INTRODUCTION

Excess mortality in patients with end-stage renal disease (ESRD) have been consistently demonstrated in registry data from both the United States and Northern Europe, **(US Renal Data System, 1999) (Raine et. al., 1992)** with a one-year mortality rate of approximately 20% **(Foley et. al., 1998).** Nonetheless, despite measures to optimize dialysis treatment, the prognosis of patients with ESRD remains poor. Cardiovascular disease is the biggest single contributor to this high mortality, which accounts for approximately 50% of all deaths **(Manjunath et. al., 2003) (Block & Port, 2000)**. And this remains one of the major outstanding issues in clinical nephrology.

Accelerated atherosclerosis in uremic patients was described back in 1970s to be the major cause of this excess cardiovascular mortality **(Lindner et. al., 1974)**. Given the high incidence of established cardiovascular risk factors (frequently referred to as ‘traditional risk factors') in patients with the chronic kidney disease (CKD), this is an attractive hypothesis. However, subsequent reports have challenged its validity **(Nicholls et. al.,**

1980) (Ibels et. al., 1979) (Burke et. al., 1978) (Thomas & Lee, 1976). While there is undoubtedly an excess of cardiovascular mortality, it remains to be shown conclusively that this is caused by accelerated atherosclerosis per se, and other factors have been implicated **(Ma et. al.,1992).**

Even after adjustment for age, diabetes, and other traditional cardiovascular risk factors in ESRD patients; there is still a higher than expected cardiovascular death rate **(Foley et. al., 2005).** Several other 'non-traditional' risk factors associated with CKD have also been implicated such as anemia, abnormalities of the calcium and phosphate metabolism, dyslipidemias, altered haemostatic factors, hyperhomocysteinemia, endothelial dysfunction, inflammation, and oxidative stress **(Foley et. al., 2005)(Safar et. al., 2004).**

One such important contributing factor to the problem is vascular calcification (VC). Calcification of cardiac tissue has been reported in nearly 60% of dialysis patients on autopsy **(Foley et. al., 2005).** These deposits have been identified in the myocardium, pericardium, conducting system, aortic and mitral valves, and small coronary arteries **(Braun et. al., 1996)(Ribeiro**

et. al., 1998). Several studies have demonstrated the presence of extensive coronary artery calcification (CAC) in dialysis patients, even from a young age **(Coates et. al., 1998)(Block et. al., 1998).** As compared with the general population, the existence of excessive arterial calcification in dialysis patients has been cited as a possible reason for the increased cardiovascular mortality in CKD **(Braun et. al., 1996)(Ribeiro et. al., 1998).** Depending on the age of the patient population examined, 54-100% of dialysis patients have some degree of CAC, with scores markedly above the general population **(Goodman et. al., 2000)(Oh et. al., 2002).**

The pathogenesis of VC in dialysis patients is poorly understood with a wide arena of multiple contributing factors incremented **(Moe and Chen, 2004).** As in the general population, calcifications of coronary arteries are part of the development of atherosclerosis. These deposits occur exclusively in atherosclerotic arteries, and are absent in the normal vessel wall. According to previous reports, arterial calcification is proportional to the overall burden of the atherosclerotic plaque but not necessary to the severity of luminal narrowing **(Moe and Chen, 2004).**

High rate of coronary events, low survival rate, and excess mortality have all been shown to correlate with CAC **(O'Rourke et. al., 2000) (Thompson and Stanford, 2005).** Patients with CAC burden above the mean were six times more likely to suffer an acute myocardial infarction or sudden death **(O'Rourke et. al., 2000).** Also it was reported that the absence of detectable CAC predicts a low likelihood of a major cardiac event within the next 2-5 years (5-10% risk) **(Thompson and Stanford, 2005).**

CAC has been also linked to the ingested quantity of calcium-containing oral phosphate binders (OPBs) **(Block , 2000)**, an observation supported by the demonstration that progressive CAC on continued calcium salt administration can be largely abolished by the use of sevelamer (Renagel®) **(Chertow et. al., 2002)**; which is a non calcium based OPB; in place of calcium-containing OPBs. In addition, cross-sectional and, to a limited extent, prospective epidemiological studies have demonstrated a U shaped relationship between all-cause and cardiovascular mortality and achieved dialysis plasma phosphate levels **(Bleyer et. al., 1998) (Levin et. al., 2001)** – an effect that persists in large datasets (e.g., the United States Renal Data System (USRDS))

even after allowance has been made for a variety of potential confounders.

Furthermore, VC alters the mechanical properties of the large arteries, this leads to a profound influence on survival for dialysis patients **(Goldsmith et. al., 2002) (London et. al., 2002).** Several studies have demonstrated that aortic stiffness is linked to patient survival **(Blacher et. al., 1998) (Blacher et. al., 1999) (Blacher et. al., 2001)**, and that calcium containing OPB ingestion is a determinant of aortic stiffness. Finally, once-rare calcification syndromes, such as calcific uremic arteriolopathy (calciphylaxis), are now being increasingly reported **(Chertow et. al., 2002)**, often as necrotizing panniculitis or ascending acral gangrene.

The natural hypothesis from these apparently unlinked observations is whether the widespread use of calcium-containing OPB to prevent uremic osteodystrophy in dialysis populations have unwittingly accelerated widespread uremic vasculopathy, increasing vascular stiffness and thereby contributing to premature cardiovascular mortality.

During the past decade, considerable progress has been made in the field of noninvasive coronary artery imaging (**Kopp et. al., 2001).** The latest generation of multislice computed tomography (MSCT) scanners; a type of spiral CT also referred to as Multidetector row CT (MDCT); enables motion-free images with submillimeter isotropic resolution and, therefore, excellent reformatted images in any plane **(Gaspar et. al., 2006).**

Calcium scoring is used for the identification and quantification of calcified lesions in the coronary arteries. The most frequently used method to quantify CAC is called Agatston scoring method**.** Agatston scoring algorithm has been introduced in 1990 based on electron beam computed tomography (EBCT) data. A threshold of commonly 130 Housenfield units (HU) is used for the identification. The score is given for each lesion by multiplication of the area (in mm with a co-factor between 1 and 4 that depends on the HU peak value in the considered lesion **(Hoffman et. al., 2003)**.

A test is considered to be positive if calcification is detected within the coronary arteries. Absolute Agatston scored of less than 10, 11-99, 100-400, and above 400 have been proposed to

categorize individuals into groups having minimal, moderate increased, or extensive amounts of calcification, respectively **(Hoffman et. al., 2003)**. A positive test in asymptomatic patients, indicates the presence of subclinical coronary artery disease (CAD). It is well established that individuals with Agatston Scores above 400 have an increased occurrence of both coronary procedures including bypass, stent placement, and angioplasty as well as coronary events including myocardial infarction and cardiac death occurring within the 2 to 5 years after the test. Individuals with very high Agatston scores (over 1000) have a 20% chance of suffering a myocardial infarction or cardiac death within a year **(Bonifacio et. al., 2001).** A test is considered to be negative if no calcifications are detectable within the coronary arteries **(Hoffman et. al., 2003)**.

Although this does not absolutely exclude the presence of atherosclerotic deposits, which might be documented by other techniques, it does indicate that there is nothing more than minimal atherosclerosis, and the risk of a coronary event over the next 2 to 5 years is very low **(Thompson et. al., 2005).**

AIM OF THE WORK

The aim of this work is to assess the prevalence of coronary artery calcification among patients on regular hemodialysis.

Also, we aim to study some of the potential risk factors that may lead to accelerated or increased risk of vascular calcifications.

Chapter 1

VASCULAR CALCIFICATION IN UREMIC PATIENTS

Contiguglia et al. showed; back in 1973; that calcium in arterial calcifications derived from vessels of uremic subjects consisted of hydroxyapatite crystals (the same as that seen in the skeleton) **(Contiguglia et. al., 1973)**. This differs from 'white-lockite' – a 'brushite' form of calcium (magnesium) phosphate, which is present in calcified stenotic regions of arteriovenous fistule and in the elastic laminae of human aorta **(Reid and Andersen, 1993)**, and calcium oxalate crystals, which are only relevant to systemic oxalosis where vessel injury, calcification and gangrene are well-described **(Galimberti et. al., 1999).**

Large elastic and medium-sized muscular arteries, and arterioles, can all calcify. Veins hardly ever undergo these changes unless injured **(Leu and Brunner, 1992)** or arterialized as after coronary artery bypass graft (CABG) and arteriovenous fistula (AVF) formation. Patients with pulmonary hypertension can develop calcification in the pulmonary arterial tree **(Smith et. al., 1969).** Calcification of arterial walls occurs at two distinct sites.

Atherosclerotic plaques typically develop associated "intimal' calcification of the vessel wall as they become mature and start to become 'complex'. This is a hallmark of advancing atherosclerosis seen in muscular arteries such as the aorta and coronaries, a process that starts in childhood and adolescence **(Starry, 2000).** 'Medial' calcification, on the other hand, is the term given to calcification of the elastic laminae of large and medium-sized arteries (particularly around fractured disorganized elastin fibres) and is responsible for the 'pipe-stem' or 'tram-line' appearances once known as Monckeberg's medial calcinosis. These two processes should more accurately be considered separately as the vascular consequences (occlusion with atherosclerosis and vascular stiffening through medial calcification) are different. Indeed, a report using plain radiography and ultrasound in hemodialysis patients showed that the presence of any VC was associated with reduced survival but that intimal lesions (seen in older patients) conferred a worse prognosis compared with medial calcification (seen in younger dialysis patients) **(London et. al., 2003).** It is likely that in some vessels (e.g., coronary arteries) intimal and medial VC can co-localize, although the promotors and risk factors for both types of VC are not identical – unlike

atherosclerosis, medial VC is not just an inflammatory process **(Proudfoot and Shanahan, 2001).**

Pathophysiology of Vascular calcification

The dialysis population is aging and this represents a higher burden of comorbid conditions as well as a higher likelihood of impaired glucose tolerance. Older age (both 'natural', and also progeria syndromes **(Baker et. al., 1981)**), diabetes, and uremia are each associated with VC **(Goldsmith et. al., 1997).** There are also sporadic or familial infantile VC conditions **(Schiffmann et. al., 1992).** VC is present even in young dialysis patients – this requires explanation and interpretation **(Byard, 1996).**

Most body calcium, approximately 99.9%, is present within bone, and plasma calcium reflects body stores very poorly. Many vital metabolic processes are poorly tolerant of major departures from the normal range and, therefore, intracellular calcium levels are kept tightly regulated. The skeleton is a highly effective buffer to keep plasma calcium in the normal range and calciotropic hormones such as parathyroid hormone (PTH) and vitamin D play

a pivotal role; thus, calcium and phosphate can be deposited into bone, or liberated from bone, as needed. It is important to note that there are PTH and vitamin D receptors in many tissues, including the vasculature **(Jones et. al., 1998).**

For many years it was thought that it was physicochemical factors alone that regulated calcification, i.e., calcium–phosphate product, and pH (alkaline pH favoring calcification) **(Mulligan, 1946);** however, there is great similarity between ossification and calcification, and ossification is a more elaborately regulated process involving the synthesis of a matrix that then becomes calcified. It is likely that a series of bone morphogenic proteins, non-collagenous bone matrix proteins and genes are involved in the VC observed in animals and humans .

There are many complex bone-synthetic pathways in the vessel wall (which resemble skeletal osteogenesis) that involve a variety of genes and proteins intimately involved in mineral metabolism. Indeed, 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 **(Ahmed et. al., 2001) (Moe et. al., 2002).** 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 of ectopic ossification as the cause of VC. Additionally, 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.

Prevalence of VC in uremic patients

Several investigators studied the prevalence of CAC in uremic patients; especially those on regular hemodialysis. Studies have shown that the prevalence of CAC in this patient population ranges from 40-84%.

Russo et.al. studied the prevalence and extent of CAC in patients with chronic kidney disease (CKD) not yet on dialysis therapy and compared them with healthy controls. This study showed that CAC was found in 40% of patients and 13% of

controls. The study group concluded that CAC is already present in the early phase of CKD; the prevalence is greater in patients with CKD than in controls, but less than that reported in dialysis patients **(Russo et.al, 2004).**

Barreto et.al. studied a total of 101 hemodialysis patients. Patients were assessed by multislice coronary tomography (MSCT) and transiliac bone biopsy. Fifty-two percent of the patients showed moderate and severe coronary artery calcification, 20% had calcium scores greater than 1000 **(Barreto et. al., 2005).**

Salgueira et.al. studied the influence of VC on cardiac morbidity and mortality in hemodialysis (HD) patients. VC was observed in 55.7% of their patients. Left ventricular hypertrophy, diastolic dysfunction, and cardiac valve calcification were significantly associated with VC. Ischemic heart disease (71.4% vs. 28.6%) and episodes of cardiac failure (0.41 vs. 0.18 per year; $P < 0.05$) appeared more frequently in the patient group with VC. VC was present in 80.6% of patients who developed episodes of heart failure. Eight patients died from cardiac disease; each of them had VC **(Salgueira et. al., 2003).**

Raggi et.al. studied calcification of the coronary arteries, aorta and mitral and aortic valves in 205 maintenance hemodialysis patients with EBCT. They compared subjects with and without clinical evidence of atherosclerotic vascular disease and showed that the median coronary artery calcium score was 595 (interquartile range, 76 to 1,600), values consistent with a high risk of obstructive coronary artery disease in the general population. The coronary artery calcium scores were directly related to the prevalence of myocardial infarction ($p < 0.0001$) and angina ($p < 0.0001$), and the aortic calcium scores were directly related to the prevalence of claudication ($p = 0.001$) and aortic aneurysm ($p = 0.02$) **(Raggi et. al., 2002).**

Furthermore, Krasniak et.al. showed that CAC were observed in 79.5% of the 73 patients examined in a similar study **(Krasniak et.al., 2007).** Also, Caro el.al. studied CAC in 44 hemodialysis patients with a 16 multidetector row CT. In this study, CAC prevalence was very high compared to other studies, accounting to that of 84% with mean calcium score of 1,580 ± 2,010 (r 0-9.844) with calcium score > 400 in 66% of patients. It was usually multiple, affecting more than two vessels in more than 50% **(Caro el.al., 2007).**