Original Article

Study on the relationship of serum fetuin-A concentration with aortic stiffness in patients on dialysis

Marc M. H. Hermans¹, Vincent Brandenburg², Markus Ketteler², Jeroen P. Kooman¹, Frank M. van der Sande¹, Ulrich Gladziwa³, Pieter L. Rensma⁴, Karlijn Bartelet⁴, Constantijn J. A. M. Konings⁵, Arnold P. G. Hoeks⁶, Jürgen Floege² and Karel M. L. Leunissen¹

¹Department of Internal Medicine and Nephrology, Academic Hospital Maastricht, Maastricht, The Netherlands, ²Department of Nephrology and Clinical Immunology, University Hospital RWTH, Aachen, Germany, ³University of Witten-Herdecke, Witten, Germany, ⁴Department of Internal Medicine and Nephrology, Elisabeth Hospital Tilburg, The Netherlands, ⁵Department of Internal Medicine and Nephrology, Catharina Hospital Eindhoven, The Netherlands and ⁶Department of Biophysics, Maastricht University, Maastricht, The Netherlands

Abstract

Background. An increase in aortic stiffness, as reflected by an increase in pulse wave velocity (PWV) or aortic augmentation index (AI) is an important predictor of cardiovascular mortality in dialysis patients. Dysregulation of calcification inhibitors, such as fetuin-A, is involved in vascular pathology in dialysis patients and fetuin-A is inversely related to mortality in dialysis patients. In this study, the relation between serum fetuin-A concentration and parameters of aortic stiffness was investigated in patients with end-stage renal disease.

Methods. In a cross-sectional study we included 131 dialysis patients, aged 62±14 years (33 on peritoneal dialysis and 98 on haemodialysis), and 41 controls, aged 60±8 years. Time-averaged pre-dialysis values of serum albumin, Ca, P and intact parathyroid hormone were included in multiregression analysis, as were high-sensitivity C-reactive protein (hsCRP), fetuin-A, age, mean arterial pressure (MAP) and dialysis modality. PWV and AI were measured with the SphygmoCor device.

Results. Mean fetuin-A concentration in dialysis patients (0.63±0.16 g/l) did not differ from controls (0.63±0.11 g/l). Median hsCRP levels in dialysis patients were higher compared with controls (4.0 vs 1.9 mg/l; P<0.0001). PWV but not AI was higher in dialysis patients than in controls (9.9 vs 7.9 m/s; P<0.0001). In univariate analysis in dialysis patients, fetuin-A levels were inversely related to both PWV (r = -0.25, P = 0.007) and AI (r = -0.26, P = 0.006), respectively. However, after correction for age, gender, MAP and diabetes mellitus, this relation lost statistical significance.

Conclusions. In a dialysis population with a relatively low level of inflammatory activity, the soluble calcification inhibitor fetuin-A could not be identified as an independent predictor of aortic stiffness as measured with PWV and AI.

Keywords: arterial stiffness; calcification; end-stage renal disease; pulse wave velocity

Introduction

End-stage renal disease (ESRD) is associated with greatly increased cardiovascular morbidity and mortality [1]. Apart from occlusive arterial disease as seen in atherosclerosis, arterial stiffening as a feature of predominant medial calcification is a hallmark of vascular pathology in dialysis patients [2,3]. Increased aortic stiffening, reflected e.g. by an increased pulse wave velocity (PWV) or aortic augmentation index (AI), is an important determinant of all-cause and cardiovascular mortality in this patient group [4,5].

Recently, abnormalities in both the calcium and phosphate metabolism have emerged as important risk factors for vascular wall calcifications and mortality, respectively [6,7]. These abnormalities were consequently found to be related to arterial stiffness, which may be explained by an interrelation between medial calcifications and vascular wall stiffness [4,6]. However, arterial stiffening is also associated with traditional risk factors, such as aging, diabetes and hypertension, and non-traditional risk factors,
such as hyperhomocysteinaemia, oxidative stress, dyslipidaemia, accumulation of glycosylation end-products and inflammation [7–9].

Recent attention has focused on the potential importance of a low serum fetuin-A concentration as a non-traditional cardiovascular risk factor in dialysis patients. This glycoprotein is a potent calcification inhibitor and absence of fetuin in fetuin-A knock-out mice results in massive extra-osseous calcification [10]. Fetuin-A, a negative acute-phase reactant, plays a pivotal role in the inhibition of Ca\(\times\)P precipitation [11,12]. In haemodialysis (HD) patients, lower fetuin-A concentrations are associated with both a higher overall and cardiovascular mortality [13,14]. At least a part of the relation between low fetuin-A levels and increased mortality appeared to be explained by the potential down-regulation of fetuin-A in inflammatory states [12,15]. Moreover, low fetuin-A levels were found to be related to increased vascular calcifications in dialysis patients [16] and to calcific uraemic arteriolopathy [10]. In peritoneal dialysis (PD) patients, Wang et al. [15] showed an inverse relationship between serum fetuin-A and valvular calcification. However, it is not clarified whether fetuin-A deficiency in dialysis patients is an independent predictor for the development of vascular stiffness as a consequence of vascular calcification in dialysis patients.

Therefore, the aim of the present study was to evaluate the association between serum fetuin-A concentrations with parameters of vascular stiffness, especially considering the presence of inflammation as a potential trigger for fetuin-A down-regulation.

Subjects and methods

Study design

The study investigated the relation between fetuin-A and markers of aortic stiffness (AI and PWV) in a cross-sectional design. In a post-hoc analysis differences with respect to fetuin-A levels and aortic stiffness between controls and dialysis patients were studied.

Subjects

A total of 131 stable dialysis patients (all but two Caucasian), undergoing HD (n = 98, 75%) and PD (n = 33, 25%), from three dialysis centres were included.

ESRD patients were eligible when they were on dialysis for >3 months. Patients with an underlying malignancy, infection or heart failure were excluded. Because fetuin-A is produced in the liver, patients with liver failure, liver cirrhosis or hepatitis B or C were excluded.

The 41 age- and gender-matched controls consisted of spouses and healthy staff members. Controls had to have a negative cardiovascular medical history, including hypertension. Cardiovascular disease (CVD) was defined as the presence or history of ischaemic heart disease, peripheral vascular disease and/or a cerebrovascular event. Hypertension was defined as a blood pressure ≥140 mmHg systolic and/or ≥90 mmHg diastolic, according to the JNC VII criteria [17] and/or the current use of antihypertensive medication. In controls, renal function was estimated by the modified MDRD formula in ml/min and expressed per 1.73 m\(^2\) body surface area [18]. Fasting plasma glucose levels ≥7.0 mmol/l were considered diagnostic for diabetes mellitus.

All participants gave their written informed consent. The study protocol was designed in adherence to the Declaration of Helsinki and approved by the ethical committees of the participating centres.

Evaluation of aortic stiffness by PWV and AI

In both groups, measurement of blood pressure, PWV and AI were done after 15 min of supine rest. The HD patients were investigated 1 h before the dialysis session. PD patients were investigated with an empty abdomen. Brachial systolic and diastolic pressures were assessed at 3-min intervals with a radial artery tonometrical device (CBM 7000; Colin Medial Instruments, San Antonio, TX, USA) on the right arm or, in case of a right-sided dialysis shunt, on the left arm. Mean arterial pressure (MAP) was calculated from the mean of three systolic and diastolic pressures as:

\[
\text{MAP} = \frac{2 \times \text{Diastolic pressure} + \text{Systolic pressure}}{3}
\]

The PWV was measured using the SphygmoCor (AtCor Medical Ltd, Moreton-in-the-Marsh, UK). Briefly, a carotid and a femoral artery waveform were obtained consecutively with a high-fidelity applanation tonometer (Millar SPT-301; Millar Instruments Inc., Houston, TX, USA). Transit time was obtained by subtraction from the delays between ECG and both pulses.

The SphygmoCor device was also used to determine the AI. The AI is a measure of the additional load to which the left ventricle is subjected as a result of wave reflection and was performed with the above-described applanation tonometer. AI is a composite parameter, because it reflects the reflective properties of the peripheral distal arterial bed and elastic properties of large arteries. PWV and AI are related but not synonymous [19]. The AI was derived from the right radial arterial pulse by means of a transfer function [20]. In our analysis, we used the AI corrected for heart rate. The methods for measuring PWV and AI have been described and evaluated elsewhere [21].

Laboratory analysis

In the patient group, serum calcium (Ca), phosphate (P) and albumin were measured using standard laboratory techniques. In the HD group, samples were taken at the start of a short-interval haemodialysis. Time-averaged values of Ca, P and albumin were calculated as the mean of the routine six weekly measurements of the previous 6 months. Calcium concentration was calculated after correction for albumin.
Intact parathyroid hormone (iPTH) was measured by a two-site chemiluminescence immunoassay (Nichols Institute Diagnostics BV, Nijmegen, The Netherlands).

In controls we measured fasting serum glucose, calcium, phosphate and creatinine.

In both groups, high-sensitivity C-reactive protein (hsCRP) and fetuin-A were measured by nephelometry. Serum was harvested by centrifugation of clotted blood. Serum samples were stored at −80°C prior to analysis. Serum analysis for hsCRP was performed by means of particle-enhanced immunonephelometry using a standard 'CardioPhase hsCRP' for 'BNII' (Dade Behring Holding GmbH, Liederbach, Germany). CRPI or CRPII assay protocols were used when appropriate. Interday precision controls revealed coefficients of variation (CV) below 6%.

The nephelometry method for fetuin-A employs the same high-specificity antibody as the enzyme-linked immuno-sorbent assay method described previously [13,22]. The nephelometric method for fetuin-A serum measurement has been evaluated in a side-by-side comparison with immunoblot analysis to exclude cross-reactivity of the antibodies with other serum proteins and proteolytic fragments of fetuin-A. Cross-reaction with fetuin-B was excluded. Serum samples were cleared by centrifugation (60 min at 15 000 g) and diluted 1:4 with 400 μl phosphate-buffered saline (N Diluent; Dade Behring Holding, Liederbach, Germany). Nephelometric assays were performed manually using an automatic nephelometer (BNII; Dade Behring Holding, Liederbach, Germany). The assay linear measurement range of human fetuin-A is 0.05–3.5 g/l. The within-run precision obtained from a 20-fold measurement of identical samples yielded a CV of 7.75%. The day-to-day precision obtained from repetitive measurements of control serum was determined as a CV of 8.2%.

Statistical analysis

The zero hypothesis to be tested was that no relation existed between serum fetuin-A levels and aortic stiffness. We estimated the sample size on the basis of the alternative hypothesis that a correlation coefficient of 0.4 exists between fetuin levels and aortic stiffness. With an alpha significance level of 0.05 and a power of 0.8, 48 patients would be needed to test this hypothesis. In order to allow for multivariate analysis, we chose to include at least 100 patients to test the hypothesis. Normally distributed variables are expressed as means±SD and non-normally distributed variables as median and range, with P<0.05 indicating significance. All continuous data were tested for a normal distribution before further statistical analysis. Not-normally distributed variables were log-transformed. Differences in frequency of nominal variables were compared using chi-square analysis. Comparison between two groups was done with unpaired Student’s t-test. Univariate and multiple regression analysis were used in dialysis patients to test the associations of variables related to PWV and AI. The variables were entered one after another with a maximum of five. Two-sided univariate analysis was used to analyse the fetuin level between the HD and PD groups with correction for confounders. Analyses were performed with SPSS for Windows® version 11.0 (SPSS, Chicago, IL, USA).

Results

The characteristics of the dialysis group and the controls are shown in Table 1. The major causes of stage 5 chronic kidney disease (CKD) were diabetes in 18%, nephrosclerosis in 30% and glomerulonephritis in 18% of the patients.

PWV was significantly higher in dialysis patients compared with controls (9.9 vs 7.9 m/s; P<0.0001), whereas AI was not significantly different.

Serum fetuin-A concentrations did not differ significantly between patients and controls (0.63±0.16 vs 0.63±0.11 g/l; P=0.59). Serum fetuin-A levels were not related to log-hsCRP, Ca, P, Ca×P product, albumin, log-iPTH and duration of dialysis (data not shown). In dialysis patients, but not in controls, serum fetuin-A concentration was inversely related to age (r = −0.24; P = 0.005). In a post-hoc analysis, fetuin-A concentration in PD patients was, also after correction for age, duration of dialysis, log-hsCRP and albumin, significantly higher than in HD patients (0.73±0.16 vs 0.60±0.15 g/l; P < 0.0001).

Predictors of PWV in ESRD

In dialysis patients, fetuin-A was significantly and inversely related to PWV (r = −0.26, P = 0.007; Figure 1). In a univariate analysis, age, MAP, presence of diabetes mellitus and fetuin-A were significant predictors of PWV, whereas gender, log-hsCRP, log-iPTH, Ca, P, the Ca×P product and dialysis modality were not. After adjustment for age, gender, MAP and diabetes, fetuin-A lost the statistical significant association to PWV (Table 2).

Predictors of AI in ESRD

Fetuin-A concentration was also significantly and inversely related to AI (r = −0.26, P = 0.006; Figure 2). Age, MAP, gender and fetuin-A were significant predictors of AI. Log-hsCRP, log-iPTH, Ca, P, presence of diabetes mellitus and the Ca×P product were not related to AI. After adjustment for age, gender, MAP and diabetes, fetuin-A lost the statistical significant association to AI (Table 2).

Predictors of PWV in controls

In contrast to the above, fetuin-A serum concentration in controls was significantly positively related to PWV (r = 0.50, P = 0.001; Figure 3). In a univariate analysis, MAP, age and fetuin-A were significant predictors of PWV, whereas gender, log-hsCRP, Ca, P and the Ca×P product were not. After correction for age, gender and MAP the significance was lost for the correlation between fetuin-A and PWV (Table 2).

Predictors of AI in controls

Fetuin-A concentration in controls was significantly positively related to AI (r = 0.37, P = 0.017; Figure 4).
In a univariate analysis, age, fetuin-A and MAP were predictors of AI. After correction for age and MAP, the significant correlation between fetuin-A and AI disappeared (Table 2).

**Discussion**

This is the first study assessing the association between serum fetuin-A levels and aortic stiffness in dialysis patients. Fetuin-A levels negatively correlated with AI and PWV in ESRD patients, but could not be identified as an independent risk factor for the development of arterial stiffness. Adjustment for (i) age, MAP and diabetes for PWV and (ii) age, MAP and gender for AI made the statistically significant correlation with serum fetuin-A disappear. Various authors have shown that serum fetuin-A levels were associated with morphological arterial parameters. In ESRD patients, a significant relation between low fetuin-A levels and coronary calcification [23], prevalence of carotid plaques [14] and valvular calcification [15] have been shown. Our study showed an absence of a relation between fetuin-A levels and a functional arterial parameter (stiffness). Interpreting the absence of such a potential independent influence of fetuin-A upon PWV and AI, it is important to point out that age and MAP are the major predictors of arterial stiffness overriding the association with fetuin-A.
In contrast to PWV, AI did not differ between patients and controls. As stated in the ‘Subjects and methods’, it is important to realize that these measures are not interchangeable. In a multivariate analysis in a study by Kelly et al. [24], AI did not even correlate with PWV. Several authors have shown that PWV and AI react differently on volume reduction with haemodialysis [25,26]. PWV did not change, or even rose, where AI decreased or even normalized after haemodialysis, compared with controls. The lower post-dialysis blood pressure, thus, had more effect on the AI than on the PWV. We hypothesize that the relative adequate blood pressure control in our patients could explain the lower AI.

In contrast to Moe, Stenvinkel, and Wang, Mehrotra et al. found a direct relationship between fetuin-A levels and coronary artery calcifications in non-dialysed patients with diabetic nephropathy in CKD stages 1–4 [22]. These data point towards a complex relation between fetuin-A and calcification in different settings. In contrast to our dialysis cohort, we found a direct relationship between fetuin-A and PWV and AI.

<table>
<thead>
<tr>
<th>Model</th>
<th>Patients</th>
<th>Controls</th>
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<tbody>
<tr>
<td></td>
<td>PWV</td>
<td>AI</td>
</tr>
<tr>
<td>(Fetuin)</td>
<td>−0.5a (−0.8 to −0.1)</td>
<td>−1.8a (−3.0 to −0.5)</td>
</tr>
<tr>
<td>(1 + Age)</td>
<td>−0.1 NS (−0.4 to 0.2)</td>
<td>−1.1 NS (−2.4 to 0.2)</td>
</tr>
<tr>
<td>(2 + Gender)</td>
<td>−0.1 NS (−0.4 to 0.2)</td>
<td>−1.1 NS (−2.2 to 0.1)</td>
</tr>
<tr>
<td>(3 + MAP)</td>
<td>0.2 NS (−0.3 to 0.3)</td>
<td>−0.7 NS (−1.8 to 0.5)</td>
</tr>
<tr>
<td>(4 + Diabetes)</td>
<td>−0.5 NS (−0.4 to 0.3)</td>
<td>−6.6 NS (−1.8 to 0.5)</td>
</tr>
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Results are expressed as regression coefficients and their 95% confidence interval. Serum fetuin-A is expressed per 0.1 g/l increase. NS, not significant.

aP < 0.01, bP < 0.05.

**Table 2. PWV and AI according to fetuin-A in dialysis patients and controls: adjusted analysis**

Fig. 2. The correlation ($R^2 = 0.07$, $P = 0.006$) between serum fetuin and AI in 114 dialysis patients.

Fig. 3. The correlation ($R^2 = 0.25$, $P = 0.001$) between serum fetuin and PWV in 39 controls.

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the control group. This analysis was not the primary goal of our study, but merely a post-hoc analysis. Therefore, the result should be interpreted with caution. Nevertheless, since fetuin-A is strongly deposited at sites of vascular calcifications [15], fetuin-A may be a functional defence system against overt unwanted calcifications in populations without or with early stages of CKD, as in our control group, but may finally become down-regulated and exhausted in the uraemic state.

In contrast to a previous study by Ketteler et al. [13], fetuin-A levels in our dialysis patients were not different from fetuin-A levels in the control group. The reason for the missing difference in fetuin-A levels between patients and controls appears to be the low mean serum fetuin-A level in spouses and healthy staff members. In the previous study by Ketteler et al., mean fetuin-A levels in dialysis patients were significantly lower than compared with controls (0.66 vs 0.72 g/l; P = 0.014), but comparable to the levels obtained in our dialysis patients. A definite explanation for this surprising finding cannot be provided. Demographic differences between our study and earlier studies might have played a role. Although all controls anamnestically had a cardiovascular negative medical history, it is quite remarkable that one-third were hypertensive according to the JNC VII criteria. Thus, they might have been less ‘healthy’ compared with the blood donors studied by Ketteler et al. Also, the renal function was decreased in some controls corresponding to stages 1–2 CKD.

Also in contrast to recent studies [13,14], in the present study no relation was seen between serum fetuin-A concentrations and hsCRP as well as between fetuin-A and serum albumin levels. Possibly, the lower level of inflammation in our study compared with earlier studies might play a role. Compared with the Ketteler cohort (mean CRP: 16.3 ± 25.1 mg/l) and the Stenvinkel cohort (mean CRP: 4.2 mg/l for patients without CVD and 13.0 mg/l for those with CVD), our ESRD patients exhibited low CRP levels (4.0 mg/l for the entire group). This interpretation is supported by cross-sectional data from non-dialysed patients in whom no drop of fetuin-A is detectable with CRP levels <10 mg/l (Ketteler and Brandenburg, unpublished data).

An interesting finding of a post-hoc analysis of our study was the significantly higher fetuin-A level in PD patients. This finding, however, requires further confirmation, especially since another study could not detect such a difference in PD vs HD patients [14].

Our study is limited by its cross-sectional nature. Parameters such as fetuin-A and CRP levels were only assessed at a single point in time instead of having time-averaged values and related to markers of arterial stiffness which develop over many years. Furthermore, arterial stiffness is a complex phenomenon and just represents a surrogate parameter of medial calcification.

In summary, in a, with respect to inflammation, relatively ‘healthy’ dialysis population, the calcification inhibitor fetuin-A was found to be inversely related to PWV and AI in univariate analyses. Statistical significance was lost after correction for confounders. Fetuin-A, therefore, appeared not to be an independent predictor of aortic stiffness in a dialysis population with a low level of inflammatory activity.

Conflict of interest statement. None declared.

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