ROLE OF CALCIUM PHOSPHATE PRODUCT IN MATURATION AND PATENCY OF NATIVE ARTERIOVENOUS FISTULA IN END STAGE RENAL DISEASE PATIENTS IN KELANTAN

DR. AZIMAH FAKRI

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF

INTERNAL MEDICINE

UNIVERSITI SAINS MALAYSIA
2017

ACKNOWLEDGEMENT

It would not have been possible to write this dissertation without the help and support of the kind people around me, to only some of whom it is possible to give particular mention here.

First and foremost, I am deeply grateful to many lectures from the Department of Internal Medicine, Nephrology Unit, Hospital Universiti Sains Malaysia for their teaching, advice, encouragement and inspiration. I would like to express my deepest appreciation to my wise principal and knowledgeable supervisor; Associates Professor Dr Azreen Syazril Adnan for his continuous encouragement, support and patience.

The good advice and help of my second supervisor, Dr Arman Zaharil Bin Mat Saad, Plastic Surgeon from Hospital Universiti Sains Malaysia, has been providing the most invaluable guidance and opinion in this dissertation, for which I am extremely grateful. Many thanks to Associates Professor Md Salzihan Md Salleh, Pathologist Hospital Universiti Sains Malaysia for continuous guidance.

Appreciation also forwarded to Miss Nurul Jannah, Research Officers in Chronic Kidney Disease Resource Centre and Miss Zulaikha, Research Officer in Unit Biostatistic for helping me in the statistical part of this dissertation.

To the staffs in the Chronic Kidney Disease Resource Centre and staffs in Pathology Lab especially En Ismail, who have helped me in many ways and shared invaluable moments and fruitful experiences during this preparation of dissertation.

Last but not least, I dedicate this dissertation to both my parents En Fakri Hassan and Pn Azizah Che Yusoff who have raised me, supported me, taught me and love me wholeheartedly. For they have sacrificed with all their endless effort to make me a better person, to endure my future and provide me opportunity to pursue my dream and destiny. To them I dedicate this thesis.

Dr Azimah Fakri

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX

APPENDIX **TITLES**

ABBREVIATION

AGEs	Advanced Glycation End Products
ADMA	Asymmetric Dimethyarginine
AVF	Arteriovenous Fistula
BBF	Brachiobasilic Fistula
BCF	Brachiocephalic Fistula
BMI	Body Mass Index
CKD	Chronic Kidney Disease
CPM	Clinical Performance Measures
CRP	C-Reactive Protein
DM	Diabetes Mellitus
DOQI	Dialysis Outcomes Quality Initiative
ESRD	End Stage Renal Disease
FFI	Fistula First Initiative
KDIGO	Kidney
LDL	Low Density Lipid
PTH	Parathyroid Hormone
RCF	Radiocephalic Fistula
RRT	Renal Replacement Therapy
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
USM HREC	USM Human Research Ethics Committee
VSMC	Vascular Smooth Muscle Cell

KAJIAN MENGENAI PERANAN KALSIUM FOSFAT PRODUK DALAM MENENTUKAN KEMATANGAN DAN PATENSI PEMBULUH DARAH FISTULA

ABSTRAK

Terdapat peningkatan pesakit penyakit buah pinggang peringkat akhir yang memerlukan rawatan hemodialisis di seluruh dunia. Akses vaskular untuk rawatan hemodialysis kekal sebagai komponen utama, tetapi malangnya, penyelenggaraan akses ini masih menjadi cabaran utama. Tempoh kematangan 'arteriovenous fistula' (AVF) adalah dipengaruhi oleh faktor-faktor pembolehubah seperti umur, kehadiran aterosklerosis, kalsifikasi vaskular kesan daripada produk kalsium fosfat dan kadar urea yang tinggi.

Kajian ini dihasilkan disebabkan kekurangan kajian mengenai kesan produk kalsium fosfat dan kesan deposit kalsium pada lapisan initima AVF (berdasarkan pemerhatian pada sampel tisu AVF) dalam menentukan kematangan dan patensi fistula. Kajian prospektif ini telah dijalankan di Pusat Sumber Penyakit Buah Pinggang Kronik, Hospital Universiti Sains Malaysia bermula Januari 2016 sehingga Mei 2016. Seramai seratus dua puluh satu pesakit yang layak terpilih dan terlibat menjalani pembedahan AVF dibawah seliaan Unit Sains Rekonstruktif. Pesakit hemodialysis yang berumur lapan belas tahun ke atas, belum pernah menjalani prosedur yang melibatkan salur darah utama (central vein) dan menjalani pembedahn AVF dimasukkan ke dalam kajian ini. Kriteria pengecualian adalah seperti penyakit vaskular periperal, stenosis salur darah vena utama, kecacatan pada bahagian tubuh untuk AVF dan penyakit vasculitis.

Di kalangan 121 pesakit yang dipilih, masing-masing mencapai tempoh kematangan normal dan lewat sebanyak 59 (48.8 %) dan 62 (51.2 %). Min umur untuk kematangan normal AVF adalah 55.7 tahun, manakala kematangan lewat AVF adalah 58.5 tahun. Produk kalsium fosfat tidak mempunyai kesan yang signifikan ke atas faktor kelewatan kematangan AVF (p value = 0.53); dengan didapati min (SD) produk kalsium fosfat adalah rendah dalam kumpulan AVF lewat matang iaitu 3.16 mmol/L (0.99) berbanding kumpulan normal kematangan AVF iaitu 3.35 mmol/L (1.28). Kesan deposit kalsium pada lapisan intima juga tidak menunjukkan keputusan yang signifikan pada faktor kematangan AVF (p value = 0.30). Terdapat 13 (21.0%) sampel tisu AVF yang mempunyai deposit kalsium yang menunjukkan kelewatan kematangan AVF, manakala sebanyak 15 (25.0%) sampel tisu AVF yang menunjukkan kematangan normal. Hubungkait antara kalsium deposit dan lewat matang AVF juga tidak menunjukkan keputusan yang signifikan, p value 0.59.

Kesimpulannya, produk kalsium fosfat tidak mempengaruhi kematangan AVF dan juga patensi AVF. Kesan kalsium deposit pada lapisan intima AVF juga tidak mempengaruhi tahap kematangan AVF. Namun, faktor konfounders dan faktor penghalang yang mempengaruhi kajian ini perlu diambil kira.

ABSTRACT

There has been an increasing trend of end stage renal disease patients requiring haemodialysis treatment worldwide. Vascular access remains the key component of haemodialysis treatment, unfortunately, the maintenance of this access remains a challenging problem. The maturity of arteriovenous fistula is influenced by variable factors such as age, presence of atherosclerosis, vascular calcification as result from calcium phosphate product and uremia.

This study had been done due to lack of study and evidence on effect of calcium phosphate product with correlation of calcium deposition in intima layer in determining AVF maturation and patency. This prospective study was performed in Chronic Kidney Disease (CKD) Resource Center, Hospital Universiti Sains Malaysia between Jan 2016 to May 2016. One hundred and twenty one patients were eligible for this study who underwent AVF creation under Reconstructive Science Unit HUSM. Patients above 18 years old with End Stage Renal Disease requiring regular hemodialysis were included in this study with no previous intervention of the central vein. Exclusion criteria included peripheral vascular disease, central venous stenosis, vascular access site deformity and vasculitic disease.

Among the selected 121 subjects, 59 (48.8%) and 62 (51.2%) achieved normal and delayed maturation respectively. The mean age for normal AVF maturation was 55.7 years, while delayed arteriovenous maturation was 58.5 years. Calcium phosphate product was not associated with delayed AVF maturation (p value 0.53); with lower mean (SD) calcium

phosphate product was observed in delayed mature AVF, 3.16 mmol/L (0.99) compare to normal mature AVF group, 3.35 mmol/L (1.28). There was no association between calcium deposition and delayed maturation of AVF (p value = 0.30). In delayed AVF group, there were 13 samples (21.0%) showed calcium deposition, whereas in normal maturation AVF group, there were 15 samples (25.0%) showed calcium deposition. Correlation between calcium depositions in intima layer with delayed maturation of AVF also showed no significant result with p value 0.59.

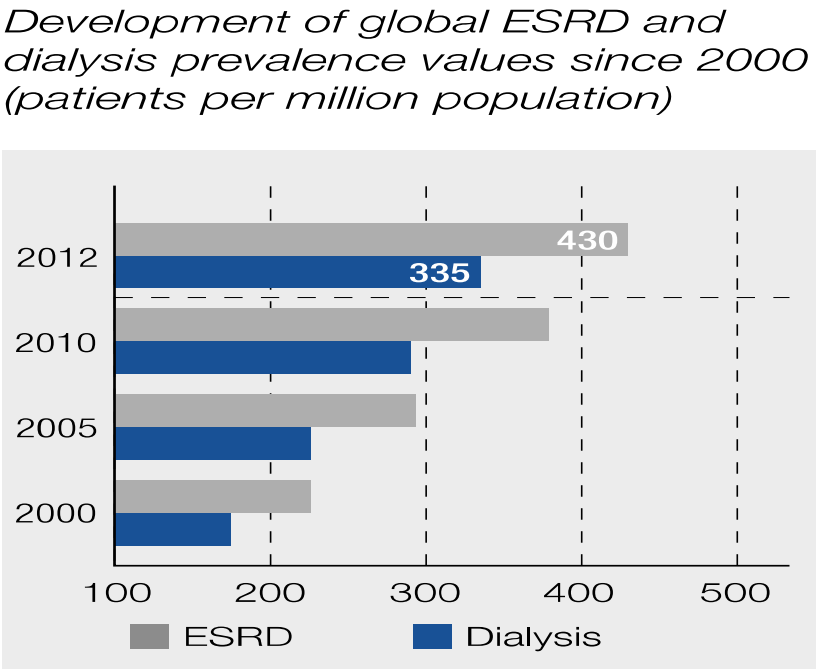
In conclusion, calcium phosphate product and calcium deposition based on tissue biopsy have no effect on AVF maturation and patency. However few considerations for confounding factors and limitation need to be taken.

CHAPTER 1: INTRODUCTION

1.1 Research Background

Statistic from National Kidney Foundation have shown that 10% of the population worldwide is affected by chronic kidney disease (CKD), and millions died each year because they do not have access to affordable treatment. According the 2010 Global Burden of Disease study, chronic kidney disease was ranked 27th in the list of causes of total number of deaths worldwide in 1990, but rose to 18th in 2010. This degree of movement up the list was second to that for HIV and AIDs. (*National Kidney Foundation*).

Fresenius Medical Care in 2012 showed globally by end 2012, 3,010,000 patients are being treated for ESRD and, with a ~7% growth rate, this figure continues to increase significantly higher rate than the world population. The growth of ESRD patients is five times the world population growth (1.3%) and continues growing beyond all normal expectations, showing no signs of reaching a steady state within the next two decades (Moeller *et al.*, 2002). Factors contributing to this growth include globally ageing, multi-morbid population, higher life expectancy of treated ESRD patients and increasing access of a generally younger patient population to treatment. This disease present a significantly challenge to 21st century global health policy.



(Fresenius Medical Care 2012)

Figure 1: Development of global ESRD and dialysis prevalence value since 2000 (patients per million population)

Renal replacement therapy (RRT) remains the most important tool for all patients with end stage renal disease (ESRD) to support their life expectancy. There are haemodialysis, peritoneal dialysis or renal transplantation. The replacement of renal function by hemodialysis (HD) demonstrated for the first time that at least the most vital functions of a complex organ could be replaced by a man-made device. The Founding Father of dialysis is the Scottish chemist Thomas Graham in 1861. The first recovery of a patient undergoing HD for acute renal failure (ARF) was reported by Kolff in 1945, paving the way for a rapidly worldwide expanding treatment of ARF with dialysis. The concept of applying HD to patients with end-stage chronic renal failure (ESRF), first pioneered by Alwall in Sweden as far back as 1948, became reality in 1960 when Scribner, Quinton et al. designed an

external arteriovenous by pass made of Teflon tubing which allowed a permanent access to the bloodstream without requirement of permanent anticoagulation. The Teflon AV shunt, later improved with the use of a silicone rubber material (Silastic) has been the cornerstone for implementing the long-term treatment of ESRF patients with maintenance HD. The next major breakthrough in this area consisted in the surgically created AV fistula performed in 1966 by Cimino, Brescia et al. which considerably reduced the complications encountered with AV shunts.

Dialysis continues to be the most frequent type of RRT because of the low rate of renal transplantation, strictly related to insufficient organ donation to meet demand. As a consequence, there is increasing demand of haemodialysis among ESRD patients and arteriovenous fistula (AVF) is widely regarded as the gold standard of vascular access. In 1997 (updated in 2001), The National Kidney Foundation Dialysis Outcomes Quality Initiative (DOQI) proposed guidelines that AVF be constructed in at least 50% of permanent hemodialysis access procedures, to improve quality of life and outcome in patients with end-stage renal disease. An additional DOQI goal was to achieve an AVF prevalence of 40% in all hemodialysis patients.

AVFs are regarded as the preferred vascular access in hemodialysis patients due to their primary patency and patient survival benefits. The prevalence of fistulas among hemodialysis patients reflects both national, regional, and local practice differences as well as patient-specific demographic and clinical factors. Increasing fistula prevalence requires increasing fistula placement, improving maturation of new fistulas, and enhancing long-term patency of mature fistulas for dialysis. Fistulas have been identified as the best outcome and

can be placed with the least expense and complication rate when compared to a catheter or graft. Several recent initiatives have focused on vascular access and ways to improve outcomes. The National Foundation for Kidney Dialysis Outcomes Quality Initiative (K-DQOL), End Stage Renal Disease Clinical Performance Measures (CPM) and Fistula First Initiative (FFI) have provided guidelines that mandate fistula access in patients on hemodialysis (Vasquez, 2009).

Several factors contribute to the creation of fistula in a patient including timing of referral for dialysis and vascular access, type of fistula placed, patient demographics, preference of the nephrologist, surgeon, and dialysis nurses, and vascular anatomy of the patient (Allon and Robbin, 2002). Vascular access should be easy to use, reliable and have minimal risk to the individual receiving haemodialysis. The ideal vascular access should provide safe and effective therapy by enabling the removal and return of blood via an extracorporeal circuit. It also improved morbidity, mortality and cost-effectiveness.

Arteriovenous fistula (AVF) is associated with the lowest incidence of morbidity and mortality compare to other modality of vascular access for hemodialysis. For maturation of AVF to occur, the main principal is vessel remodeling. This means that AVF must be of adequate size to allow for successful repetitive cannulation and provide good blood flow to support the hemodialysis prescription.

Based on United States Renal Data System (USRDS), AVF prevalence has risen to 42% as of June 2006. The unanticipated consequence of aggressive AVF placement is the

high proportion of AVFs failure to mature. Approximately 25-30 years ago, only 10% of new AVFs failed to mature, in subsequent years, it has increased to 20-50% (Allon and Robbin, 2002). Hence, there is a challenging issue need to maintain a good quality access as the fundamental of the treatment of haemodialysis treatment.

The non- matured AVF has emerged as the major obstacle to dialysis patients. Fistula failure is classified as primary failure and secondary failure. Primary failure is defined as the failure of the fistula prior to its first cannulation while secondary failure is defined as failure after a radiologic intervention such as angioplasty or stent or surgical revision (Hammes, 2011). It is well known from several studies that there is significant primary failure rate for all AV fistulas after its placement (Schild, 2004; Biuckians, 2008; December 2008). The common causes of fistula failure include inflow problems due to inadequate arterial supply, anastomotic stenosis that result from trauma during creation, or outflow problems of the venous segment. Outflow problems may occur because of underlying fibrosis of the vein (Hammes, 2011).

Numerous studies have been performed in the attempt to correlate the factors responsible for failure of AVF maturity. Various predicting factors have been studied in this matter and the understanding of the pathogenesis of AVF non maturation is imperative to achieve the KDOQI AVF goals. The importance of identifying potential patient factors predisposing to failure of AVF maturity may provide opportunities in improving dialysis vascular access outcomes. This will guide clinicians into deciding the optimal timing and patient selections prior vascular access creation. Hence will benefit patients and reduce

potential complications from the unfavorable surgical interventions.

Various clinical factors will be considered including patients' demographic factors, comorbidities and biochemical factors related to the Delayed of AVF maturity. In view of disturbances in mineral and bone metabolism are common complications in CKD; these will be the alarming factors in influencing the arteriovenous fistula maturation together with the risk of vascular calcification and reduced arterial compliance (Moe and Chen, 2004).

1.2 Rationale Of The Study

This study is designed to evaluate the role of calcium phosphate product in arteriovenous fistula maturation and patency in End Stage Renal Disease patient in Kelantan. There is high prevalence of delayed arteriovenous maturation and multiple postulated factors contributing to it including comorbidities, type of fistula and biochemical levels especially calcium phosphate product level. We hope this study will provide valuable information on calcium phosphate product level in influencing the arteriovenous fistula maturation while enabling us to further improve the outcome of the created AVF.

The study findings will help in identifying the acceptable calcium phosphate product in achieving an effective and good quality vascular access in ESRD patients. In long term, the cost effectiveness of AVF placement will be improved hence reducing the unwanted complications.

This study will also provide local data in Kelantan and develop new information about the microfactors (calcium phosphate product) that can potentially delay arteriovenous fistula maturation. It is hoped through the gained information will provide a useful guidance in patients selections for vascular access placement and subsequently preventing unnecessary AVF failure.

CHAPTER 2: LITERATURE REVIEW

2.1 CKD MBD and vascular calcification

As kidney function declines, there is progressive deterioration in mineral homeostasis with disruption of normal serum and tissue concentrations of calcium, phosphorus and changes in circulating levels of hormones including parathyroid hormone (PTH), 25-hydroxyvitamin D (25 (OH)D), 1,25-dihdroxyvitamin D (1.25$(OH)_2$D) and other vitamin D metabolites, fibroblast growth factor-23 (FGF-23), and growth hormone. Later, kidney fails to respond adequately to PTH, downregulation of vitamin D receptor and resistance to the actions of PTH. These minerals and endocrine functions are critically important in regulation of bone formation and recently there has been an increasing concern on extraskeletal calcification that may result from the therapies used to correct these abnormalities (KDIGO 2009).

KDIGO 2009 has come out with new term CKD Mineral and Bone Disorder (CKD MBD) in order to describe the broader clinical syndrome encompassing mineral, bone and vascular abnormalities that developed as a complication of Chronic Kidney Disease. The definition of CKD-MBD is a systemic disorder of mineral and bone metabolism due to CKD by either one or combination of the following :

a) abnormalities of calcium, phosphorus, PTH, or vitamin D metabolism
b) abnormalities in bone turnover, mineralization, volume, linear growth or strength

c) vascular or other soft tissue calcification

The mechanism of vascular calcification in CKD and ESRD remain an active area of research. Calcification is an important part of atherosclerosis process and generally develops within intimal layer of vessel wall (Lee T *et al,* 2013). In CKD population, coronary artery and generalized vascular calcification is exceedingly more prevalent and more severe compare with that normal population. Hence, cardiovascular event is the leading mortality in ESRD patients (Lee T *et al,* 2013).

Longitudinal studies have shown that the progression of vascular calcification seems to be modifiable by the choice of phosphate binder. Few studies comparing treatment between calcium containing phosphate binders and non calcium phosphate binders showed inconsistency of results in terms of calcification progression. Some showed calcification progress with calcium containing phosphate binders, but treatment with non calcium based phosphate binders was associated with lack of calcification progression (Russo *et al, Kidney Int* 2007). Therefore, calcification of fistula also contributed partly by type of phosphate binder used.

2.2 Factors Affecting Arteriovenous Fistula Maturation

The factors affecting maturity of AVF are postulated by multifactorial including (Lok et al, 2006):

a) sociodemographic factors; age and gender
b) comorbidities such as diabetes mellitus, cardiovascular disease, hypertension, atherosclerotic disease and peripheral vascular disease
c) adequacy of vessels and type of fistula
d) biochemical; calcium phosphate product, hyperphosphatemia, uremia which result in vascular calcification

Several studies have provided some insights into the problem of delayed AVF maturity, unfortunately there is still limited understanding behind the failed AVF. Thrombosis and/or delayed in maturity are reasons of primary AVF failure, however risk factors for primary failures are not limited to the site and vascular diameters (Rodriguez *et al.*, 2000). A study conducted from 1997 to 1999 showed the primary failure rate between 10-20% (Pavcnik *et al.*, 2008). A recent meta-analysis has demonstrated 15.3% primary failure rate for native AVF (Irish *et al.*, 2009).

Delayed maturation of fistulas will lead to prolonged dependence on dialysis catheters. This phenomenon is well known to be associated with the risk of infection, central

venous thrombosis or stenosis and social inconvenience to the patients. In this study, we are focusing on role of calcium phosphate product in maturation and patency of AVF. There are few studies on these factors, but each study has its own limitation, end points and clinical factors consideration. Identifying the role of calcium phosphate product risk factor in delaying maturation or fistula failure will help the clinicians in order to provide suitable treatment as primary and secondary prevention.

2.2.1 Calcium Phosphate Product On Vascular Calcification

The presence of increased vascular calcification in patients with CKD has been known, however the extent to which vascular calcification impacts on cardiovascular disease and mortality has recently been appreciated. Studies have reported increased coronary artery calcification and the extent of vascular calcification in CKD compared with the general population. There is a relationship between increased vascular calcification and loss of bone mineral content, with recent experimental studies revealing the mechanisms link these two processes (Davies and Hruska, 2001; Moe, 2006).

The mechanisms of vascular calcification in CKD and ESRD remain an active area of research. Vascular calcifications classically occur in two locations, the intima and the media. Intimal calcifications are part of the atherosclerosis process. They are limited to large and medium-sized conduit arteries. Although they are not specific to CKD, calcifications of coronary arteries are twofold to fivefold more frequent in CKD patients than in age-matched individuals with angiographically proven coronary artery disease (Reynolds *et al.*, 2005). Medial calcifications occur in elastin fibers around vascular smooth muscle cell (VSMC) in the absence of atherosclerosis are seen primarily in CKD or diabetes.

Local and systemic calcium-regulatory proteins as well as inhibitory extracellular factors are also involved in the pathogenesis of vascular calcification. Vascular calcification is the end result from an imbalance between cellular mediators which promote and inhibit

mineralization. In addition to proper homeostasis of vascular mineralization, the current research to date has demonstrated that one major mechanism involved in regulation of vascular calcification is vascular smooth muscle cell (VSMC) damage (Shroff and Shanahan, 2007). Normally, VSMCs play an important role in inhibiting calcification, but in pathologic environments such as kidney disease their normal function is compromised and they develop osteogenic phenotypic changes that favor deposition of minerals that leads to calcification (Shroff and Shanahan, 2007). It is also hypothesized that when VSMCs become apoptotic, they release apoptotic bodies which accumulate calcium and initiate the calcification process (Proudfoot *et al.*, 2000; Shroff and Shanahan, 2007; Shroff *et al.*, 2008). Furthermore, VSMCs have been reported to bud matrix vesicles from their plasma membranes. These small membrane bound particles form a microenvironment capable of concentrating calcium and phosphate and allowing for crystal nucleation (Proudfoot *et al.*, 2000; Reynolds *et al.*, 2004; Shroff and Shanahan, 2007).

In patients with chronic kidney failure, increased serum calcium phosphate product and hyperphosphatemia are important contributors to the higher incidence of arterial calcifications and cardiovascular events. Hyperphosphatemia, by accelerating the progression of secondary hyperparathyroidism, increases serum PTH and bone loss. High PTH itself induces increases in intracellular calcium and abnormal lipid metabolism that promote soft tissue calcifications. Phosphorus-induced and PTH induced bone loss elevates calcium phosphate product and, most likely, the expression of factors that mediate the strong association between bone loss and arterial calcification, such as bone-associated proteins.

Direct effects of phosphorus on vascular pathology include the regulation of vascular cell proliferation as well as the induction of the expression of the osteoblast-specific bone-forming proteins, Cbfa-1 and osteocalcin. The control of serum phosphorus levels in patients with uremia may reduce vascular calcification not only by decreasing calcium-phosphate product but also by reducing serum PTH, thus ameliorating the active and yet incompletely understood processes common for vascular calcification and bone loss. In the last 10 years, several mechanisms have been proposed for phosphate regulation of vascular calcification, involving not only deposition of calcium and phosphate in the vasculature, but also direct activation of genes associated with osteoblastic functions in vascular smooth muscle cells (Jakoby IV and Semenkovich, 2000).

Recent studies have demonstrated phosphate regulation of vascular calcification and provided some insights into the mechanisms for phosphate induction of metastatic calcifications. *In vivo* studies by Kuro-o *et al.*, in the KLOTHO-gene mutant mice with a phenotype that resembles human aging, demonstrated that, in the presence of normal serum creatinine, albumin, cholesterol, and triglyceride levels and with only a mild increase in serum calcium levels (from 9.5 to 10.6 mg/dl), a two-fold increase in serum phosphate levels resulted in increased calcium phosphate product as well as the development of vascular calcifications and osteoporosis that were unrelated to malnutrition, abnormal lipid metabolism, or chronic renal failure (Kurosu *et al.*, 2005). Hyperphosphatemia was the main determinant of the increased calcium phosphate product. In the study by Jono *et al.*, 2000 which assessed the contribution of hyperphosphatemia *per se* on vascular calcification, the study demonstrate that, high phosphorus levels in the incubation media (2.0 mmol/L

phosphate) enhanced calcification in human aortic smooth muscle cells. In conclusion, elevation of calcium phosphate product and high phosphate *per se* could induce vascular calcification.

The calcium crystal that accumulates in the vasculature and contributes to vascular calcification is calcium apatite, which is the mineral found in bone, and the discovery of bone-related factors in vessels when calcified adds stronger evidence to the intimate relationship between bone disorder and vascular calcification. With high bone turnover, associated with elevated parathyroid hormone (PTH) levels, the egress of phosphate from bone is increased and this condition is associated with increased vascular calcification. There are many studies have been reported a positive relationship between vascular calcification and serum calcium, phosphate and calcium phosphate product (Goodman WG, Goldin J, Kuizon BD *et al*, NEJM 2000, London GM, Guerin AP, Marchais SJ *et al* NDT 2003.). Vascular calcification induced by calcium and phosphate excess and uraemia, are the major risk factors and is independently associated with cardiovascular events and death. This is well demonstrated in the Figure 3 as follow. The optimal control of mineral metabolism, especially hyperphosphatemia with non-calcium based phosphate binders, has been shown to be effective to reduce vascular calcification, and attenuation of arterial stiffness (Toussaint and Kerr, 2007).

Vascular calcification, previously thought to be precipitation of excess minerals at sites of damage, is now recognised as an active process involving a complex interaction of

inducers and inhibitors with dysregulation of the normal equilibrium, as in CKD, resulting in the development of vascular calcification. The extent of vascular calcification and the degree of arterial stiffening, closely interrelated are independent predictors of cardiovascular mortality in both the general and CKD populations. There is emerging evidence that vascular calcification is an active cell-mediated process, exacerbated by both uraemia and abnormalities in mineral and bone metabolism and it has been well reported that increased serum levels of calcium, phosphate and PTH are associated with increased mortality as well as greater vascular calcification (Toussaint and Kerr, 2007).

The calcium–phosphorous product is considered a theoretical indicator of the risk of mineral crystallization in soft tissues. However, as deposition and dissolution of calcium salts is a dynamic process, there is no absolute level of calcium–phosphorous below which precipitation will not occur. KDIGO CKD MBD 2009, indicated a threshold product of 4.18 $mmol^2/L^2$ based on a comparison of patients with and without visceral calcifications, suggesting that this level represents the saturation product of the two ions.

Velentzas *et al.* mentioned that inflammation might trigger calcium deposition in the arteries. In this context it should also be considered that CRP, being a member of the pentraxin family, binds to damaged tissue in a calcium dependent manner and shows membrane association with multiple calcium ions. Moreover, CRP binds to enzymatically degraded LDL (E-LDL) particles in normal atherosclerotic lesions, inducing complement activation and promoting the development and progression of the atherosclerotic lesion. There is a general consensus about CRP being a marker of cardiovascular risk both in non-

uraemic and uraemic subjects. It could also be a promoter of the progression of atherosclerotic lesions.

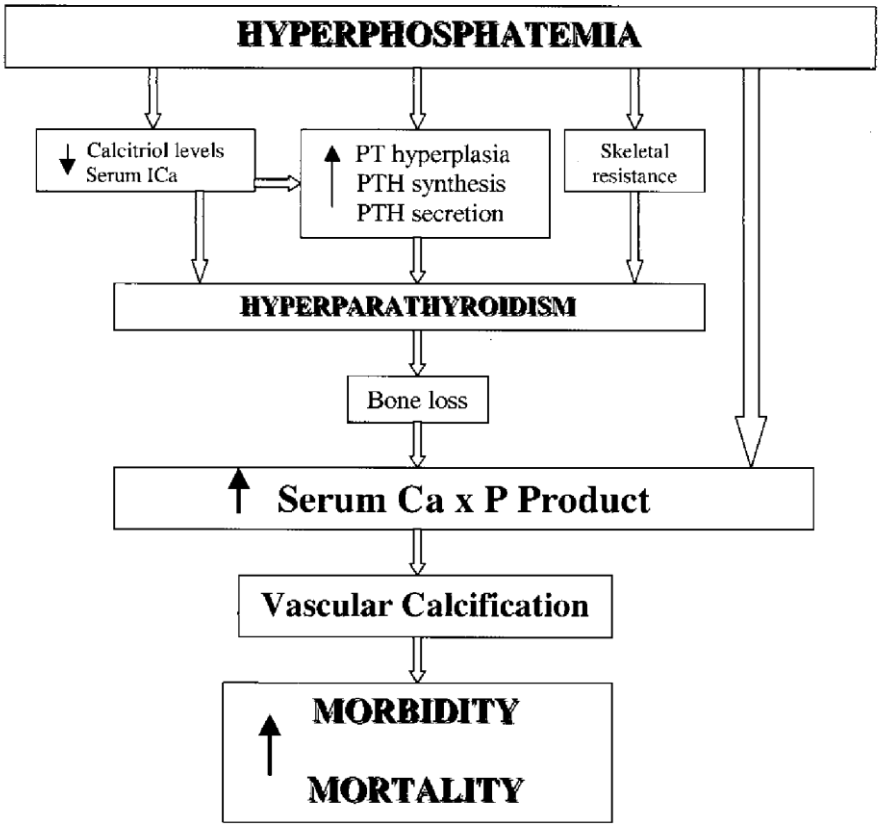


Figure 2: Impact of hyperphosphatemia on vascular calcification

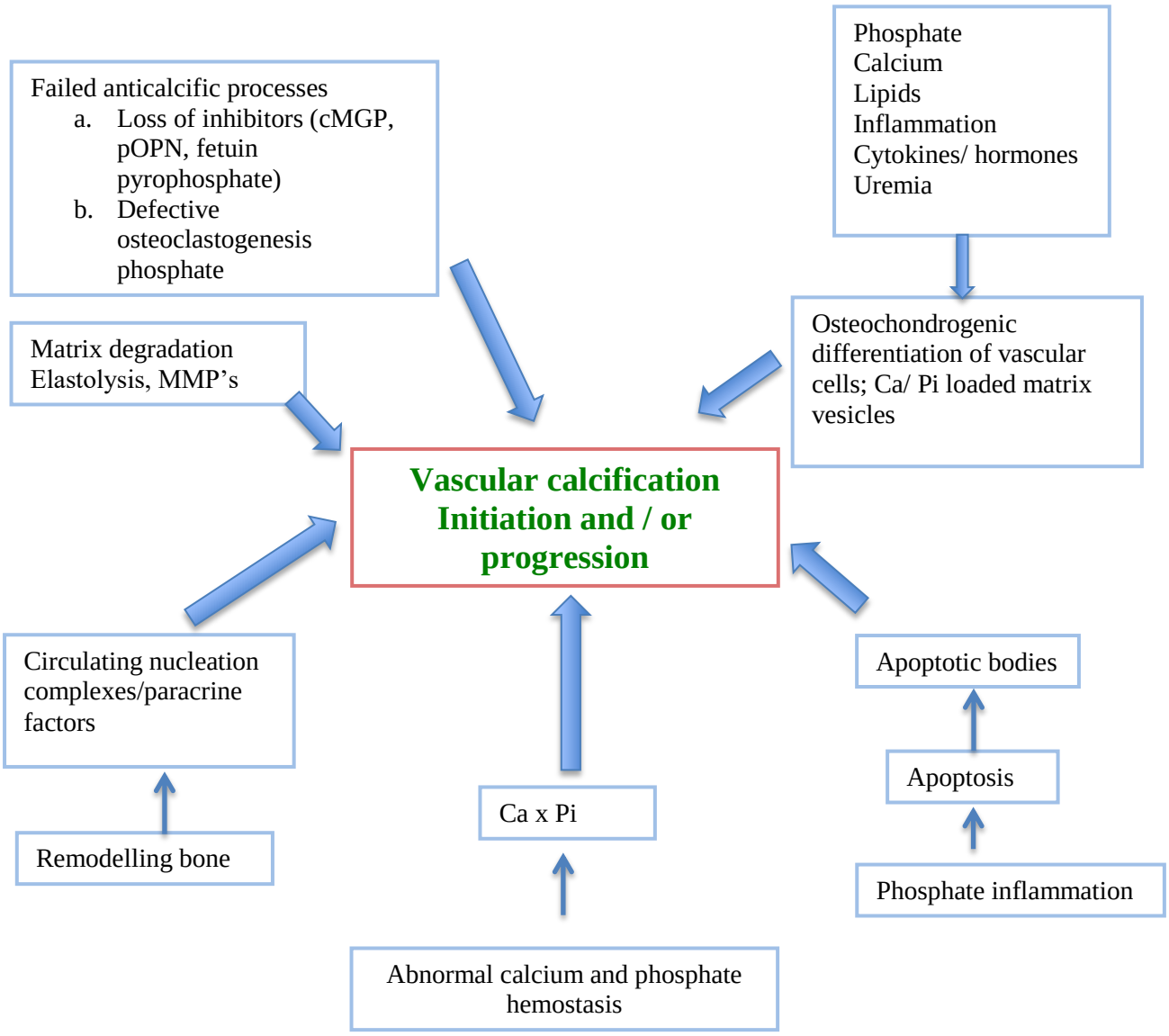


Figure 3: Pathogenesis of vascular calcification

Adapted from Kidney International; Official Journal of International Society of Nephrology, Dec 2008

2.2.2 Adequacy Of Vessels And Types Of Arteriovenous Fistula (AVF)

In order for AVF to mature, there must be sufficient delivery of intra-access blood flow and pressure, depending upon on adequate cardiac output / systemic blood pressure. A good quality feeding (arterial) vessel is mandatory, which will be able to transmit a high pressure to an accepting, unrestricted (i.e. no anastomotic stenosis), compliant and distensible outflow (venous) vessel (Lok *et al.*, 2006). Adequacy of the fistula flow rate depends on the pressure gradient and the total resistance in the fistula circuit including the proximal artery, fistula anastomosis, and the downstream vein. Hence, both coronary artery disease and peripheral vascular disease were predictors of failure to mature, each indicating diseased inflow and outflow in reference to the anastomosis, respectively.

Haemodialysis patients have vascular properties that impair dilatation. Their arteries may develop increased intimal and medial thickening, medial calcification and increased stiffness with decreased flow-mediated dilatation. Artery is generally narrower than the vein hence, the artery is the chief source of vascular resistance in a new fistula. Thus, arterial dilatation plays an important role in fistula maturation. These observations have led to suggestions that vascular compliance should be assessed preoperatively. So far, there are many factors influencing the maturation of fistula and one of the commonest cause is arterial elasticity (Morad, 2012).

Radiocephalic fistula (RCF) is the first recommended fistula; however it often fails to mature in the elderly patient with underlying vascular disease, particularly in diabetics (Miller,1999; Rodriquez, 2000). The second recommended fistula is the brachiocephalic fistula (BCF) due to high failure rate of RCF. The third recommended fistula is the brachiobasilic fistula (BBF), which usually involves a two steps surgical procedure and may be difficult to cannulate given the medial location of the basilic vein.

AVF have a relatively high rate of primary failure, due to either normal thrombosis or failure of the draining vein to dilate adequately to mature. The rate of primary failure may be substantially higher in forearm, as compared to upper arm fistulas (brachiocephalic or brachiobasilic). Primary failure rate has been reported as 66% in forearm fistulas, as compared with 41% among upper arm fistulas (Allon *et al.*, 2001). Similarly, Hakaim, Nalbandian and Scott observed a 70% non-maturation rate for forearm fistulas among diabetic dialysis patients, as compared with 22% for upper arm fistulas (Ethier *et al.*, 2008). In the recent study done in Alor Setar, it concludes that established peripheral arterial disease, distally placed fistula, lower mean arterial pressure and absence of post operative immediate thrill were significantly associated with premature failure of autogenous AVF (Najmi *et al.*, 2012).

Based on multiple previous studies, we can conclude that distally placed fistula has higher primary failure rate than proximal placed fistula. It is likely associated with the anatomically larger diameter and feasible vessels in the proximal of the upper arm.

2.3 Assessment Of Arteriovenous Fistula (AVF) Maturation

Several changes are critical for successful maturation of a new AVF. There must be adequate dilatation of vessels to a calibre large enough to be cannulated repeatedly with two large bore dialysis needles. Furthermore, the blood flow rate in the draining vein must increase sufficiently to accommodate the dialysis blood flow required to deliver adequate dialysis. To avoid vein collapse and recirculation, the access blood flow should exceed the desired dialysis blood flow by at least 100 mL/min. The mean dialysis blood flow varies substantially among countries: about 400 mL/min in the United States, 300 mL/min in Europe, and 200 mL/min in Japan (abstract; Dykstra et al, *J Am Soc Nephrol* 11:182A, 2000). These differences also mean that the definition of a mature fistula can vary among countries. Finally, the fistula must be superficial enough for the landmarks to be appreciated and permit safe cannulation without infiltration.

There is marked variation in the published literature regarding the definition of a successful fistula. The definitions have included presence of a thrill or bruit, ability to use the fistula for at least one dialysis session, or ability to use the fistula reproducibly for dialysis for at least one month with a dialysis blood flow > 350 mL/ min.

Fistula maturation is usually assessed subjectively by means of physical examination. A mature fistula will typically have an easily palpable superficial vein of adequate diameter that facilitates easy cannulation. It will have a uniform thrill to auscultation and palpation

that indicates adequate blood flow without stenosis. The accessible draining vein needs to be more than 10 cm long to allow for rotation of needle sites and adequate distance between the cannulating needles. If the draining vein is too tortuous or too deeply located in the subcutaneous tissues, it will be difficult to cannulate (Morad, 2012).

Timing of first cannulation of an arteriovenous fistula remains a controversial subject, but has been the subject of a few investigations reported in the literature recently. Data from DOPPS show that a functional fistula should have an outflow vein that can be successfully cannulated 1 month postoperatively.

According to National Kidney Foundation. *Am J Kidney Dis. 2006;48(suppl 1):S1-S322,* the assessment for maturation of AVF by definition is by which a fistula becomes suitable for cannulation and the parameters included are volume flow, wall thickness, and vascular diameter. In general, a mature fistula should be a minimum of 6 mm in diameter with discernible margins when a tourniquet is in place, less than 6 mm deep from skin and have a blood flow greater than 600 ml/min. As per the K/DOQI guidelines, all AVFs should be routinely evaluated at 4 weeks. By 4 weeks, normal postoperative edema will have resolved and it is expected that the majority of fistulae with have reached maximum flow. For evaluation for nonmaturation, it will be taken as 6 weeks after surgical creation if it does not meet the above criteria (Group, 2009).

CHAPTER 3: OBJECTIVE OF STUDY

3.1 General Objective

To determine the effect of calcium phosphate product in maturation and patency of native arteriovenous fistula among End Stage Renal Disease patients in Kelantan

3.2 Specific Objectives

1. To determine the association of calcium phosphate product with maturation of arteriovenous fistula
2. To determine the mean of calcium phosphate product in patency of arteriovenous fistula
3. To evaluate the association of calcium with microcalcification present in arteriovenous intima layer
4. To determine correlation between calcium deposition on biopsy with delayed maturation

3.3 Research Questions

1. Do calcium phosphate product delayed arteriovenous fistula maturation in ESRD patients in Kelantan?

2. Do patency of arteriovenous fistula in ESRD patients in Kelantan depend on level of calcium phosphate product ?

3. Do level of calcium level correlate with product deposition in intimal layer of AVF that lead to vascular calcification?

3.4 Research Hypothesis

The mean measurement of calcium phosphate product is an indicator of vascular calcification, which delayed the maturation of arteriovenous fistula in chronic kidney disease patients.

There was no research hypothesis needed for the first objectives since they are answerable by simple descriptive analysis.

Null Hypothesis

The calcium phosphate product is not significantly associated with delayed maturation and patency of arteriovenous fistula in ESRD patients.

Alternative hypothesis

The calcium phosphate product is significantly associated with delayed maturation and patency of arteriovenous fistula in CKD patients.