Asymmetric dimethylarginine,C-reactive protein and carotid intima media thickness in chronic renal failure patients on regular hemodialysis

Thesis

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Abstract:

The most common cause of death in dialysis patients is cardiovascular disease (CVD). Although controversial, this may be due in part to the presence of excess vascular calcification (VC) particularly in the form of extensive coronary artery calcification (CAC), which can be observed even in very young dialysis patients, this may contribute to premature cardiovascular (CVD) disease and the markedly increased mortality observed in the dialysis population. ADMA increases oxidative stress and potentiates monocyte binding, which are two key processes in the genesis and evolution of atherosclerosis.

Key words:

- Asymmetric dimethylarginine (ADMA)
- CRP
- Carotid IMT

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Contents

List of Abbreviations

1,25(OH)2D3	1,25 dihydroxy vitamin D
AB	Apoptotic bodies
ACEI	Angiotensinconverting enzyme inhibitors
ADMA	Asymmetric dimethyl arginine
AGE	Advanced glycation end products
AHSG	Alpha2-heremann Schmitt glycoprotein
ARBs	Angiotensin receptor blockers
AT	Angiotensin
BMI	Body mass index
BMP-7	Bone morphogenetic protein 7
CAC	Coronary artery calcification
CAD	Coronary artery disease
Cbfa1	Core-binding factor alpha(1)
CCA	Common carotid artery
cGMP	Cyclic glycosyl monophosphate
CIMT	Carotid intima media thickness
CKD	Chronic kidney disease
CLI	Critical limb ischemia
CRF	Chronic renal failure
CRP	C-reactive protein
CVCs	Calcifying vascular cells
CVD	Cardiovascular disease
DDAH	Dimethyl arginine dimethyl amino hydrolases
ECA	External carotid artery
EDRF	Endothelium-derived relaxing factor
EPC	Endothelial progenitor cells
ESRD	End stage renal disease
FFA	Free fatty acid
FIMT	Femoral artery intima media thickness
FMC	Fetuin mineral complex
FXR	Farnesoid X receptor
GFR	Glomerular filtration rate
HD	Hemo dialysis
HDL	High density lipoprotein
HPLC	High performance liquid chromatography

List of Abbreviations

ICA	Internal carotid artery
IGT	Impaired glucose tolerance
IL-6	Interleukin-6
IMT	Intima-media thickness
IRAS	Insulin resistance atherosclerosis study
LDL	Low density lipoprotein
L-NMMA	L-N-monomethyl-L-arginine
LPL	Lipoprotein lipase
MGP	Matrix Gla protein
MV	Micro vesicles
NASCET	North American symptomatic carotid End-Arterectomy trial
NGT	Normal glucose tolerance
NO	Nitric Oxide
NOS	Nitric oxide synthase
OPB	Oral phosphate binders
PRMTs	Protein arginine methyl transferases
PTH	Parathromone hormone
PTHrP	Parathromone hormone related peptide
RANKL	Receptor activator of NF-KB ligand
RAS	Renin angiotensin system
RRT	Renal replacement therapy
SAH	S-adenosyl-homocysteine
SAM	S-adenosyl-methionine
SDMA	Symmetric dimethyl arginine
TIAs	Transient ischaemic attacks
TNF	Tumor necrosis factor
VC	Vascular calcification
VLDL	Very low-density lipoprotein
VSMC	Vascular smooth muscle cell

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Vascular calcification

INTRODUCTION

The most common cause of death in dialysis patients is cardiovascular disease (CVD). Although controversial, this may be due in part to the presence of excess vascular calcification (VC), particularly in the form of extensive coronary artery calcification (CAC), which can be observed even in very young dialysis patients , **[London GM et al.,2003] .**This may contribute to premature cardiovascular (CVD) disease and the markedly increased mortality observed in the dialysis population , **[Goldsmith D et al., 2002].**

The presence of CAC in the dialysis population appears to correlate in part with the ingested quantity of calcium-containing oral phosphate binders (OPB). This may be supported by the demonstration that progressive CAC is attenuated by the substitution of sevelamer (Renagel), a non-calcium based phosphate binder, for calcium-containing OPBs, **[Chertow GM et al.,2002]**.Such calcification, however, was commonly noted in patients with renal disease during periods when calcium-containing OPBs were not yet available. This suggests that additional clinical factors are also associated with VC and CAC in the dialysis population.

Calcium can be deposited into either the medial or intimal layers of the vasculature. The relationship between adverse cardiovascular outcomes and the particular type of calcium deposition is a source of considerable controversy in the nephrology community.

Some investigators contend that calcium deposition in the medial layer, a common finding in dialysis patients, is not clearly associated with adverse cardiovascular outcomes, **[Goodman et al.,2000];** by comparison, others feel that such deposition is associated with stiffening of the vasculature, resulting in significantly adverse cardiovascular outcome, **[McCullough PA et al .,2004].**

Intimal deposition , a relatively less common lesion in patients with chronic kidney disease, is principally associated with atherosclerotic plaques; in patients with normal renal function, such plaques are clearly associated with myocardial infarction and thrombotic events, **[Hunt JL et al .,2002].** However, in those with chronic kidney disease, the association between intimal lesions and these adverse outcomes is less clear.

In general, it is likely that, among patients with renal disease, both intimal and medial calcifications are co-localized in the coronary, aortic, and ilio-femoral circulations.

DEFINITION OF VASCULAR CALCIFICATION

All large elastic, medium-sized muscular arteries and arterioles can calcify. By comparison, veins hardly ever undergo these changes, unless injured or arterialized, **[Leu HJ and Brunner UL, 1992]**. The latter can occur after coronary artery bypass grafting or arteriovenous fistula formation. Patients with pulmonary hypertension may also develop calcification in the pulmonary arterial tree, **[Smith JC et al., 1969].**

Contiguglia SR et al., 1973 found that calcium in arterial calcifications derived from vessels of uremic subjects was shown to consist of hydroxyapatite crystals, this form of calcium deposition, which is the same as that found in the skeleton, differs from other types of vessel calcification.

Calcification of arterial walls occurs at two distinct sites, the intimal and medial layers; medial calcification is the term given to calcification of the elastic laminae of large and medium-sized arteries (particularly around fractured disorganized elastin fibers). This is responsible for the "pipe-stem" or "tram-line" appearances once known as Monckeberg's medial calcinosis.

BIOLOGY

It was previously thought that physicochemical factors alone, such as the calcium-phosphate product and pH (with alkaline pH favoring calcification) regulated the process of vascular calcification

However, it has become clear that there is a great similarity between ossification and vascular calcification, with ossification being an elaborately regulated process that involves the synthesis of a matrix that then becomes calcified. There are many complex bone-synthetic pathways and proteins in the vessel wall that are intimately involved in mineral metabolism and resemble skeletal osteogenesis. As examples, various bone-related proteins, such as osteonectin, osteopontin, PTH, PTH-related peptide, osteroprotegerin, and bone morphogenic protein, can be found in complex atherosclerotic plaques as well as sites of medial arterial calcification **, [Cozzolino M et al.,2006].**

Clinical and histological descriptions of structures resembling bone and even bone marrow within vessel walls as well as the presence of calcium in the hydroxyapatite form are suggestive that ectopic ossification is the cause of VC. In addition, matrix vesicles are seen in vessel walls in proximity to calcified areas (in bony ossification they serve as the focus for ossification initiation) and immunocytochemistry has demonstrated the presence of characteristic bone-related proteins, such as collagen I and a number of noncollagenous bone matrix proteins.

Genetics

Many animal models suggest that genetic factors are important in the tendency to calcify. In the mouse, for example, one genetic element that contributes to aortic and myocardial calcification is the Dyscalc locus, [**Colinayo VV et al., 2002].**

Additional models of vascular calcification include the MGP-null, Apolipoprotein-E-null, , carbonic anhydrase II deficient, desmin-null, and osteoprotegerin-null animals, **[Massy ZA et al .,2005]**,by comparison, there is a paucity of evidence concerning the role of genetic factors in humans, with many studies in patients without renal disease being underpowered .One large investigation concluded that, after adjusting for multiple risk factors, 42 percent of the residual variation in CAC quantity in patients with coronary disease was attributable to genetic factors, **[Peyser PA et al., 2002].**

In patients without renal failure, mutations in the ecto-nucleotide pyrophosphate/phosphodiesterase 1 enzyme and polymorphisms within well known genes and functionally characterized cellular and extracellular proteins (such as E-selectin, ACE I/D gene, and TNF genes)

have been linked with a genetic susceptibility to VC,**[Rutsch F et al .,2003]** . Thus far no study has attempted to examine the associations of any of these genetic alterations with vascular calcification in renal failure patients.

Biochemistry

The exact biochemical mechanisms underlying vascular calcification in renal insufficiency are poorly understood. This is largely a result of the difficulties posed in interpreting cross-sectional data because of the large number of confounding factors

The following are some of the biochemical alterations possibly associated with vascular calcification in renal failure:

- **Hyperphosphatemia**

Hyperphosphatemia can change the phenotype of human aortic vascular smooth muscle cells in vitro from contractile to secretory in a fashion dependent upon normal sodium-phosphate co transport .This leads to up regulation of many genes associated with matrix mineralization, **[Jono S et al .,2000].**

It was noted that elevated both calcium and phosphate levels have synergistic effect than the isolated elevation of any of them, Therefore ,in the presence of increased P, even modest increases in Ca can substantially exacerbate calcification, which is induced by nucleation of BCP(bony calcium phosphate) in vesicles that are released from both viable and apoptotic VSMC, [**Joanne L et al ., 2005]**.The main effect of elevated calcium and phosphate product is its effect on the vascular smooth

muscle cell (VSMC) which might be affected in the following ways:

-VSMCS can resist calcification in the presence of serum but loses this ability in the absence of serum which emphasizes the role of systemic inhibitors of calcification.

-In the presence of hyperphosphatemia and hypercalcemia VSMCS undergo the following processes all of which aid in vascular calcification:

1]Apoptosis with the release of apoptotic bodies (AB).

It was shown that apoptosis and AB release can initiate VSMC calcification in vitro, and abundant AB have been demonstrated in calcified atherosclerotic plaques.

2]Increased formation of micro vesicles (MV)

MV are released by viable VSMC, particularly in the presence of elevated levels of extracellular Ca and P. Vesicle release by VSMC has been described in vivo, in a number of conditions, including atherosclerosis, hypertension, and Ca overload induced by vitamin D_3 toxicity, under normal physiologic conditions, MVdo not calcify as a result of the presence of mineralization inhibitors derived from both cells serum. However, in the presence of raised extracellular concentrations of Ca and P, if serum proteins are limiting or the action of endogenous inhibitors is compromised, then vascular damage is exacerbated, vesicle release is potentiated, and MV can nucleate bony calcium phosphate (BCP), **[Moe SM et al ., 2004] .**

3] Change into chondrocyte or osteocyte like cells: in the presence of huge number of microvesicles containing large amount of nucleated calcium, VSMCS change into