progressive increase in arterial stiffness.

In this study, serum phosphate correlated closely with plasma fetuin-A. However, interestingly, there was no significant relationship found between CRP and fetuin-A. This may be explained by the large proportion of patients in whom inflammatory markers were not elevated (71.7% patients CRP <5 mg/l).

As expected, age correlated closely with C–F PWV (r = 0.608, P < 0.001). However, it appears that the rate of change over 1 year is affected to a greater extent by fetuin-A, blood pressure and by baseline PWV. This finding is compatible with the additional mortality risk attributable to CKD (in part via abnormal calcium regulatory protein metabolism) and hypertension over and above the ageing process.

One of the problems associated with this analysis, unpublished at the time of study conception, is the association of type 2 diabetes with increased circulating concentration of fetuin-A [22,23]. Type 2 diabetes is not only associated with increased cardiovascular risk per se but also with increased arterial stiffness due to a variety of mechanisms that may be unrelated to fetuin-A. These opposing relationships have the potential to confound the natural disease process.
any association between fetuin-A and arterial stiffness in the wider population, and for this reason, analysis of the cohort was performed separately on non-diabetic and diabetic populations.

The finding of increased fetuin-A in our diabetic cohort, whilst consistent with the findings of other groups [30], must, however, be interpreted with caution due to potential confounders and small sample size.

Studies examining the role of fetuin-A in arterial stiffening in CKD have shown varied results. Among non-diabetic children receiving renal replacement therapy, fetuin-A was an independent predictor of baseline aortic PWV [31]. However, in a study of elderly dialysis patients, this relationship lost significance after correction for age, gender, MAP and diabetic status [32]. Another study of a heterogeneous CKD stage 4 and dialysis population found no association between fetuin-A and change in vascular calcification [11], whilst a study of fetuin-A and aortic pulse wave velocity in adults with normal renal function also found no independent relationship [33].

Additional evidence for the link between fetuin-A and arterial stiffness is found in the association of fetuin-A with cardiovascular mortality in dialysis patients [13]. However, this association was not upheld in the only published study examining this relationship in stage 3 and 4 CKD [34]. There are several possible reasons for this. The 822 people followed up by this group were relatively young (mean age 52 years) and healthy, with a previous history of coronary artery disease in only 9.7% of their study population. This study found adult polycystic kidney disease (ADPKD, which was heavily represented at 40.7% of patients) was associated with a higher fetuin-A concentration. This demographic contrasts with our non-diabetic group where the average age was 67.7 years, 4.1% had ADPKD and 37% had a history of coronary artery disease.

Inflammation and CRP are associated with adverse cardiovascular and total mortality rates in the general [35], dialysis [36] and pre-dialysis CKD populations [37]. Since fetuin-A is a negative acute-phase reactant [38], the finding in this study of a relationship between low fetuin-A and progressive arterial stiffness, independent of CRP, is striking. This underlines the possible role of active calcification in the aetiology of arterial stiffness in the CKD stage 3 and 4 population. Given the clear link between arterial stiffness and survival in the dialysis population [39], and recent evidence that PWV is an independent risk factor for progression to end-stage renal failure [40], strategies which target the determinants of arterial stiffening (e.g. fetuin-A concentration) may, therefore, have the potential to influence cardiovascular morbidity. Other than the negative acute-phase reactant properties of fetuin-A, the factors controlling the synthesis of fetuin-A and entry and exit of fetuin-A from the blood pool are not well understood but may have the potential for therapeutic intervention.

The identification of biomarkers predictive of progressive arterial stiffness has significant clinical potential given the large numbers of patients with CKD, the important role of arterial stiffening in both cardiovascular mortality and progression of renal failure, and emerging evidence regarding potential pharmacological manipulation of fetuin-A concentrations [41]. The identification of those patients at highest risk of cardiac events and progression may allow individual tailoring of blood pressure and metabolic management.

The plausibility of a link between reduced fetuin-A and progression of arterial stiffness is strengthened by evidence that alteration of fetuin-A levels may attenuate progression of arterial pathology. Use of the non-calcium-containing phosphate binder, sevelamer, has been linked to a slowing in the progression of aortic calcification in haemodialysis patients [42]. A recent study in non-diabetic stage 4 CKD patients demonstrated a significant increase in fetuin-A after treatment with sevelamer [43]. However, the mechanism for this increase in fetuin-A is not currently understood and the long-term clinical relevance of this change is yet to be demonstrated.

The finding in this study of an interaction between diabetic status and fetuin-A on change of C–F PWV adds weight to the concept that the pathophysiology of increased aortic stiffness seen in diabetic CKD patients may be driven by different processes than in the non-diabetic population. These may include deposition of advanced glycation products, calcification of more extensive atherosclerotic plaque or development of additional de novo atherosclerosis.

In contrast, however, the independent relationship between lower baseline concentration of fetuin-A with increased aortic stiffening in non-diabetic patients is consistent with the biological activity of the protein, i.e. that reduction in calcification inhibitor is associated with increased aortic stiffening. A putative mechanism for this is via increased medial calcification; however, no imaging or histological examination was performed in this study to support this.

In summary, we have shown that fetuin-A is an independent risk factor for change in arterial stiffness in non-diabetic patients with CKD stages 3 and 4. In order to develop therapeutic strategies targeting arterial calcification and stiffening, a more thorough understanding of the biology of calcification regulatory proteins—synthesis, excretion and the determinants of their flux from blood and bone—is required.

Acknowledgements. M.F. has received a travel grant from Genzyme. The other authors have no conflict of interest. Mr. Winston Banya provided statistical support.

Conflict of interest statement. None declared.
References


Received for publication: 14.5.09; Accepted in revised form: 1.12.09

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