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ORIGINAL ARTICLE

Low serum fetuin-A concentration predicts poor outcome only in the presence of inflammation in prevalent haemodialysis patients

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ABSTRACT

Background Fetuin-A, a negative acute phase protein that inhibits vascular calcification, has a controversial association with mortality in chronic kidney disease (CKD) patients. Chronic inflammation, which is common in CKD, may promote vascular calcification.

Materials and methods We investigated the impact of inflammation on the relationship between serum fetuin-A and mortality (42 months) in 222 prevalent haemodialysis (HD) patients.

Results Serum fetuin correlated negatively with comorbidity score (assessed by Davies score) and circulating inflammatory markers. Patients with low fetuin-A levels (< median) had higher mortality (Hazard ratio 'HR' 2·2; CI 1·4–3·5, $P < 0·001$), but this association was lost after adjustment for age, gender, comorbidities score, dialysis vintage and inflammation (CRP > median). In inflamed patients with low fetuin a significantly independent association with mortality (HR 2·3; CI 1·2–4·5, $P = 0·01$) was observed compared to non-inflamed patients with high fetuin-A, after adjusting for the same variables. Non-inflamed patients with low fetuin-A and inflamed patients with high fetuin-A did not have increased mortality compared to non-inflamed patients with high fetuin-A.

Conclusions The results show that low levels of serum fetuin-A are associated with increased mortality in HD patients only in the presence of inflammation. This suggests that coexistence of a low serum fetuin-A level and low-grade inflammation exerts an additive effect on the risk of death in HD patients.

Keywords Fetuin-A, haemodialysis, inflammation, mortality.

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Introduction

The prevalence and extent of vascular calcification is a strong predictor of cardiovascular disease (CVD) events and all-cause death in chronic kidney disease (CKD) patients [1,2]. The pathogenetic mechanism(s) of vascular calcification are still not well defined. Recent attention has focused on the potential role of a low serum fetuin-A concentration as a cardiovascular risk factor in CKD patients. Fetuin-A, a negative acute phase reactant, plays a pivotal role in the inhibition of $Ca \times PO_4$ precipitation and is thereby considered as a potent inhibitor of vascular calcification [3].

Clinical studies suggest that low serum fetuin-A concentration is a predictor of poor outcome in CKD stage 5 patients [4–7]. This in contrast to the findings of Ix *et al*. [8] who showed in CKD stage 3–4 patients that low serum fetuin-A level did not associate with poor clinical outcome. Although these reports in CKD stage 5 do not necessarily infer causality, they are fully compatible with a role for fetuin-A in clearing insoluble remnants and link fetuin-A deficiency with an inflammatory state. On the other hand, it has been speculated that chronic inflammation may promote vascular calcification [9,10]. For instance, it has been demonstrated that activation of monocytes and macrophages enhance *in vitro* vascular calcification via two independent mechanisms: cell to cell interaction and production of soluble factors, such as tumour necrosis factor (TNF)-α [11]. Moreover, low fetuin-A levels were related to increased vascular calcification and inflammation in dialysis patients [6]. Altogether there seems to be some consensus that vascular calcification is, indeed, a part of an active cell-mediated inflammatory process [9,10] and that the presence of inflammation may contribute to progression of arterial calcification [12]. In CKD stage 5 patients, the serum fetuin-A

concentration is significantly lower than in healthy individuals and is associated with increased levels of C-reactive protein (CRP) and with enhanced cardiovascular mortality [4–7]. Thus, it is possible that the combination of inflammation and low fetuin-A in an additive way may aggravate the vascular calcification process and worsen clinical outcome. Therefore, in this study, we investigated the impact of inflammation on mortality in prevalent haemodialysis (HD) patients divided into high and low serum fetuin-A levels, respectively.

Patients and methods

Patients

The study was performed at five dialysis units in Stockholm and at Uppsala Academic Hospital in Uppsala. This is a post hoc analysis from a cross-sectional study with a follow-up that originally aimed at investigating the variability of inflammatory markers in patients undergoing prevalent haemodialysis ($n = 254$) over time. The protocol is explained in more detail elsewhere [13]. Recruitment of the patients occurred from October 2003 through March 2004. All patients who were currently receiving regular therapy at any of the units were invited to participate; six patients declined, and one patient with HIV infection was excluded. The 247 eligible patients were then followed for 12 weeks, during which time the concentration of CRP was measured weekly. Because the aim of the original study was to investigate the variability of inflammatory markers, patients were excluded from the study if available for fewer than six of these weekly CRP measurements. Eleven patients were excluded because insufficient baseline clinical information was available, seven were excluded because of insufficient CRP measurements, and one patient died. The remaining 228 patients were further followed for assessment of overall and cardiovascular mortality in relation to biochemical markers. For the present study, six more patients were excluded because no serum fetuin-A was available, which left 222 patients for inclusion in this analysis. The current study is limited only to baseline values.

Each patient's medical chart was thoroughly reviewed by a nephrologist (SSJ), who extracted data pertaining to underlying kidney disease, history of CVD, other comorbid conditions and survival data. Thus, the causes of renal failure were diabetic nephropathy ($n = 41$), chronic glomerulonephritis ($n = 39$), polycystic kidney disease ($n = 27$), hypertensive nephrosclerosis ($n = 31$), pyelonephritis ($n = 17$), interstitial nephritis ($n = 9$), and other ($n = 32$) or unknown aetiologies ($n = 25$). The comorbidity score of each patient was determined at baseline according to the Davies comorbidity index [14]. The index includes seven specified comorbidity domains for patients receiving renal replacement therapy: malignancy, ischaemic heart disease, peripheral vascular disease (including cerebrovascular disease), left ventricular heart failure, diabetes mellitus, systemic collagen vascular disease, and other significant pathology (e.g. cirrhosis). The patients were assigned a risk score based on the presence or absence of the indexed comorbidities: low (0 comorbidities), medium (1–2 comorbidities) and high (3–7 comorbidities).

One hundred and forty-one patients (64%) had clinical signs of ischaemic cardiac disease, cerebrovascular disease, peripheral vascular disease and/or left ventricular dysfunction and were grouped as CVD. Of these 141 patients, 68 patients had ischaemic heart disease including 48 who had a history of acute myocardial infarctions, 50 patients had angina pectoris (stable or unstable), seven patients had undergone percutaneous transluminal coronary angiography, 23 patients had undergone coronary artery by-pass graft and 46 patients had left ventricular dysfunction (based on clinical criteria and/or echocardiography). Forty-three patients had suffered from cerebrovascular disease (stroke and/or history of transient ischaemic attack). Thirty-five patients had clinical signs of peripheral atherothrombotic vascular disease and four patients had a history of an aortic aneurysm.

Patients were treated with HD three times a week (4–5 h per session) using bicarbonate dialysate and high-flux (26%) or low-flux (74%) dialysis membranes: One hundred and thirty-eight patients were dialysed with polyamide, 12 with cellulose (1·7 and 1·3 m^2, respectively, Gambro, Lund, Sweden), 71 with polysulphone (1·8–2·4 m^2, Fresenius, Hamburg, Germany) and one with a polyacrylonitrite membrane (1·3 m^2, Hospal, Industrie Meyziev; France). The average Kt/V in these patients was 1·53 ± 0·33, which is a dimensionless number representing the dialysis adequacy. In this formula K is the dialyser clearance of urea, t the dialysis time and V the patient's total body water. Patients had different vascular accesses, i.e. 127 patients (57%) had an arteriovenous fistula, 42 (20%) had a central dialysis catheter and 51 (23%) had a graft. Most patients were on antihypertensive medications (β-blockers; $n = 110$, calcium channel blockers; $n = 57$, and angiotensin-converting enzyme inhibitors/angiotensin receptor blockers; $n = 72$), as well as other commonly used drugs in terminal CKD, such as phosphate and potassium binders, and vitamin B, C, and D supplementation. Seventy-two patients were also on lipid-lowering medication (statins).

The study protocols were approved by the Ethics Committee of Karolinska Institute at Huddinge University Hospital, Stockholm and by the Ethics Committee at Uppsala University Hospital, Uppsala, Sweden, and informed consent was obtained from each patient.

Laboratory analyses

After collection of blood samples plasma and serum were separated within 30 min and samples were kept frozen at –70 °C pending analyses, if not analysed immediately. Serum fetuin-A was measured by a sandwich immunoenzymometric assay using two polyclonal human fetuin-A antibodies (Epitope Diagnostics, Inc., San Diego, CA, USA). The serum concentrations of interleukin

(IL)-6 and TNF-α were quantified on the Immulite® automatic analyser (Diagnostic Products Corporation, Los Angeles, CA, USA). Plasma pentraxin (PTX)-3 concentration was measured by using commercially available enzyme-linked immunosorbent assay (ELISA) kit (Perseus Proteomics Inc., Tokyo, Japan). High sensitivity (hs)-CRP (nephelometry), urea, haemoglobin, Ca, PO_4, creatinine, fibrinogen, and albumin (bromcresol purple) concentrations were analysed using routine methods at the Department of Laboratory Medicine, Karolinska University Hospital, Huddinge or Uppsala Academic Hospital. Serum cholesterol and triglyceride levels were analysed by means of standard enzymatic procedures (Roche Diagnostics GmbH, Mannheim, Germany). High-density lipoprotein (HDL) cholesterol level was determined after precipitation of apolipoprotein (apo) B–containing lipoproteins by using phosphotungstic acid.

Nutritional status

Subjective global nutritional assessment (SGA) was used to evaluate the overall protein-energy wasting (PEW), as described previously [15]. The evaluation was performed by only one investigator who was blinded to other clinical details of patients. Body mass index (BMI) was calculated as weight in kg (height in m)$^{-2}$.

Statistical analyses

All variables were expressed as mean ± SD or as median (range), unless otherwise indicated. Statistical significance was set at the level of $P < 0{\cdot}05$. Comparisons between two groups were assessed for continuous variables with the Student's unpaired *t*-test, Mann-Whitney test or χ^2 test, as appropriate. Differences among more than two groups were analysed by analysis of variance (ANOVA) using one-way ANOVA or Kruskal-Wallis test, as appropriate, followed by a post-*hoc* test if ANOVA was significant. Spearman's rank correlation (ρ) was used to determine correlations of fetuin-A concentration with other variables. Survival analyses were made with the Kaplan-Meier survival curve or the Cox proportional hazard model. The univariate and multivariate Cox regression analysis are presented as hazard ratio (HR) 95% confidence intervals (CI). The statistical analysis was performed using statistical software SAS version 9·1·3 (SAS Campus Drive, Cary, NC, USA 27513) or JMP IN® release 5·1 (SAS Inc., Campus Drive, Cary, NC, USA).

Results

Table 1 shows the characteristics of the 222 prevalent HD patients in this study. The HD patients had significantly lower fetuin-A concentrations [0·173 (0·008–0·647) ng mL^{-1}] compared to our healthy control reference [0·549 (0·350–0·950) g L^{-1}] [5]. Median levels of fetuin-A did not differ between males and females (0·171 vs. 0·176 g L^{-1}), respectively. However, patients with DM ($n = 58$) had significantly lower median levels of fetuin-A than patients without DM (0·150 vs. 0·180 g L^{-1}, $P < 0{\cdot}001$). Patients with signs of PEW (SGA > 1; 48%) had a significantly lower median of fetuin-A than patients with normal nutritional status (0·160 vs. 0·185 g L^{-1}, $P < 0{\cdot}001$). Moreover, patients with CVD (64%) had a significantly lower median fetuin-A level than patients without clinically manifest CVD (0·164 vs. 0·181 g L^{-1}, $P < 0{\cdot}01$).

Table 1 Characteristics of 222 prevalent haemodialysis patients

Age (years)	63 ± 14
Body mass index (kg m^{-2})	24 ± 5
Dialysis vintage (months)	28 (1–378)
Male/female (*n*)	123/99
Diabetes mellitus (%)	26
Cardiovascular disease (%)	64
Wasting (SGA > 1) (%)	53
Davies score (0, 1, 2; %)	19, 56, 25
Urea (mmol L^{-1})	23 ± 6
Creatinine (μmol L^{-1})	770 ± 211
Albumin (g L^{-1})	35 ± 5
Fetuin-A (g L^{-1})	0·173 (0·008–0·647)
C-reactive protein (mg L^{-1})	6·5 (0·2–151·0)
Pentraxin-3 (ng mL^{-1})	10·6 (2·4–75·1)
Interleukin-6 (pg mL^{-1})	8·6 (0·9–102·0)
TNF-α (pg mL^{-1})	13·9 (5·5–57·7)
Fibrinogen (g L^{-1})	4·5 ± 1·4
Haemoglobin (g L^{-1})	119 ± 13
Calcium (mmol L^{-1})	2·51 ± 0·22
Phosphate (mmol L^{-1})	1·87 ± 0·57
Ca × PO_4	4·7 ± 1·5
Total cholesterol (mmol L^{-1})	4·4 ± 1·1
Triglycerides (mmol L^{-1})	1·9 ± 1·0
HDL-cholesterol (mmol L^{-1})	1·4 ± 0·5

Values expressed as mean ± SD, median (average) or %, unless noted otherwise.
Abbreviations: SGA, subjective global assessment; TNF, tumour necrosis factor; HDL, high-density lipoprotein.

As shown in Fig. 1, the fetuin-A concentration was associated with the Davies comorbidity score and patients with 'high risk' of comorbidities (0·146 g L^{-1}) had significantly lower median levels of fetuin-A ($P < 0{\cdot}0001$) compared to patients having 'medium risk' of comorbidities (0·173 g L^{-1}) or patients with 'low risk' of comorbidities (0·204 g L^{-1}). Table 2 shows that fetuin-A concentration correlated negatively with age, hsCRP, PTX-3, IL-6,

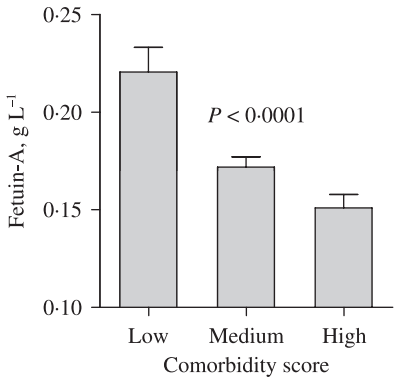

Figure 1 Concentrations of serum fetuin-A in relation to the presence of comorbidities, as assessed by Davies *et al*. [14]. The grades of comorbidities are classified as 'low risk' when there was no comorbidity ($n = 43$), 'medium risk' when one or two ($n = 124$, and 'high risk' when three or more comorbidities were present ($n = 55$). The *P*-value refers to Kruskal-Wallis test. The post-*hoc* test (Tukey-Kramer) was statistically significant ($P < 0{\cdot}05$) between the groups.

Table 2 Correlations of serum concentration of fetuin-A and pertinent variables.

	ρ	*P*-value
Age (years	–0·18	0·005
C-reactive protein (mg L^{-1})	–0·29	< 0·0001
Dialysis vintage (months)	0·02	ns
Pentraxin-3 (ng mL^{-1})	–0·28	< 0·0001
Interleukin-6 (pg mL^{-1})	–0·35	< 0·0001
TNF-α (pg mL)	–0·15	< 0·05
Albumin (g L^{-1})	0·34	< 0·0001
Creatinine (μmol L^{-1})	0·17	0·01
Calcium (mmol L^{-1})	–0·06	ns
Phosphate (mmol L^{-1})	–0·05	ns
Ca × PO_4	–0·07	ns
Total cholesterol (mmol L^{-1})	0·19	< 0·01
Triglycerides (mmol L^{-1})	0·18	< 0·01
HDL-cholesterol (mmol L^{-1})	–0·09	ns

Abbreviations: SGA, subjective global assessment; TNF, tumour necrosis factor; HDL, high-density lipoprotein.

TNF-α, HDL-cholesterol, and positively with s-albumin s-creatinine, cholesterol and triglycerides. There were no significant correlations between fetuin-A concentrations and dialysis vintage, calcium or phosphate levels.

Survival was determined after a median follow-up of 31 (range, 3 to 42) months. No patient was lost to follow-up. Within the follow-up period 85 (38%) of patients died. Median level of serum fetuin-A was significantly ($P < 0{\cdot}0001$) lower in non-survivors (0·155 g L^{-1}) than in survivors (0·183 g L^{-1}).

In the first analysis, patients were divided into two groups according to the median of serum fetuin-A concentration (0·173 g L^{-1}) for comparison of survival between the two patient groups (below vs. above median). Whereas 27% of the patients died within the group with serum fetuin-A above the median level, 50% of the patients died in the group with fetuin-A below the median level ($P < 0{\cdot}001$). Moreover, patients with low fetuin-A levels showed a significantly higher mortality rate (HR 2·2; CI 1·4–3·5, $P < 0{\cdot}001$) compared to the patients with high fetuin-A levels. This difference in mortality lost its significance ($P = 0{\cdot}08$) after adjustment for age, gender, comorbidity risk groups, dialysis vintage and CRP (HR 1·5; CI 0·95–2·5).

In order to study the influence of inflammation on the relationship between fetuin-A and survival, the patients were divided into four groups based on the median of fetuin-A (0·137 g L^{-1}) and the median of CRP concentrations (6·5 mg L^{-1}), as follows: *Group I* ($n = 69$) included patients who had high fetuin-A and low CRP (reference group); *Group II* ($n = 40$) included patients who had high fetuin-A and high CRP; *Group III* ($n = 45$) included patients who had low fetuin-A and low CRP; *Group IV* ($n = 68$) included patients who had low fetuin-A and high CRP. The patient characteristics of the four groups are shown in Table 3.

The percentages of deaths in the four patient groups were 19, 38, 44% and 54%, respectively. In the non-adjusted analysis (Fig. 2), patients in *Group II* (HR 2·3; CI 1·1–4·9, $P < 0{\cdot}01$), *Group III* (HR 3·0; CI 1·5–6·0, $P < 0{\cdot}001$) and *group IV* (HR 3·7; CI 2·0–6·9, $P < 0{\cdot}0001$) had significantly higher mortality compared to *Group I*. This relationship remained significant after adjustment for age, gender, Davies score and dialysis age, but *only* for the patients with low fetuin-A and high CRP (*Group IV*), and *not* for the other patient groups (Table 4).

Similarly, when patients were divided into four groups based on the median of fetuin-A and IL-6 (Fig. 3), the adjusted and non-adjusted survival rates for each of the three patient groups, compared to *Group I*, were similar to the results seen when patients were grouped based on the median of fetuin-A and CRP.

Discussion

This study demonstrates that a low serum concentration of fetuin-A is related to comorbidity and is an independent predictor of all-cause mortality in prevalent HD patients but only in the presence of inflammation. Several clinical studies have shown that low fetuin-A levels are associated with poor outcome in CKD stage 5 patients [4–7]. In accordance, the current study demonstrated that low fetuin-A was associated with mortality. However, this

Table 3 Characteristics of 222 prevalent haemodialysis patients based on the presence of low and high fetuin-A and low and high CRP levels

	Group I *n* = 69	Group II *n* = 40	Group III *n* = 45	Group IV *n* = 68	*P*-value
Age (years)	58 ± 15	66 ± 13	64 ± 13	66 ± 13	< 0·01
Body mass index (kg m^{-2})	24 ± 5	26 ± 7	24 ± 5	24 ± 5	ns
Dialysis vintage (months)	28 (3–160)	32 (1–378)	24 (3–180)	29 (1–269)	ns
Male/female (*n*)	42/27	21/19	20/25	40/28	ns
Diabetes mellitus (%)	12	23	42	32	< 0·05
Cardiovascular disease (%)	49	70	65	74	< 0·05
Wasting (SGA > 1) (%)	22	60	50	62	< 0·0001
Davies score (0, 1, 2; %)	37,50,13	18,65,17	14,58,28	6,56,38	< 0·0001
Urea (mmol L^{-1})	24 ± 6	23 ± 7	24 ± 6	23 ± 5	ns
Creatinine (μmol L^{-1})	819 ± 216	781 ± 204	739 ± 231	728 ± 191	ns
Albumin (g L^{-1})	37 ± 4	34 ± 3	36 ± 3	32 ± 5	< 0·00001
Fetuin-A (g L^{-1})	0·225 (0·174–0·340)	0·210 (0·174–0·647)	0·138 (0·008–0·173)	0·128 (0·019–0·173)	
hs-CRP (mg L^{-1})	2·5 (0·2–6·5)	17·5 (6·7–88·5)	2·5 (0·2–6·5)	22·0 (6·6–151·0)	
Interleukin-6 (pg mL^{-1})	5·5 (0·9–17·5)	10·0 (2·9–35·5)	7·0 (1·7–30·8)	16·1 (2·7–102·0)	< 0·0001
Pentraxin-3 (pg mL^{-1})	9·2 (2·4–70·0)	9·9 (4·7–75·1)	10·0 (4·2–25·5)	14·4 (6·1–63·2)	< 0·001
TNF-α (pg mL^{-1})	12·2 (5·5–39·6)	15·1 (6·9–23·1)	12·7 (7·0–48·0)	15·1 (7·7–57·7)	< 0·01
Fibrinogen (g L^{-1})	3·8 ± 0·9	5·3 ± 1·4	3·9 ± 0·9	5·3 ± 1·5	< 0·01
Haemoglobin (g L^{-1})	121 ± 13	118 ± 14	120 ± 12	117 ± 13	ns
Calcium (mmol L^{-1})	2·51 ± 0·23	2·48 ± 0·23	2·56 ± 0·16	2·50 ± 0·23	ns
Phosphate (mmol L^{-1})	1·90 ± 0·59	1·87 ± 0·62	1·93 ± 0·53	1·81 ± 0·63	ns
$Ca \times PO_4$	4·7 ± 1·4	4·6 ± 1·5	4·9 ± 1·4	4·5 ± 1·6	ns
Total cholesterol (mmol L^{-1})	4·7 ± 1·0	4·3 ± 1·0	4·3 ± 1·1	4·2 ± 1·1	< 0·05
Triglycerides (mmol L^{-1})	2·1 ± 1·3	1·9 ± 1·1	1·6 ± 0·9	1·7 ± 0·9	ns
HDL-cholesterol (mmol L^{-1})	1·4 ± 0·6	1·3 ± 0·5	1·5 ± 0·5	1·4 ± 0·4	ns
Death during follow-up (%)	19	38	44	54	< 0·001

Patients were divided into four groups, based on the median of fetuin-A (0·173 pg ml) and the median of CRP (6·5 mg L^{-1}), as follows: *Group I*, patients with high fetuin-A and low CRP (this group was used as a reference group); *Group II*, patients with high fetuin-A and high CRP; *Group III*, patients with low fetuin-A and low CRP and *Group IV*, patients with low fetuin-A and high CRP. Values are presented as mean ± SD, median (average) or % of patients, unless noted otherwise. Abbreviations: SGA, subjective global assessment; TNF, tumour necrosis factor; HDL, high-density lipoprotein.

association was lost when survival analysis was adjusted for several confounders, including inflammation. Indeed, inflammation markers were significantly associated with low serum fetuin-A in the current study. The association between fetuin-A deficiency and inflammation markers has been reported repeatedly in CKD patients [4–6]. Nonetheless, Hermans *et al*. [7] showed that a low serum fetuin-A level was related to increased all-cause mortality in an association independent of inflammation and other confounders in dialysis patients. The anti-inflammatory property of fetuin-A is supported by different findings in the literature, such as inhibition of cytokine production by macrophages [16], protection against TNF [17], antifibrotic activity [18,19], and inhibition of apoptosis of vascular smooth muscle cells [20]. As inflammation may be an important cause of low fetuin-A levels, genetic alterations may have an effect on the circulating amounts of this protein. It has been reported that several polymorphisms exist in the AHSG gene [21]. We have reported that CKD patients carrying the 256Ser allele had lower fetuin-A levels than 256Thr allele carriers, and CKD patients with a genetic propensity for low fetuin-A levels in the presence of inflammation had a higher mortality rate than CKD patients carrying the 256Thr allele with signs of inflammation [5].

Table 4 Cox proportional hazard analysis of factors that predicted all-cause mortality among prevalent HD patients

	Hazard ratio	95% Confidence limits		*P*-value
Age (50–70 vs. < 50 years)	4·4	1·5	12·6	< 0·01
Age (> 70 vs. < 50 years)	7·9	2·8	22·1	< 0·0001
Sex (male vs. female)	0·8	0·5	1·3	0·45
Comorbidity (medium vs. low)	2·7	1·1	6·9	< 0·045
Comorbidity (high vs. low)	5·0	1·9	13·0	< 0·001
Dialysis vintage (high vs. low)	1·8	1·2	2·8	< 0·01
Group (II vs. I)	1·8	0·8	3·8	0·13
Group (III vs. I)	1·9	0·9	3·9	0·08
Group (IV vs. I)	2·3	1·2	4·5	0·01

Likelihood Ratio = 75, $P < 0{\cdot}0001$. To study the coexistence effect of inflammation and serum fetuin-A on mortality, patients were divided into four groups based on the median levels of fetuin-A (0·173 pg mL^{-1}) and CRP (6·5 mg L^{-1}), as follows: *Group I*, patients with high fetuin-A and low CRP (this group was used as a reference); *Group II*, patients with high fetuin-A and high CRP; *Group III*, patients with low fetuin-A and low CRP and *Group IV*, patients with low fetuin-A and high CRP.

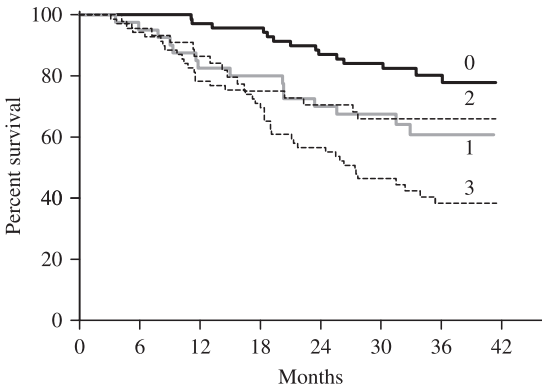

Figure 2 Kaplan-Meier curves showing differences in all-cause mortality in four haemodialysis patient groups. The patients were divided into four groups based on the median levels of fetuin-A and C-reactive protein (CRP), as follows: *Group 0* ($n = 69$) were the patients with high fetuin-A and low CRP and used as a reference; *Group 1* ($n = 40$) were the patients with high fetuin-A and high CRP; *Group 2* ($n = 45$) were the patients with low fetuin-A and low CRP and *Group 3* ($n = 68$) were the patients with low fetuin-A and high CRP. Long rank test $\chi^2 = 20$; $P = 0{\cdot}0002$).

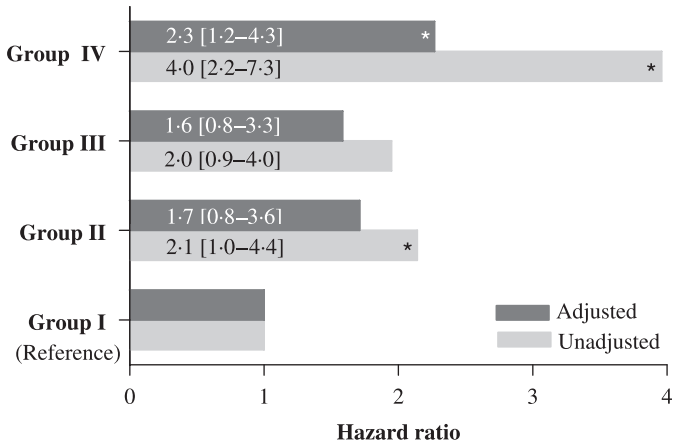

Figure 3 Hazard ratios of the four haemodialysis patients groups. The patients were divided into four groups based on the medians of serum fetuin-A and interleukin (IL)-6, as follows: *Group I* ($n = 69$) were the patients with high fetuin-A and low IL-6 and used as a reference; *Group II* ($n = 40$) were the patients with high fetuin-A and high IL-6; *Group III* ($n = 44$) were the patients with low fetuin-A and low IL-6 and *Group IV* ($n = 69$) were the patients with low fetuin-A and high IL-6 levels. The adjusted analysis was adjusted for age, gender, comorbidities, assessed by Davies risk groups, and dialysis vintage. The likelihood ratio for non-adjusted analyses was 24, $P < 0{\cdot}0001$ and for adjusted analysis was 75, $P < 0{\cdot}0001$.

As the mechanism for extraossoeus calcification is complex and not well known, it is possible that inflammation may participate in the vascular calcification process. Recent evidence suggests that vascular calcification is not only a passive degenerative process but also involves active inflammation [6,22,23]. In an attempt to evaluate whether coexistence of both low fetuin-A and inflammation had an additive effect on survival, we found that the presence of inflammation, indeed, amplified the link between fetuin-A and mortality. In the current study, patients with evidence of inflammation and low fetuin-A levels had a significantly higher mortality compared to inflamed patients with high fetuin-A levels.

Interestingly, patients with low fetuin-A and no evidence of inflammation did not show a significantly higher mortality rate. Thus, our study is the first one suggesting that inflammation has an additive detrimental effect on the link between low serum fetuin-A levels and mortality in HD patients. The exact mechanism of this additive effect at present is unknown. Nonetheless, our findings suggest that inflammation is a possible missing link in this association and recent studies suggest that an elevated CRP level is closely related to coronary artery calcification [12] and carotid plaques [24]. Recently, Ix *et al*. [8] showed that low fetuin-A did not associate with mortality in 822 CKD stage 3–4 patients followed-up for 9·5 years. However, in this study the fetuin-A levels were in fact similar to the levels in the general population. As Ix *et al*. [8] did not report data on inflammatory markers it is not clear whether these patients were inflamed or not. Therefore, it is possible that the characteristics of these patients could be an additional explanation for the observed lack of association between fetuin-A levels and mortality in those CKD stage 3–4 patients.

Another finding in the present study is that low serum fetuin-A was associated with comorbidities, as assessed by the Davies score. The exact mechanism is not fully clear. However, low fetuin-A levels have been reported, as in this study, in CKD patients with CVD or DM, both of which are disease domains in the Davies score. Another explanation could be that fetuin-A is an acute-phase protein and thereby may reflect the degree of severity of pre-existing cellular or organ damage of diverse nature and therefore that low serum fetuin-A levels may represent the aggregate of comorbidities present in these patients. Fetuin-A is a multifunctional molecule and it has been implicated in several diverse functions. As chronic inflammation and the uremic milieu *per se* may contribute to fetuin-A depletion, it is plausible that the deficiency of fetuin-A may represent a 'common denominator' of morbidity in CKD patients.

Some limitations of the present study should be considered, starting with the cross-sectional nature of this analysis. Second, the classification of CVD included only patients with clinically significant disease, which may limit and underestimate the true prevalence of CVD. Finally, the mortality classification was based on death certificates, which can be biased and inaccurate [25]. Thus, in the present study we have relied on the robust end-point of all-cause mortality instead of cardiovascular mortality.

In summary, coexistence of low serum fetuin-A levels and low-grade inflammation exerts an additive effect on the risk of death in prevalent HD patients. Thus, simultaneous measurements of fetuin-A and inflammation markers could be useful for the prediction of clinical outcome in CKD patients.

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