Fetuin-A, Valve Calcification, and Diabetes
What Do We Understand?

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In the current issue of *Circulation*, Ix et al demonstrate an inverse correlation between mitral and aortic valve calcification and serum fetuin-A levels in a cross-sectional study of 970 patients with coronary artery disease and without renal disease. Increased serum fetuin was significantly associated with diabetes mellitus, hypertriglyceridemia, serum albumin, and body mass index, with a weaker association with serum C-reactive protein, low-density lipoprotein cholesterol, and serum calcium. The link between aortic stenosis and fetuin held only in those without diabetes. The data highlight the complex interactions of fetuin with inflammation, insulin resistance, and tissue calcification. Furthermore, the results, though intriguing, underscore our lack of understanding of the cellular mechanisms of calcification in diverse pathological substrates, such as the degenerating aortic leaflet and mitral annulus.

Fetuin is a member of the cystatin superfamily of cysteine protease inhibitors, originally discovered as the major component of fetal bovine serum. It is a carrier for growth factors, binds to and inactivates transforming growth factor (TGF)-β and bone morphogenic protein, and is a major component of mineralized bone. Fetuin-A is also an acute-phase glycoprotein, produced in adults primarily in the liver, and is a powerful circulating inhibitor of hydroxyapatite formation. Mice that lack fetuin-A exhibit extensive soft-tissue calcification, which is accelerated on a mineral-rich diet, which suggests that fetuin-A acts to inhibit calcification systemically.

In addition to its effects on mineralization, fetuin-A inhibits insulin receptor autophosphorylation and tyrosine kinase activity in vitro and in vivo. Fetuin-null knockout mice demonstrate improved insulin sensitivity and resistance to diet-induced obesity, and it has been postulated that the absence of fetuin in mice may contribute to the improvement of insulin sensitivity associated with aging.

Clinically, serum fetuin-A is decreased in patients with moderate to severe chronic kidney disease, especially dialysis patients. Ix et al have demonstrated in this same cohort of 970 patients that no relationship exists between fetuin-A and renal function in patients without significant renal impairment.

Patients with severe renal disease have an inverse relationship between serum fetuin-A and Agatston score, mitral annular and peripheral vascular calcification, and overall survival. Despite the fact that fetuin-A is an acute phase reactant, these studies demonstrate an inverse correlation between serum fetuin-A and C-reactive protein in hemodialysis patients. Genetic polymorphisms affect the level of serum fetuin-A and the risk of vascular calcification in end-stage renal disease patients. So far, the study of Ix et al is the first to relate valvular calcification with serum fetuin in patients with normal renal function.

A link between vascular calcification and serum fetuin has not been shown in patients with normal renal function. Serum fetuin-A has been shown to be increased in patients with peripheral vascular disease as measured by intimal-medial thickness, although no measurements of calcification were performed. Serum fetuin has also been positively associated with carotid arterial stiffness, independent of known atherogenic factors in healthy subjects. Therefore, fetuin’s relationships with tissue calcification and atherogenesis are divergent, which reflects its diverse actions.

The pathology of tissue calcification is determined by systemic factors as well as by local tissue changes, primarily of inflammation and cell death, which result in local alterations in pH and matrix that promote the deposition of calcium in the form of hydroxyapatite crystals. Hyperphosphatemia is recognized as a major risk factor for cardiovascular disease mortality in end-stage renal disease patients. In vitro studies show that an elevated phosphate level stimulates smooth muscle cell phenotypic transition and mineralization. Osteopontin, on the other hand, appears to block vascular calcification in that it prevents calcium phosphate crystal growth and induces cellular mineral resorption.

In vitro studies of valvular calcification have primarily focused on cultured interstitial cells obtained from human heart valves. Because these cells are generally derived from aortic valve leaflets, the findings may not be applicable to mitral annular calcification. Cultured interstitial cells calcify in a growth medium supplemented with organic phosphate to form calcified nodules that express osteogenic markers. This process is blocked by inhibitors of alkaline phosphatase.

Osteopontin has been demonstrated within calcified human aortic valves, in the vicinity of macrophages. However, the role of fetuin has not been studied in human valve tissues, nor has the cellular morphology of valvular calcification been studied in detail. For example, fibrin may be a common substrate of calcification within human aortic valves, although the mechanisms of tissue calcification, other than in smooth muscle cells, have not been studied. In vitro studies of vascular calcification have largely focused on matrix vesicles...
formed by altered smooth muscle cells, which adapt a phenotype reminiscent of osteoblasts as extracellular initiators of calcification. Programmed cell death (apoptosis) may be a contributor to physiological mineralization and, if it occurs after tissue injury, may induce ectopic mineralization and mineralization-related differentiation events in the injured area and surrounding areas. Furthermore, the degree of calcification of matrix vesicles is influenced by exposure to calcium and phosphate, other mineralization inhibitors such as matrix Gla protein, and inhibitors of matrix Gla protein such as warfarin, as well as by fetuin-A. Matrix Gla protein potentially acts to regulate calcium deposition by the binding of calcium ions and crystals, inhibition of bone morphogenetic protein, binding to extracellular matrix, and regulation of apoptosis. The mechanisms of action of fetuin are less understood. However, confocal microscopy and electron microscopy–immunogold labeling with fetuin-A suggest that the uptake of the serum protein fetuin-A by vascular smooth muscle cells is a key event in the inhibition of vesicle-mediated calcification.

Morphologically, cardiovascular calcification is heterogeneous, and may primarily affect the media and elastic laminae of muscular arteries (Monckeberg’s medial calcification), the media of arterioles in renal failure patients, especially those of diabetes mellitus (calciphylaxis), and intimal lesions of atherosclerosis. Valvular calcification involves the annulus of the mitral valve and the leaflets of the aortic valve (Figure 1). The morphological changes in each of these scenarios are quite distinct (Figure 2), and the role of fetuin or other calcification inhibitors has not been studied in human tissue samples. The microscopic patterns of calcification are quite varied and include microscopic calcium in areas of apoptotic cell death, of both smooth muscle cells and inflammatory cells, plate-like calcification of matrix and collagen, calcification of lipid pools, and ossification with bone and marrow formation. The differences in calcification patterns of mitral and aortic valves may explain clinical differences that relate to serum fetuin levels as in the current study.

Aortic leaflet calcification is an invariable component of aortic stenosis in adults, but imaging techniques, primarily echocardiography, are able to detect valve calcification well before a significant gradient is present. Several risk factors, in addition to age, have been associated with aortic valve calcification: hypertension, smoking, hypercholesterolemia, female sex, and diabetes mellitus. The pathogenesis of aortic valve calcification involves altered shear stress, nodules with myofibroblast calcification, inflammation, lipid accumulation, T-lymphocytes and interleukin-2 receptors, the renin-angiotensin system, and activation of matrix metallo-

Figure 1. Gross and radiographic patterns of valvular calcification. A, Aortic stenosis, trileaflet valve. Notice thickened free edges and nodular thickening in the sinus (arrowhead). B, Mitral annular calcification. The calcification is at the left ventricular myocardium adjacent to the annulus, primarily under the posterior leaflet. C, Postmortem radiograph of patient with renal failure. Note calcification that extends from mitral annulus onto aortic valve, with calcifications of aortic root as well as right atrium.
proteinases (reviewed in Allison et al). A role of fetuin in calcification of the aortic valve has not yet been established at a cellular level.

The report by Ix et al in this issue of Circulation does not provide information about congenital malformations of the study population; therefore, it is unknown whether fetuin’s relationship with aortic valve calcification is modified by underlying structural compromise. Congenitally, bicuspid aortic valves account for \( \approx 50\% \) of resected aortic valves, calcify at a younger age than trileaflet valves, and have formed the basis for the study of mechanisms of calcification. Computerized digital modeling of congenital bicuspid aortic valve has shown hemodynamic alterations that include excessive folding and creasing, extended areas of leaflet contact, significant morphological stenosis, and asymmetrical flow patterns and turbulence. Morphological studies of human bicuspid valves show, in addition to a high rate of calcification, decreased acid mucopolysaccharides. Mutations in the NOTCH1 gene are associated with familial and nonfamilial bicuspid valve. The NOTCH signaling pathway has been linked to a molecular pathway for aortic valve calcification, as NOTCH1 has been found to repress activation of Runx2, a transcription factor critical for osteoblast cell fate that is upregulated in calcified human aortic valves.

Mitrval annular calcification differs not only in morphology, but also in its risk-factor profile when compared with aortic valve calcification. Risk factors for mitral annular calcification include those classically associated with atherosclerosis and systemic calcification, as well as local factors, likely related to physical effects on the annulus or changes in matrix. Most studies show a link with female sex, and imaging studies have shown a link with not only hypertension, but also with diabetes mellitus, hypercholesterolemia, and osteoporosis. An increased risk exists with chronic renal failure, which accelerates after the institution of dialysis, by mechanisms that are unclear. The major local factor that accelerates calcification of the mitral valve is mitral valve prolapse, which is associated with increased proteoglycan matrix in the valve and annulus, as well as altered stresses on the chordal apparatus. How these factors result in annular calcification is largely unknown, as is the potential role of fetuin in patients with and without mitral valve prolapse or other systemic risk factors of calcification. Pathologically, the microscopic calcification of mitral annulus may be caused by calcium deposition on cellular degradation products, probably released from apoptotic or necrotic interstitial cells. The role of extracellular matrix, especially proteoglycans such as decorin, has been shown in tissue localization studies of vascular calcification, but not yet in studies of human valves.

In the study by Ix et al in this issue of Circulation, the presence of diabetes mellitus was found to negate any effect of serum fetuin on aortic valve calcification, but not mitral annular calcification. This difference may in part be the result of underlying cellular substrates, and annular calcification does not appear to arise from apoptotic smooth muscle cells, but rather calcified extracellular matrix. The effect of diabetes mellitus on vascular and valvular calcification is well documented but may reflect increased coronary plaque burden in diabetic individuals as opposed to increased rates of intimal calcification per plaque area. In fact, angiographic stenosis, when correlated with calcium burden as determined by electron-beam computed tomography, did not show any difference in diabetic versus nondiabetic patients. Because serum fetuin is in itself strongly associated with diabetes mellitus, it is likely that fetuin-related differences in vascular calcification within the diabetic group may be more difficult to demonstrate than in those without diabetes.

The observations by Ix et al are provocative and raise questions about fetuin’s role at a cellular and systemic level. Do the different types of cellular substrates in valvular and vascular calcification signify different actions of fetuin in, for example, inflammation, in contrast to the aortic specimen in A and B.
example, Monckeberg’s medial calcification versus athero-
sclerotic calcification? Does fetuin’s involvement with insu-
lin resistance indicate that its role in calcification pathways
may be modified in diabetic patients, as is apparently the case
in patients with renal failure? We will not know the answers
to these questions until studies that correlate serum fetuin are
complemented with tissue localization of fetuin in diverse scen-
arios of tissue calcification.

Disclosures

None.

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