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Fetuin-A and atherosclerotic calcified plaque in patients with type 2 diabetes mellitus

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Abstract

Fetuin-A is a multifunctional circulating glycoprotein. Among its roles, inhibition of ectopic calcification is a prominent feature. Low fetuin-A levels in dialysis patients are associated with cardiovascular mortality, possibly via accelerating vascular calcification. However, except for dialyzed conditions, a correlation between fetuin-A levels and vascular calcification remains controversial. Furthermore, any inhibitory effect of fetuin-A on atherosclerotic calcified plaques (CPs) remains unclear compared with its effect on medial artery calcification that is often found in dialyzed patients. Therefore, we examined the association between fetuin-A levels and atherosclerotic CPs. For this study, 416 consecutive patients with type 2 diabetes mellitus and without renal dysfunction were examined. We measured serum fetuin-A levels and investigated for the presence of CP in the common carotid and femoral arteries using ultrasonography. Fetuin-A levels were significantly lower in patients with CP than those without CP (262.6 ± 56.7 and 281.5 ± 64.6 μg/mL, respectively; $P = .001$). Multivariate logistic regression analysis showed that fetuin-A levels were inversely associated with the presence of CP (odds ratio = 0.753; 95% confidence interval, 0.608-0.933; $P = .010$). These results suggest that fetuin-A may inhibit the calcification of atherosclerotic plaques independently of the dialyzed condition.

1. Introduction

Vascular calcification has recently received much attention because of its relationship with cardiovascular disease. Although the mechanisms that regulate ectopic calcification, including in the vasculature, remain unclear, it has been hypothesized that calcification inhibitors are present in serum [1]. Among these, fetuin-A (α2-Heremans Schmid glycoprotein) is a good candidate [2]. In vitro data showed that fetuin-A inhibited apatite formation using primary osteoblast cultures and salt precipitation assays [3]. In support of these results, fetuin-A–deficient mice developed ectopic calcifications of various tissues [4]. Based on these findings, any association of fetuin-A with vascular calcification is under intense investigation in humans. However, it is still controversial whether or not fetuin-A is actually an inhibitor against vascular calcification.

Vascular calcification is very common in dialysis patients. Interestingly, several reports have shown that low fetuin-A levels were associated with high morbidity and mortality for dialysis patients, possibly through accelerated vascular calcification [5-8]. A very recent report has demonstrated that fetuin-A levels were inversely correlated with coronary artery calcification in hemodialysis patients, as quantified by multidetector computed tomography (CT) [9]. On the other hand, vascular calcification is also often found in advanced atherosclerosis, especially in diabetic patients. Considering that fetuin-A was shown to be a strong calcification inhibitor from experimental results, it is predicted that there would be an inverse association of fetuin-A levels with vascular calcification, even in non-dialyzed patients. Mehrotra et al [10] first reported an association between fetuin-A levels and coronary artery calcification in nondialyzed patients with diabetic nephrop-

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athy. Contrary to expectations, they observed that fetuin-A levels were positively related to coronary artery calcification. Interestingly, no associations between fetuin-A and coronary artery calcification have been reported in similar predialysis diabetic nephropathy patients [11] or in patients with normal real function [12]. Moreover, no associations between fetuin-A levels and calcifications in coronary and carotid arteries were found, although 4 single nucleotide polymorphisms for the α2-Heremans Schmid glycoprotein (fetuin-A) gene were associated with coronary artery calcification in type 2 diabetes mellitus patients [13].

Vascular calcification is typically classified into 2 types: medial artery calcification, which is predominant in dialysis and/or diabetic patients [14], and calcified atherosclerotic plaque, which often underlies some diseases, such as hypertension, diabetes, and dyslipidemia [15]. Although advanced imaging technology, such as multidetector CT, makes it possible to quantify vascular calcification, it cannot precisely distinguish intimal from medial calcification [14]. Because vascular calcification was evaluated by quantitative CT in all the previous studies cited above [10-13], discordant results may have derived from a mix of intimal and medial calcifications. Among various metabolic disorders, medial calcification is a prominent feature in patients with diabetes. Thus, both types of calcification could frequently coexist under diabetic condition, resulting in inconsistent findings.

To clarify such confusion in diabetic patients, we mainly focused on calcified plaques, but not medial artery calcification, in the carotid and femoral arteries using ultrasonography. We examined if serum fetuin-A levels were associated with the presence of calcified plaques for 416 patients with type 2 diabetes mellitus and without renal dysfunction.

2. Research design and methods

2.1. Subjects

A total of 416 patients with type 2 diabetes mellitus (261 men and 155 women) were consecutively selected for the present study from patients who visited our diabetes center at Osaka City University Hospital. A diagnosis of diabetes was based on a prior history of diabetes or on the American Diabetes Association criteria [16]. Patients with serum creatinine levels greater than 1.2 mg/dL (106 μmol/L) were excluded. *Hypertension* was defined by systolic blood pressure of at least 140 mm Hg, by diastolic blood pressure of at least 90 mm Hg, or by current use of antihypertensive medications. *Hypercholesterolemia* was defined as total cholesterol level greater than 220 mg/dL or current use of a lipid-lowering treatment. The study protocol was approved by the local ethics committee, and informed consent was obtained from all participants.

Of 416 diabetic patients, 135 were treated with dietary therapy alone, 73 with sulfonylureas, 8 with α-glucosidase inhibitors, 10 with biguanide, 7 with insulin secretagogues, 1 with thiazolidinediones, 74 with insulin, and 108 with combination therapies of these drugs. One hundred twenty-seven patients were receiving 3-hydroxy-3-coenzyme A reductase inhibitors, and 11 received fibrates as lipid-lowering therapies. There were 145 patients using a calcium channel blocker and 123 patients using an angiotensin-converting enzyme inhibitor or an angiotensin II receptor blocker.

2.2. Biochemical analyses

Plasma glucose was measured by the glucose oxidase method, hemoglobin A_{1c} (HbA_{1c}) was measured by high-pressure liquid chromatography (reference range, 4.0%-5.8%), and plasma insulin was measured by immunoradiometric assay (Insulin Riabead II kit; Dainabot, Tokyo, Japan). Serum creatinine, serum total cholesterol, triglycerides, and high-density lipoprotein cholesterol levels were measured by standard laboratory methods. Serum fetuin-A was measured by a commercial enzyme-linked immunosorbent assay kit (BioVender Laboratory Medicine, Modrice, Czech Republic) as previously reported [17-19]. Fetuin-A concentrations were normally distributed (data not shown). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from fasting plasma glucose (FPG) and insulin levels according to a report by Matthews et al [20], using the following formula: HOMA-IR = fasting insulin in microunits per milliliter × FPG in millimoles per liter/22.5.

2.3. Ultrasonography

Ultrasonographic examinations of the common carotid artery (CCA) and the femoral artery (FA) used an ultrasonic phase-locked echo-tracking system, which was equipped with a high-resolution real-time 13-MHz linear scanner (ProSound 6500, Aloka, Tokyo, Japan), as described elsewhere [21-23]. The bilateral carotid arteries were scanned at the level of the bifurcation and the CCA; scanning included −4 cm of the CCA, the carotid bulb, and 1 cm each of the internal and external carotid arteries. The bilateral FAs were scanned distal to the inguinal ligament at the site where the artery divides into the superficial and the profound FAs, including −4 cm proximal and 1 cm distal from the flow divider. Calcified plaque was carefully identified by the presence of an acoustic shadow in hard plaque by 2 examiners (Fig. 1). If calcified plaque was detected at any site among the 4 sites for bilateral CCA or FA, the subject was categorized into a calcified plaque group (CP group). If not, the subjects were defined as a no calcified plaque group (non-CP group).

2.4. Statistical analysis

All results are given as means ± SD, unless otherwise indicated. Unpaired t tests and χ^2 tests were used, where appropriate. We used multivariate logistic regression analysis to adjust for risk factors. The dependent variable was the presence of calcified plaque. The independent

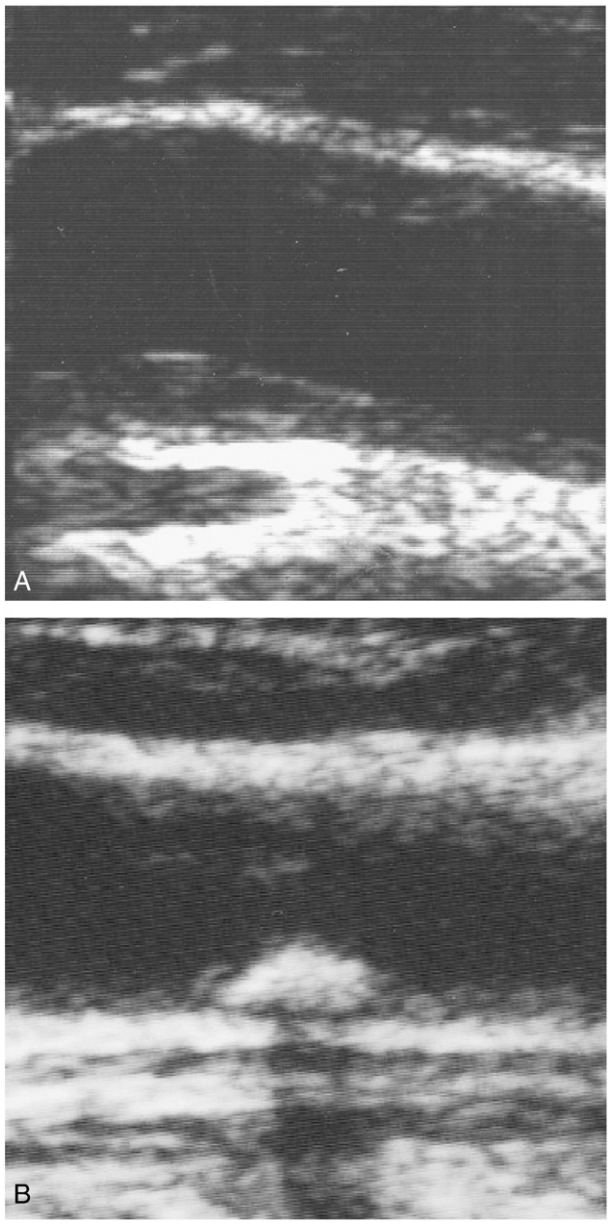

Fig. 1. Representative atherosclerotic calcified plaque. A, Atherosclerotic plaque without calcification. B, Typical atherosclerotic calcified plaque with an acoustic shadow.

variables were age, sex (male = 1), duration of diabetes, smoking, hypertension, hypercholesterolemia, and serum fetuin-A levels. Odds ratios (ORs) are given with 95% confidence intervals (CIs). Statistical analysis used Stat View 5 system (SAS System, Cary, NC) for Windows. P values < .05 were considered statistically significant.

3. Results

The clinical and laboratory characteristics of the study population are summarized in Table 1. Age, known duration of diabetes, and systolic blood pressure were significantly higher in the CP group than the non-CP group. In contrast, body mass index and HbA_{1c} were significantly lower in the CP group than the non-CP group. There were no significant differences for serum creatinine, total cholesterol, FPG, HOMA-IR, or medications.

Table 1
Clinical subject characteristics

	Non-CP group	CP group	Total
Age	64.0 ± 6.2	65.8 ± 6.9*	64.8 ± 6.6
Sex (male-female)	135:88	126:67	261:155
Duration (y)	10.2 ± 8.2	14.7 ± 9.8*	12.3 ± 9.2
BMI (kg/m^2)	25.0 ± 4.0	23.3 ± 3.3*	24.2 ± 3.8
SBP (mm Hg)	131 ± 18	139 ± 21*	135 ± 20
sCre (mg/dL)	0.76 ± 0.19	0.78 ± 0.20	0.77 ± 0.19
TC (mg/dL)	195.5 ± 36.0	197.2 ± 36.6	196.3 ± 36.3
FPG (mg/dL)	136 ± 37	138 ± 43	137 ± 40
HOMA-IR	3.0 ± 2.2	2.7 ± 2.0	2.8 ± 2.1
HbA_{1c} (%)	8.6 ± 1.6	8.1 ± 1.7*	8.4 ± 1.7
ACEI/ARB (%)	66 (30.0%)	57 (29.5%)	123 (29.6%)
Statins (%)	76 (34.1%)	51 (26.4%)	127 (30.5%)
Medications for diabetes (%)	153 (68.6%)	128 (66.3%)	281 (67.5%)

All values are the mean ± SD or number. Because HOMA-IR was not normally distributed, log-transformed HOMA was used for statistical analysis. Medications for diabetes are as follows: insulin, oral hypoglycemic agents, and combination therapies of these drugs. BMI indicates body mass index; SBP, systolic blood pressure; sCre, serum creatinine; TC, total cholesterol; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

* $P < .05$ compared with non-CP group by unpaired t test.

The overall mean serum level of fetuin-A was 272.5 ± 61.7μg/mL, with a range of 144.3 to 483.8 μg/mL. In the CP group, fetuin-A levels were significantly lower than in the non-CP group (262.6 ± 56.7 and 281.5 ± 64.6μg/mL, respectively; $P = .001$) (Fig. 2). Next, we categorized all subjects into fetuin-A quartiles: less than 225 μg/mL (first quartile), 225 to 270 μg/mL (second quartile), 270 to 310 μg/mL (third quartile), and greater than 310 μg/mL (fourth quartile). The prevalence of CP was as follows: first

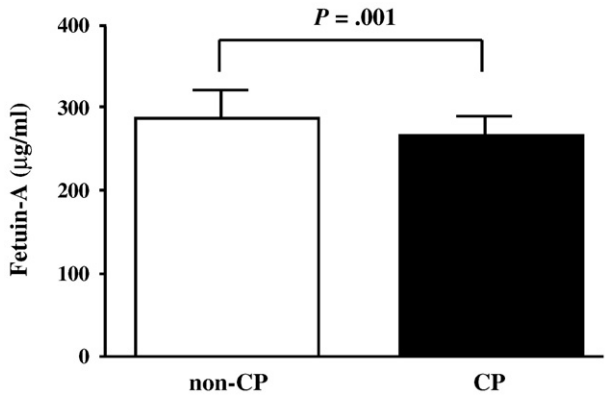

Fig. 2. Serum fetuin-A levels in CP and non-CP patients. Serum fetuin-A levels were significantly lower in the CP group than in the non-CP group (262.6 ± 56.7 and 281.5 ± 64.6 μg/mL, respectively; $P = .001$).

Table 2
Odds ratios for clinical factors, including fetuin-A, for the presence of calcified plaque

	OR	95% CI	P
Age (per 1-SD increase)	1.104	0.884-1.378	.382
Male sex	0.689	0.406-1.169	.168
Duration of diabetes	1.050	1.025-1.075	<.0001
Smoking	2.057	1.235-3.427	.006
Hypertension	1.650	1.049-2.596	.030
Hypercholesterolemia	0.954	0.625-1.455	.826
Fetuin-A (per 1-SD increase)	0.753	0.608-0.933	.010
$R^2 = 0.081$, $P < .0001$			

Results from multivariate logistic regression analysis. One-SD increase in age = 6.6 years; 1-SD increase in fetuin-A = 61.7 μg/mL.

quartile, 56.0%; second quartile, 49.1%; third quartile, 48.5%; and fourth quartile, 33.0%. Fetuin-A levels were inversely associated with the prevalence of CP ($P = .007$). We have also examined the fetuin-A levels with or without macrovascular diseases such as coronary artery disease, cerebrovascular disease, and arteriosclerosis obliterans. Interestingly, serum fetuin-A level was significantly lower in patients with macrovascular diseases (259.0 ± 59.2 μg/mL) than in those without macrovascular diseases (281.8 ± 61.5 μg/mL, $P = .0003$), suggesting that lower fetuin-A levels might reflect the presence of advanced atherosclerosis with calcification.

To explore the OR for the ability of fetuin-A to predict the presence of CP, multivariate logistic regression analysis was performed. As shown in Table 2, serum fetuin-A levels were independently associated with the presence of atherosclerotic CP. A 61.7-μg/mL (1-SD) increase in serum fetuin-A concentration was associated with an OR of 0.753 (95% CI, 0.608-0.933; $P = .010$). Duration of diabetes, smoking, and the presence of hypertension were significant, positive contributors for CP.

4. Discussion

To our knowledge, this is the first study focusing on an association between fetuin-A and atherosclerotic calcified plaques under nondialysis conditions. We demonstrated an inverse association of serum fetuin-A levels with the prevalence of calcified plaques, as evaluated by ultrasonography. Multivariate logistic regression analysis also revealed that fetuin-A levels were inversely related to the presence of atherosclerotic calcified plaques, independent of known risk factors for atherosclerosis. These results suggest that fetuin-A may be an inhibitor of atherosclerosis-related vascular calcification in patients with type 2 diabetes mellitus and without renal dysfunction.

In vitro and in vivo results have clearly demonstrated that fetuin-A is a calcification inhibitor [3,4]. Recent clinical findings support this concept and suggest that fetuin-A could be a predictor of cardiovascular mortality in dialysis patients who often have accompanying medial artery calcification [5-8]. However, except for the dialyzed condition, there are some matters of dispute regarding the role of fetuin-A as a calcification inhibitor. First, in nondialyzed patients, the association between fetuin-A and vascular calcification remains controversial. One report showed an unexpected positive association between fetuin-A and coronary artery calcification [10]. Yet, 3 other reports found no significant associations [11-13].

Second, it remains unclear whether or not fetuin-A can inhibit atherosclerotic calcified plaque, but not medial calcification. One possible explanation for these discordant findings between fetuin-A and coronary artery calcification might be due to the relatively small sample sizes (fewer than 100 subjects in 3 studies) [10-12]. Furthermore, the evaluation methods used for vascular calcification might lead to confusion. All 4 studies used highly technological CT, such as multidetector CT [10-13]. Although these methods are valuable with respect to quantification of vascular calcification, they cannot completely distinguish between intimal and medial calcification [14]. On this point, the presence of diabetes should also be considered, as diabetes is known to be an accelerating factor not only for plaque calcification, but also medial calcification, comparable to uremia [14]. In addition, we have to pay attention to a possible biphasic effect of fetuin-A on atherosclerosis. Other than being an inhibitor of vascular calcification, fetuin-A may have an atherogenic effect [18] through exacerbation of insulin resistance [17]. As 2 of the above studies targeted diabetic patients without dialysis [10,11], the presence of diabetes could have resulted in their incompatible findings.

Very recently, it was reported that fetuin-A could inhibit intimal calcification, rather than medial calcification, in atherosclerotic lesions using fetuin-A/apolipoprotein-E double-negative mice [24]. These results suggest that, when not restricted to medial calcification under dialyzed conditions, fetuin-A can act as a calcification inhibitor against atherosclerotic vascular lesions. On the other hand, an association of fetuin-A with ectopic calcification in the cardiovascular system, valve calcification, was previously reported for nondialyzed patients. Ix et al [25] found an inverse association of fetuin-A with mitral valve calcification in a large number of patients (N = 970) with coronary heart disease and without severe kidney function impairment. A recent longitudinal, prospective study also showed that low fetuin-A levels were associated with the progression of aortic valve calcification independent of renal function [26]. Taken together, fetuin-A appears to be an inhibitor of ectopic calcification, including atherosclerotic calcified plaque, regardless of a dialyzed or a nondialyzed condition.

There are several limitations to this study. First, this was a cross-sectional study, although the number of subjects was relatively large. Second, we used ultrasonography to detect vascular calcification. We could precisely detect atherosclerosis-based calcified plaques. However, it is very hard to

distinguish intimal calcification from medial calcification using ultrasonography, which means that precise evaluation of simple medial calcification is very difficult. Unfortunately, this method does not provide quantitative estimations for vascular calcification. Moreover, it is now recognized that development of atherosclerosis in the carotid artery is generally correlated with coronary artery disease [27]. However, we still cannot determine if there is a direct association of fetuin-A with calcification in coronary arteries in the absence of dialyzed conditions.

In conclusion, we found that fetuin-A levels were inversely associated with the prevalence of calcified plaque independent of known risk factors for atherosclerosis in subjects with diabetes and without impaired renal function. Prospective studies are needed to determine if low fetuin-A levels are causally related to calcification in the atherosclerotic plaque by attempting simultaneous quantification of vascular calcification. Furthermore, recent studies suggest the involvement of a protein-mineral complex, including fetuin-A, termed as a *calciprotein particle* [28] or *fetuin-mineral complex* (FMC) [29,30], with calcification inhibitory features. Although the existence of FMC in human serum has not been confirmed, it provides a diverse point of view for considering the function(s) of fetuin-A. Additional studies are needed to clarify the significance of FMC in human serum.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2009.10.005.

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