Introduction

In chronic kidney disease (CKD) patients, calcification prevalence, degree, and progression are greater than in general populations, and progress with declining kidney function. As in the general population, the presence and magnitude of arterial calcification (AC) are strongly associated with cardiovascular morbidity and mortality ***(Schoppet et al., 2008)***.

However, strong data supporting the reduction of AC impact on patients' outcomes are uncertain and limited. Despite these limitations, the KDIGO recommendations suggest that ''patients with CKD stages 3–5 with known vascular and or valvular calcifications be considered at highest cardiovascular risk, and screening for calcifications is recommended for several patient groups'' including those with systemic atherosclerosis, diabetes or pronounced mineral and bone disorders, because arterial and valvular calcifications progress rapidly in these individuals and screening could help orient their management ***(KDIGO, 2009).***

Probably the most revolutionary and fascinating findings in the field of vascular calcification regulation are associated with Fetuin-A (the α_2–Heremans–Schmid protein). This protein has been well known for decades as a negative acute-phase reactant, strongly down regulated in the course of sepsis ***(Schaefer et al., 2003).***

Fetuin-A accounts for approximately 50% of the capacity of serum to inhibit the spontaneous apatite formation from solutions supersaturated in calcium and phosphate. The inhibition is achieved by rapid formation of soluble colloidal Fetuin-A calcium phosphate complexes, termed calciprotein particles (CPPs) ***(Ralf et al., 2009).***

Fetuin-A is predominantly synthesized in liver. It is secreted into the blood stream and deposited as a non collagenous protein in mineralized bones and teeth. During fetal life, there is high serum concentration of Fetuin-A. Its level declines following infection, acute or chronic inflammatory states and malignancy. Recent data have shown a relationship between vascular calcification and endothelial dysfunction (ED) in vascular disease. Therefore, low serum Fetuin-A level could be one of the contributing factors for the development of ED in chronic renal disease (CRD) patients ***(Brandenburg et al., 2010).***

In CKD patients, serum levels of Fetuin-A are significantly lower than in healthy control subjects and in patients with a functioning renal graft. In addition, in CKD patients, serum levels of Fetuin-A are lower among those with diabetes, with higher serum levels of C- reactive protein (CRP), and with malnutrition and cardiovascular disease. Wang and colleagues from Hong-Kong found that Fetuin-A levels

decrease composites that are increased by the malnutrition–inflammation–atherosclerosis–calcification syndrome ***(Wang et al., 2005).***

Chronic liver disease may be a factor reducing the level of Fetuin-A. It is not known if hepatitis C virus infection in hemodialysis patients is a contributing factor that affects Fetuin-A level or not, influencing vascular calcification in return.

Aim of the Work

This study will be performed in order to elicit the possible relation between serum Fetuin-A as a natural calcification inhibitor and vascular calcification in hepatitis C seropositive hemodialysis patients.

Vascular Calcification (Types and Mechanisms)

Vascular calcification is often encountered in advanced atherosclerotic lesions and is a common consequence of aging. Calcification of the coronary arteries has been positively correlated with coronary atherosclerotic plaque burden, increased risk of myocardial infarction, and plaque instability. Chronic kidney disease (CKD) patients have two to five times more coronary artery calcification than healthy age-matched individuals. Vascular calcification is a strong prognostic marker of cardiovascular disease mortality in CKD patients ***(Jono et al., 2006).***

In CKD stages 2–4 and in incident dialysis patients, respectively, it was demonstrated that coronary artery calcification preceded the dialysis stage in a high percentage of patients, emphasizing the fact that diagnostic, preventive, and therapeutic measures should be initiated in early CKD stages ***(Tomiyama et al., 2006).***

Therefore **the Kidney Disease Improving Global Outcomes (KDIGO) recommendations** were as follows:

However, strong data supporting the reduction of arterial calcification (AC) impact on patients' outcomes are uncertain and limited. Despite these limitations, the KDIGO recommendations

suggest that ‘‘patients with CKD stages 3–5 with known vascular and or valvular calcifications should be considered at highest cardiovascular risk, and screening for calcifications is recommended for several patient groups’’ including those with systemic atherosclerosis, diabetes or pronounced mineral and bone disorders, because arterial and valvular calcifications progress rapidly in these individuals and screening could help orient their management ***(KDIGO, 2009).***

Although AC screening is not currently recommended in general populations , the presence and magnitude of vascular and⁄or valvular calcifications represents a higher cardiovascular risk in CKD and end stage renal disease (ESRD) patients, and once present, they rarely regress ***(Goodman et al.,2004).***

Therefore, the primary goals for CKD and ESRD patients are prevention and stabilization of existing calcifications, and KDIGO considers that, ‘‘It is reasonable to use information from calcification screening to guide the management of mineral and bone disorders in some patients’’. That statement is not graded, as there is limited evidence that slowing AC progression impacts on mortality. The ‘‘specific’’ preventive measures for CKD or ESRD patients should include controlling mineral and bone disorders, serum calcium (Ca) and phosphate levels, and control of parathyroid gland activity, including hyper- and hypoparathyroidism ***(KDIGO,2009).***

The presence and severity of calcifications strongly predicts morbidity and mortality of cardiovascular aetiology in subjects with CKD. Evidence from randomised clinical trials on the impact of interventions in reducing the progression of vascular calcifications on mortality is still limited. Because of all of this, although screening for the random detection of cardiovascular calcification in all patients with CKD cannot currently be recommended, it could be justified in patients with significant hyperphosphataemia, those receiving calcium-based phosphorus binders in high doses, patients on the waiting list for a kidney transplant or in other cases on the doctor's judgment ***(Bellorin-Font et al.,2013).***

Types of Vascular Calcification:

Calcification may generally develop within large and small arteries, the myocardium, and at heart valves. Arterial calcification localizes either in the intima or the media of the vessel. Intimal plaque calcification is a common feature of atherosclerosis; medial calcification is restricted to the smooth muscle cell layer and especially to the elastic lamina ***(Vliegenthart et al., 2002).***

A cross sectional study in hemodialysis patients, stratifying subjects to the two types of vascular calcification, revealed that patients with intimal calcification were older and carried 'traditional' risk factors(e.g., smoking, dyslipidemia) before the start of dialysis ***(London et al., 2003).***

In contrast, those patients with predominant medial calcifications were nearly 20 years younger on average and characterized by a long duration of dialysis treatment and an increased incidence of disturbances of the Ca × P balance. Both groups showed evidence of chronic inflammation (based on elevated CRP levels) when compared with dialysis patients with no evidence of vascular calcification ***(Kalantar-Zadeh et al., 2006b).***

Intimal Calcification (Atherosclerosis):

Atherosclerosis is a chronic, immunoinflammatory, fibroproliferative disease of large and medium sized arteries, fuelled by lipids (Figure 1).

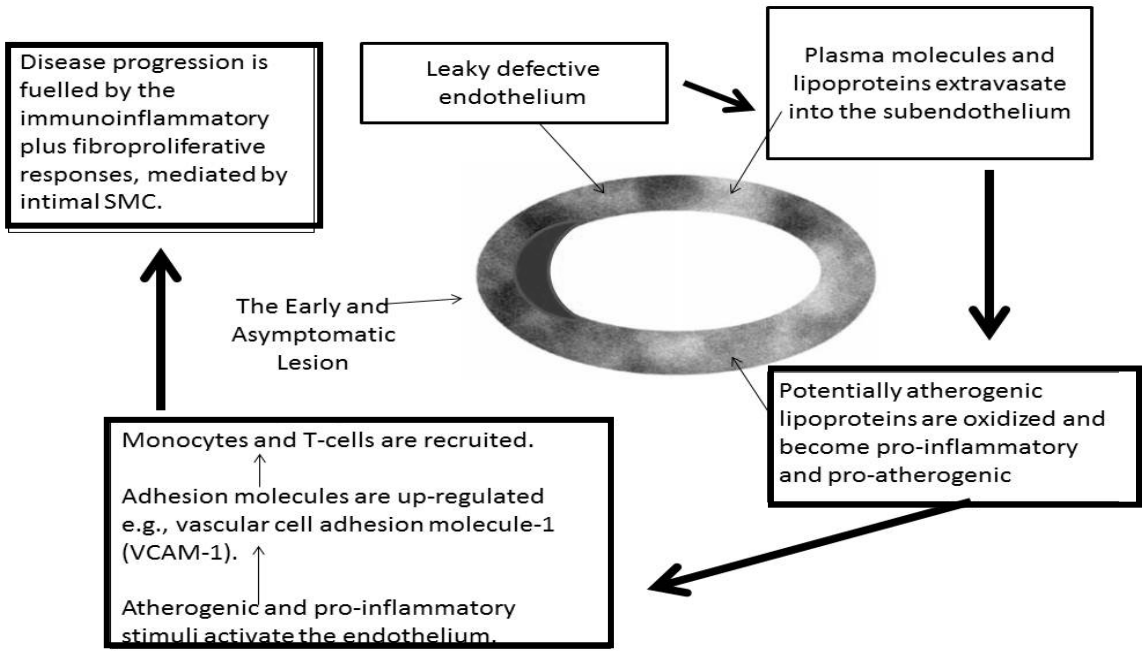

Figure (1): Key Cellular and Molecular Processes in Endothelial Dysfunction ***(Mizobuchi et al., 2009).***

Major cell players are endothelial cells, leukocytes, and intimal smooth muscle cells (SMC). The cellular and humoral activity may be responsible for destabilizing the plaque and initiating atherothrombotic events. Focal calcification within atherosclerotic plaques is common, increases with age, and is due to both active (osteogenic) and passive (cellular necrosis) processes ***(Mizobuchi et al., 2009).***

Atherosclerosis refers to intimal lesions, histologically classified as type I to type VI along a continuum of minimal changes to clinically significant lumen stenosis. A type I lesion contains enough atherogenic lipid protein to form scattered macrophage foam cells; type II lesions consist of the foam cells with lipid-laden vascular smooth muscle cells (VSMCs) and fatty streaks; type III lesions show extracellular lipid droplets and disruption to the intimal SMCs; type IV is a disruptive atheroma with characteristic lipid core; type V lesions add fibrous connective tissue layers to the lipid core and may calcify (type Vb) or fibrose (type Vc); and type VI lesions demonstrate fissures, hematoma or thrombus. Morbidity and mortality is due largely to types IV and V lesions that disrupt the surface ***(Stary et al., 1995).***

Atherosclerotic plaque occurs within the intimal layer. Calcification of the lesions is common, but exhibits a patchy, discontinuous course along the artery. Arterial intimal

calcification (AIC) is advanced atherosclerosis. Plaques and occlusion develop and the lesions impinge on the lumen:

- Advanced disease ⟶ compromised blood flow ⟶ tissue ischemia ⟶ necrosis

- Plaque rupture ⟶ thrombus formation ⟶ arterial occlusion ⟶ acute ischemic events. (***Coll et al.,2010)***

Medial Calcification (Monckeberg's Sclerosis):

Monckeberg's sclerosis occurs in the medial wall (or tunica media). Calcification increases vascular stiffness and reduces vascular compliance. AMC (arterial medial calcification) is typically less occlusive of the arterial lumen than AIC, but causes:

Vascular stiffening

↓

Systolic hypertension

↓

Widened pulse pressure and higher pulse wave velocity (PWV)

↓

Left ventricular hypertrophy (LVH)

↓

Reduced coronary perfusion during diastole

(Kanbay et al.,2009)

Calciphylaxis/Calcific Uremic Arteriolopathy (CUA):

It refers to a potentially life-threatening calcification entity of ESRD, characterized by subcutaneous small vessel media calcification, panniculitis, tissue ischemia, dermal necrosis and ulcerating, painful wounds. Sepsis and amputation are among the morbidities of this obliterative disease. The muscles of the lumbar area and lower limbs are affected. No single treatment approach is superior; aside from management of secondary hyperparathyroidism (SHPT), adjunctive strategies have been studied for their role in ulcer healing (hyperbaric oxygen) and reduction of the vascular calcium load (sodium thiosulfate) ***(Rogers and Coates, 2010).***

Mechanism of Vascular Calcification in CKD Patients:

Normally, mesenchymal stem cells differentiate to adipocytes, osteoblasts, chondrocytes, and VSMC. In the setting of CKD, diabetes, aging, inflammation, and multiple other toxins, these VSMC can dedifferentiate or transform into osteo/chondrocytic-like cells by upregulation of transcription factors such as RUNX-2 [Runt-related transcription factor 2 also known as core-binding factor subunit alpha-1 (CBF-alpha-1)] and MSX2 [Muscle segment homeobox 2]. These transcription factors are critical for normal bone development and thus their upregulation in VSMC is indicative of a phenotypic switch. These osteo/chondrocytic-like VSMC then become calcified in a process similar to bone formation. These cells lay down collagen

and non collagenous proteins in the intima or media and incorporate calcium and phosphorus into matrix vesicles to initiate mineralization and further mineralize into hydroxyapatite. The overall positive calcium and phosphorus balance of most dialysis patients feeds both the cellular transformation and the generation of matrix vesicles. In addition, the extremes of bone turnover in chronic kidney disease (low and high or adynamic and hyper -parathyroid bone, respectively) will increase the available calcium and phosphorus by altering the bone content of these minerals. Ultimately, whether an artery calcifies or not depends on the strength of the army of inhibitors (I) standing by in the circulation (Fetuin-A) and in the arteries (PPI pyrophosphate, MGP matrix Gla protein, and OPN osteopontin as examples) as shown in Figure (2) ***(Moe and Chen, 2008).***

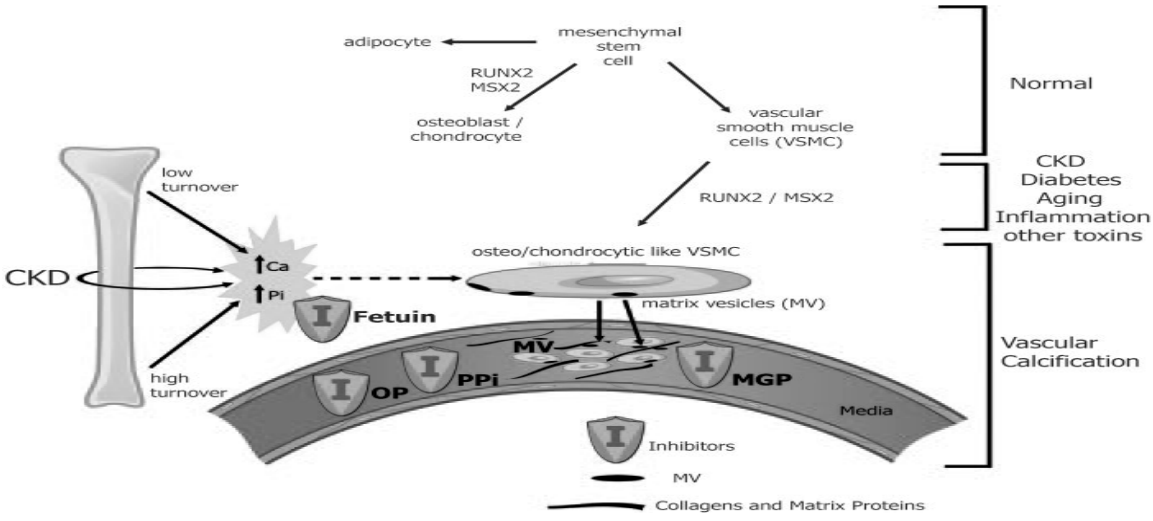

Figure (2): Mechanism of vascular calcification in CKD patients.

Relationships between bone and vascular modifications in CKD and hemodialysis patients:

Relationships between bone and vascular modifications were also observed in CKD and ESRD patients. In dialysis patients, coronary artery calcification score was found to be inversely correlated with vertebral bone mass. In addition, a high systemic arterial calcification score combined with bone histomorphometry suggestive of low bone activity was observed in hemodialysis patients. Arterial stiffening and low spine bone mineral density (BMD) or calcaneal osteopenia were significantly associated in CKD and hemodialysis patients ***(Toussaint et al., 2008).***

Risk Factors of Vascular Calcification

Certain risk factors, such as age, cigarette smoking, diabetes mellitus, and/or glucose intolerance, are thought to contribute to both atherosclerotic calcification and medial wall calcification (Table 1). Disturbances in lipid metabolism, male sex, and hypertension aggravate the atherosclerotic process, but there is little evidence that these represent risk factors for medial wall calcification ***(Goodman et al., 2004).***

Table (1): Risk Factors for Vascular Calcification in CKD.

Risk Factor	Intimal/Atherosclerotic	Calcif.Medial/Monkeberg's Calcification
Dyslipidemia	Yes	No
Advanced age	Yes	Yes
Elevated BP	Yes	Medial lesions worsen BP
Male	Yes	No
Smoking	Yes	No
Inflammation	Yes (local)	Yes (systemic mediators)
DM/glucose intolerance	Yes	Yes
Reduced GFR	No	Yes
Hypercalcemia	No	Yes
Hyperphosphatemia	Yes	Yes
PTH abnormalities	No	No
Vitamin D intake	No	Yes
Duration of dialysis	No	Yes

(Goodman et al., 2004)

The Risk Factors

1-Hypercalcemia and hyperphosphtemia:

Two different mechanisms are proposed to explain the occurrence of vascular calcification in calcium-phosphate disorders: (a) a passive, direct calcium-phosphate precipitation in the vasculature, and (b) an active induction of the expression of bone-associated genes in VSMCs ***(Covic et al., 2010).***

Under normal phosphate conditions, HSMC (human smooth muscle cell) express smooth muscle lineage markers, including SM 22α and SMα-actin. After treatment with elevated phosphate, there is a dramatic loss of these smooth muscle cell lineage markers, and simultaneous gain of osteogenic markers such as core-binding factor-1 (cbfa-1) and osteocalcin ***(Steitz et al., 2001).***

Yang and colleagues showed that increase in either calcium level, phosphorus level, or both can induce mineralization of human smooth muscle cells in vitro via enhancing the sodium dependent phosphate cotransporter-dependent mineralization pathway. Hyperphophatemia and long-term elevated calcium level upregulate the type III sodium-dependent phosphate cotransporter, Pit-1 ***(Yang et al., 2004).***

Interestingly, phosphonoformic acid, an inhibitor of this system, completely blocks calcium- as well as phosphorus-induced mineralization in human smooth muscle cells as shown in Figure (3) ***(Shioi and Nishizawa, 2009).***