

# High Fetuin-A Level as a Protective Factor to Abdominal Aortic Calcification in Indonesian Regular Hemodialysis Patients

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## Abstract

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**BACKGROUND:** Although the prevalence of cardiovascular disease decreases in the general population, this pattern is not followed in hemodialysis patients. Hence cardiovascular events still occur in 50% of cases resulting in hemodialysis patients. One of the risk factors is vascular calcification. The pathogenesis is not yet fully understood, but recent years studies have shown that vascular calcification in chronic kidney disease (CKD) occurs as a result of the interaction of stimulatory and inhibitory factors. One of the inhibitory factors is Fetuin-A. Until now there has been no data on levels of Fetuin-A as a risk factor for abdominal aortic calcification in Indonesia.

**AIM:** To determine the effect of Fetuin-A levels on abdominal aortic calcification in regular hemodialysis patients.

**METHODS:** This study was a cross-sectional study on 76 regular hemodialysis patients at Rasyida Renal Hospital Medan. Fetuin-A level was examined by enzyme-linked immunosorbent assay (ELISA). Assessment of abdominal aortic calcification was done by lateral lumbar X-ray.

**RESULTS:** Most patients (68.4%) had abdominal aortic calcification, in both layers; intima and media (44.7%). Abdominal aortic calcification was associated with Fetuin-A level and age. Multivariate analysis showed that high Fetuin-A levels were significantly associated with abdominal aortic calcification.

**CONCLUSION:** High Fetuin-A level appeared to be a protective factor against abdominal aortic calcification in regular hemodialysis patients in Indonesia.

## Introduction

The life expectancy of hemodialysis patients is still very low. According to the United State Renal Data System in 2010, the 5-year cumulative life expectancy for chronic hemodialysis patients in North America is around 25% compared to 2005 which was only 20% [1]. Muzasti in 2011 found that the 5 years of survival rate for regular hemodialysis patients in Medan, Indonesia, was only 37.8% [2]. A study by Sibarani et al., in Medan, Indonesia, showed that the 1-year survival rate of maintenance hemodialysis patients was 63.4% [3]. One of the main causes of

this low survival in various countries is cardiovascular disease. Despite the declining prevalence in the general population, this pattern is not followed in hemodialysis patients. Hence cardiovascular events still occur in 50% of cases of mortality in hemodialysis patients [1].

One risk factor for high mortality from cardiovascular disease in hemodialysis patients is vascular calcification. The pathogenesis is very complex and not fully understood [4]. However, research in recent years has shown that vascular calcification in CKD occurs as a result of the interaction of stimulatory and inhibitory factors. One of the inhibitory factors is Fetuin-A. Research in dialysis

patients shows that the lower the level of Fetuin-A, the more extensive the vascular calcification that occurs [5]. The role of Fetuin-A in physiology is still under study. However, several reports have shown that Fetuin-A is a multifunctional protein that can work as a major vascular calcification inhibitor by inhibiting 50% calcium phosphate salts formation [6]. Until now in Indonesia, there are no data regarding the levels of Fetuin-A as a risk factor for vascular calcification in hemodialysis patients.

Therefore, this study was conducted to determine the association of Fetuin-A levels and abdominal aortic calcification in regular hemodialysis patients.

## Methods

### Study design

This study was a cross-sectional study on 76 regular HD patients conducted in April 2018 at Rasyida Renal Hospital Medan. Each medical record was reviewed to verify the diagnosis and to obtain all relevant demographic and clinical data. Inclusion criteria were outpatients with age  $\geq 18$  years, on hemodialysis for  $\geq 36$  months independently of aetiology, and willing to undergo a lateral examination with lumbar X-ray and were taken a blood sample for laboratory examination. Patients who refused to continue the research and with lack of medical records were excluded.

### Assessments

Lateral lumbar X-rays interpretation was performed by a radiologist who did not know the patient's clinical condition. Grading of calcification was assessed using abdominal aortic calcification scores where the scores in the abdominal aortic segment both the anterior and posterior walls in front of the one to fourth lumbar vertebrae (L1-L4) were summed up. Patients were told to have no calcification if the score was 0, mild calcification if the score was 1-4, severe calcification if the score was above 4 [7]. Blood samples were taken 5-10 minutes before the hemodialysis procedure. Then the levels of Fetuin-A serum were examined by ELISA technique.

### Statistical analysis

All data were analysed with statistical SPSS 22.0 software using univariate, bivariate and multivariate analysis. To assess numerical data distribution, the Kolmogorov-Smirnov test was used. Categorical and numerical variables were analysed using an independent T-test or Mann Whitney U test,

and two categorical variables were analysed using the Chi-Square test or physic test. The multivariate analysis used logistic regression. A p-value  $< 0.05$  is considered significant.

## Results

From the 76 respondents, 47 patients (61.8%) were male, while the rest 29 (38.2%) were female. Patients aged between 25-78 years (mean  $54.39 \pm 11.32$  years) with a mean BMI of  $24.16 \pm 4.49$  kg/m<sup>2</sup> (range 16.73 to 42.67). Most patients underwent hemodialysis for 10 hours a week (71.1%) and have undergone hemodialysis for  $73.24 \pm 35.11$  months.

The medical history showed that almost all patients had hypertension (75%). Only 14 patients (18.4%) had diabetes. A history of cardiovascular disease was known in only 15.8% of patients. Twenty-four patients (31.6%) reported previously or currently smoking.

On laboratory examination, the median (mean  $\pm$  SD) levels of calcium, phosphate, calcium phosphate product and Fetuin-A were  $9.80$  ( $9.74 \pm 0.73$ ) mg/dL,  $5.5$  ( $5.47 \pm 0.61$ ) mg/dL,  $52.91$  ( $53.54 \pm 8.59$ ) mg<sup>2</sup>/dL<sup>2</sup> and  $235$  ( $254.08 \pm 112.50$ ) pg/ml.

Examination of lateral lumbar images showed that vascular calcification was found in 52 patients (68.4%) with an average abdominal aortic calcification score of  $6.67 \pm 6.34$ . The highest incidence for calcification was found in both layers both intima and media (44.7%) followed by tunica media in only (15.8%), whereas the calcification of the tunica intima was found in 2 patients (2.6%). Based on the grading, it was found that the majority of respondents experienced severe vascular calcification (52.6%). Clinical characteristics of these study participants are presented in (Table 1).

**Table 1: Demographic and clinical characteristics of the study participants**

Variables	n (%), median (min-max), mean $\pm$ SD
Gender: Male vs female	47 (61.8%) vs 29 (38.2%)
Age (years):	57 (25-78), $54.39 \pm 11.32$
$\leq 60$ vs $> 60$	50 (65.7%) vs 26 (34.2%)
BMI (kg/m <sup>2</sup> )	$23.37$ (16.73 – 42.67), $24.16 \pm 4.49$
$< 23$ vs $\geq$	34 (44.7%) vs 42 (55.3%)
Dialysis duration (months):	67 (36-231), $73.24 \pm 35.11$
$\leq 60$ vs $> 60$	35 (46.1%) vs 41 (53.9%)
Dialysis hours per week: 10 vs 12	54 (71.1%) vs 22 (28.9%)
Renal failure etiology:	
diabetic vs hypertension	14 (18.4%) vs 57 (75%)
History of cardiovascular disease: yes	12 (15.8%)
Tobacco-smokers: yes	24 (31.6%)
Abdominal aorta calcification scores	6 (0-19), $6.67 \pm 6.34$
The degree of vascular calcification:	
None vs mild vs severe	24 (31.6%) vs 12 (15.8%) vs 40 (52.6%)
Location of calcification:	
Intima vs media vs intima + media	2 (2.6%) vs 12 (15.8%) vs 34 (44.7%)
Calcium	$9.80$ (8 – 11), $9.74 \pm 0.73$
Phosphate	$5.5$ (4.1 – 6.8), $5.47 \pm 0.61$
CaxP	$52.91$ (33.60 – 69.36), $53.54 \pm 8.59$
Fetuin-A	$235$ (78 – 756), $254.08 \pm 112.50$

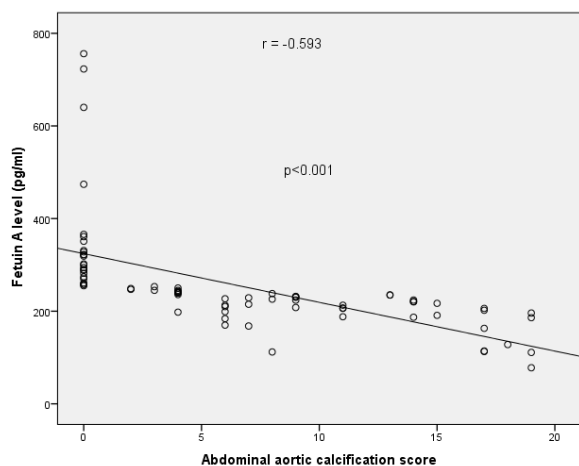
To determine the relationship of various factors with abdominal aortic calcification, bivariate analysis was performed. Table 2 shows that, even though statistically not significant, male sex, higher BMI and longer dialysis vintage were associated with more vascular calcification. Patients who suffer from diabetes, hypertension, are smoking and have a history of the cardiovascular disease also, had more vascular calcifications than non-DM patients, normotensive patients, non-smokers and those with no history of cardiovascular disease, although the statistical differences were not significant ( $p > 0.05$ ). The same appeared for higher calcium and phosphate levels; it was more likely to experience vascular calcification than lower calcium levels and phosphate levels.

**Table 2: Association of demographic and clinical characteristics with vascular calcification**

Variables	Calcification (+)	Calcification (-)	p
Gender; male	66%	34%	0.556
Age	59.04 ± 9.68	52.25 ± 11.46	0.014*
BMI	24.73 ± 4.99	22.94 ± 2.87	0.053
Diabetes: yes	71.4%	28.6%	0.789
Hypertension: yes	68.4%	31.6%	1.000
History of cardiovascular disease: yes	91.7%	8.3%	0.059
Tobacco-smokers: yes	75.0%	25.0%	0.402
Dialysis duration:	83.96 ± 42.47	68.29 ± 30.34	0.070
Calcium	9.85 ± 0.61	9.70 ± 0.78	0.366
Phosphate	5.55 ± 0.61	5.44 ± 0.61	0.478
CaxP	54.72 ± 7.82	53.0 ± 8.95	0.422
Fetuin-A	205.48 ± 41.81	359.38 ± 143.12	< 0.001*

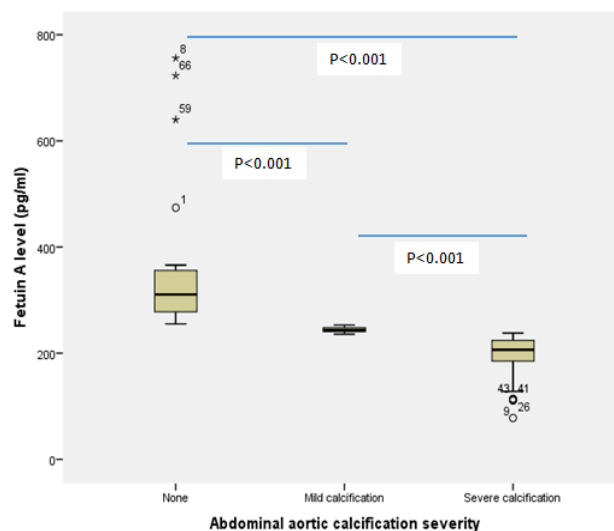
The results of the statistical analysis in table 2 showed that the only significant variables for vascular calcifications were Fetuin-A levels ( $p < 0.001$ ) and age ( $p = 0.014$ ).

To determine the correlation and strength between Fetuin-A levels and abdominal aortic calcification scores, a correlation test was conducted. The Kruskal Wallis test in Figure 1 showed that there was a significant negative correlation between Fetuin-A levels and abdominal aortic calcification score ( $p < 0.001$ ), meaning that the lower the level of Fetuin-A, the higher the abdominal aortic calcification score. The value of  $r = -0.60$  indicates that the strength of the correlation between the two variables is moderate.



**Figure 1: Serum Fetuin-A levels correlated negatively with abdominal aortic calcification score**

Figure 2 shows that there is a significant negative correlation between the levels of Fetuin-A and the degree of calcification ( $p < 0.001$ ), where the more severe the calcification, the lower the level of Fetuin-A.



**Figure 2: Serum Fetuin-A levels based on abdominal aortic calcification severity**

To determine the most predominant variables associated with significant abdominal aortic calcification, a multivariate analysis was performed, including age, BMI, history of cardiovascular disease, dialysis vintage, and Fetuin-A levels

Statistical analysis showed that Fetuin-A levels were protective, and older age and higher BMI were risk factors for developing abdominal aortic calcification.

**Table 3: Multivariate logistic regression analysis**

Variable	OR	95% CI	P
Fetuin-A	0.98	0.96 – 0.99	0.001
History of cardiovascular disease: yes	6.13	0.97 – 38.92	0.054
Age	4.44	1.12 – 17.68	0.034
BMI	1.28	1.05 – 1.57	0.014

## Discussion

Aetiology of CKD in Indonesia is different from other countries. In the United States, diabetes is the leading cause of CKD with a proportion reaching 37% followed by hypertension (24%), and the rest by other diseases. While in Indonesia, the main cause of CKD based on Indonesian Renal Registry (IRR) data is hypertension (44%), followed by diabetes (22%) and other diseases. The results of this study are consistent with the IRR data. The high rate of hypertension in this study is likely because patients

are late referrals, where secondary hypertension has occurred due to kidney failure, so it is difficult to recognise whether hypertension occurs as a cause or as a result of CKD [8], [9]. Limitation of this study is that there is no biopsy done in patients to determine the aetiology of CKD, only by history taking of hypertension and consumed anti-hypertension drugs.

In contrast to developed countries, all patients received dialysis services 3 times a week (4 hours/session), but in this study, most patients received dialysis services only twice a week (5 hours/session). This is because hemodialysis facilities in Indonesia are strongly influenced by the financing system, namely using national health insurance (JKN), which is generally done twice a week [8], [9].

This study proves that the prevalence of vascular calcification in chronic hemodialysis patients is quite high, i.e. 68% with a median (range) abdominal aortic calcification score of 5 (0-19). Publication in recent years shows that the prevalence of vascular calcification varies greatly from 60-100%, depending on the location of the examination and the diagnostic method used and in the area where the study was conducted [10]. London et al. reported 68% of 202 hemodialysis patients in France had arterial calcification determined by radiography and echocardiography [11]. An Australian study of 137 hemodialysis patients found that the prevalence of abdominal aortic vascular calcification was 90% [12] while international multicenter reports from America, Canada, Puerto Rico, Spain, and the United Kingdom showed that with the radiographic method, the prevalence of abdominal aortic calcification was 77.8% and with echocardiography, all patients (100%) had calcifications in the aortic and mitral valves [13].

Variation in this prevalence of vascular calcification is strongly influenced by the characteristics of the sample study. When the results of this study were compared with the results of previous studies, all of them found that older age was associated with vascular calcification [7], [14]. Reaven and Sack's study of 245 participants using EBCT found that patients over 61 years experienced severe aortic calcification [15]. This study also shows the same thing, that is the older the patient, the more severe the degree of vascular calcification. This is because the process of atherosclerosis has started since childhood, and continues to form atheroma with various lesions that form calcification. So, age is considered to be an important determinant of the presence of calcification [15].

Several studies have shown that abdominal aortic calcification is more common in patients undergoing long-term dialysis. For example, the Goldsmith et al., the study of 38 patients who underwent hemodialysis for 10-25 years, had radiographically increased the prevalence of vascular calcification from 39% at the start of dialysis to 92% after 16 years of dialysis with an average onset of 9.7

years after starting dialysis [16]. Kawaguchi et al. found that the average duration of dialysis in patients with grade 1 aortic abdominal calcification was 41 months, while patients with grade 3 calcification, had been on dialysis for 68 months [15].

This study shows that Fetuin-A levels are associated with vascular calcification, where the higher the level of Fetuin-A, the lower the degree of vascular calcification. These results are supported by previous studies which proved that low levels of Fetuin-A in hemodialysis patients were independently associated with the extent of vascular calcification [17].

Several limitations of our study should be emphasized. First, our study population is a single centre with a lower prevalence of diabetes, so it has not described the condition of all hemodialysis patients in Indonesia. Second, our study method is cross-sectional and observational, so that it cannot describe the causal relationship between various variables with vascular calcification and thirdly; fetuin-A levels were measured once at the inclusion.

Even so, for the best of our knowledge from the current body of evidence, this study was the first study in Indonesia to prove that high levels of Fetuin-A are a protective factor against vascular calcification. Further research is needed to extend the role of Fetuin-A in preventing the occurrence of vascular calcification.

In conclusion, high serum fetuin-A levels are shown to be protective factors and are associated with low severity of abdominal aortic calcifications in regular hemodialysis patients in Indonesia.

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