FACTORS ASSOCIATED WITH DELAYED ARTERIOVENOUS FISTULA MATURATION IN CHRONIC KIDNEY DISEASE PATIENTS

BY

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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 FAKTOR-FAKTOR BIOKIMIA YANG MEMPENGARUHI KEMATANGAN AWAL 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 dan penyakit mineral tulang.

Kajian ini bertujuan untuk menilai faktor-faktor yang mempengaruhi kematangan akses vaskular, termasuk penyakit sedia ada dan sosio demografi. Kajian retrospektif ini telah dijalankan di Pusat Sumber Penyakit Buah Pinggang Kronik, Hospital Universiti Sains Malaysia dari tahun 2004 hingga 2014. Satu ratus rekod perubatan pesakit yang telah menjalani pembedahan AVF terlibat dalam kajian ini, dengan tumpuan diberikan kepada faktor-faktor yang berkaitan dengan kematangan AVF. Pesakit hemodialysis yang berumur dua puluh lima tahun keatas, belum pernah menjalani prosedur yang melibatkan salur darah utama (central vein) dan menjalani pembedahn AVF untuk kali pertama 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 100 pesakit yang dipilih, masing-masing mencapai tempoh kematangan awal dan lewat sebanyak 49 (49 %) dan 51 (51 %). Min (SD) umur untuk kematangan awal AVF adalah 58.51 (11.27%) tahun, manakala kematangan lewat AVF adalah 57.61 (0.89%)

tahun. Kebanyakan pesakit adalah tidak merokok dan bangsa Melayu. Di kalangan kematangan lewat AVF, 27 (52.8 %) adalah pesakit kencing manis dan 47 (92.2 %) adalah pesakit tekanan darah tinggi. Tiada perbezaan yang signifikan dalam produk kalsium and fosfat pada awal dan lewat kematangan AVF (nilai p: 0.092) ; walau bagaimanapun , nilai produk kalsium dan fosfat didapati lebih tinggi pada kumpulan yang mencapai tempoh kematangan yang lewat. Penyakit kencing manis, tahap HbA1c dan tahap LDL didapati dengan ketara mempengaruhi lewat kematangan AVF dengan nilai p: <0.05 masing-masing. LDL didapati menjadi faktor peramal kematangan lewat (nisbah ods: 2.63; 95% CI: 1.62, 4.40; nilai p: < 0.001).

Kesimpulannya, pelbagai faktor mempengaruhi kematangan AVF. Penyakit kencing manis, tahap HbA1c dan tahap LDL didapati mempengaruhi kematangan AVF. Pesakit dengan kenaikan 1 g/dL LDL didapati menyebabkan lewat kematangan AVF sebanyak 2.67 kali ganda.

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 and bone mineral disease.

This study is aimed to evaluate the biochemical factors that influence the vascular access, including comorbidities and the socio-demographics. This retrospective study was performed in Chronic Kidney Disease (CKD) Resource Center, Hospital Universiti Sains Malaysia between 2004 to 2014. One hundred medical records of patients underwent native arteriovenous fistula (AVF) creation were reviewed focusing on the associated factors and arteriovenous fistula maturity. Patients above 25 years old with chronic kidney disease stage 4 and above, with no previous intervention of the central vein and for first time AVF creation were included in this study. Exclusion criteria included periperal vascular disease, central venous stenosis, vascular access site deformity and vasculitic disease.

Among the selected 100 subjects, 49 (49%) and 51 (51%) achieved normal and delayed maturation respectively. The mean (SD) age for normal AVF maturation was 58.51(11.27%) years, while delayed arteriovenous maturation was 57.61 (0.89%) years. Majority of the subjects were non-smoker and Malay. Among delayed AVF maturation, 27 (52.8%) were patients with diabetes mellitus and 47 (92.2%) were patients with hypertension. Calcium phosphate product was insignificantly associated with delayed AVF

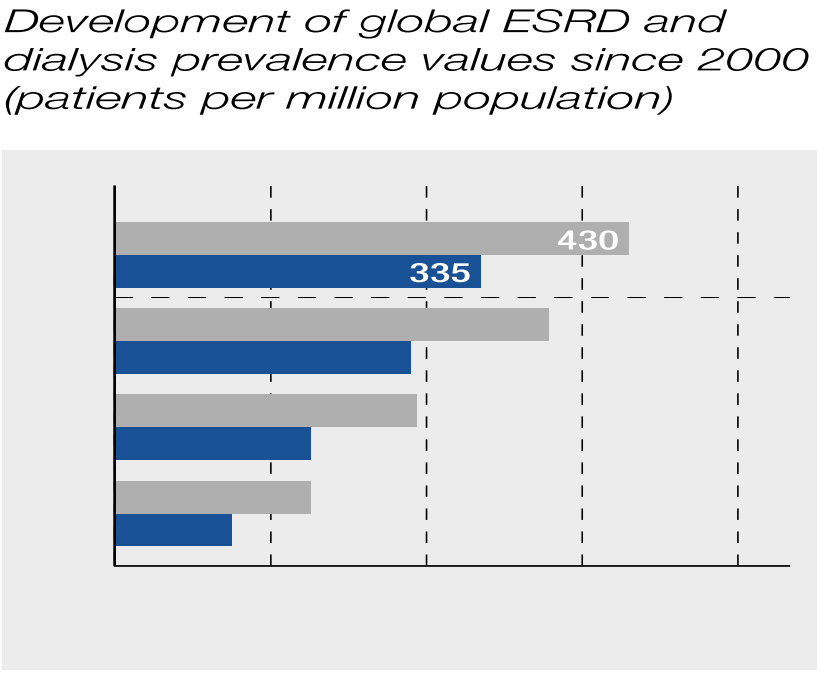
maturation (p-value: 0.092); despite higher mean (SD) calcium phosphate product was observed in delayed mature AVF than normal mature AVF group. Diabetes mellitus, HbA1c and LDL were found to be associated with delayed AVF maturation with p-value < 0.05 respectively. Whereas, LDL has statistical significance at the level of multivariable analysis in delayed AVF maturation with adjusted OR of 2.67 (95% CI: 1.62, 4.40); p-value < 0.001.

In conclusion, impact on AVF maturation is multifactorial. Diabetes mellitus, HbA1c and LDL were found to be associated with delayed arteriovenous fistula maturation. A Chronic Kidney Disease subject who has increasing LDL level by 1 g/dL has 2.67 times of delayed AVF maturation.

CHAPTER 1: INTRODUCTION

1.1 Research Background

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. 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 widely 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. Therefore, regional and network indicators promote the placement of AVF. 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).

Whether a patient receives a fistula depends on several factors: 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.

As the number of newly diagnosed end stage renal disease increases, the demand for hemodialysis grows proportionately. The arteriovenous fistula is preferred for long-term hemodialysis vascular access since it has the best long-term primary patency rate, requires the fewest interventions of any type of access and most importantly, AVF are associated with the lowest incidence of morbidity and mortality. Once a fistula is created, it must develop to the point that it is usable. For maturation 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 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.

AVF non-maturity has emerged as the major obstacle to dialysis patients. Fistula failure may be classified as primary defined as a fistula, which fails prior to cannulation and secondary, 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 a significant primary failure rate for all AV fistulas that are placed (Schild, 2004; Biuckians, 2008; Dember 2008). Causes of normal fistula failure are due to inflow problems from inadequate arterial supply, anastamotic stenosis which may 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 the AVF maturity. Various predicting factors have been studied in this matter and the understanding of the pathogenesis of AVF nonmaturation 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 to decide the optimal timing and patient selections on vascular access creation. Thus will benefit patients and reduce potential complications from the unfavorable surgical interventions.

Various clinical factors will be identified, 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; they will be the alarming factor in influencing the arteriovenous fistula maturation 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 correlate between the association factors and the delayed arteriovenous fistula maturation in chronic kidney disease patients in HUSM. The main focus of this study is to evaluate the influence of sociodemographic data, comorbidities and biochemical factors on the arteriovenous fistula maturity. Since there is high prevalence of delayed arteriovenous maturation and yet the factors were not well known, we hope that with this study, it will provide valuable information on the factors in influencing the arteriovenous fistula maturation and enable us to improve the outcome of fistula.

Besides, the results of this study may help in identifying the predictor factors of delayed arteriovenous fistula maturation, hence justification for the execution of this study is for the purpose of achieving effective vascular access in CKD patients requiring heamodialysis. In a long term, this will improve the cost effectiveness of AVF placement and at the same time, reduce the unwanted complications.

The benefits of this study are to provide local data in HUSM and develop new information about the associated factors with delayed arteriovenous fistula maturation. Hopefully with the information gained, it provides a guide for the selection of patients in vascular access placement and subsequently prevents unnecessary failure.

CHAPTER 2: LITERATURE REVIEW

2.1 Factors Affecting Arteriovenous Fistula Maturation

Arteriovenous fistula (AVF) creation is feasible in majority of cases including diabetics and elderly patients. However, 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). Several studies provided insights into the problem of AVF delayed maturity unfortunately there is a relatively limited understanding of why fistulas fail.

The maturity of AVF influenced by multifactorial, pietro ravani *et al.,* 2004 stated that the important finding of the study is the negative effect of cardiovascular disease on AVF survival. In the present series, presence of cardiovascular disease conferred 83% and two fold increase in probability of primary and final AVF failure, respectively. The explanation for this may be complex and multifactorial. Whether the presence of cardiovascular disease has a direct effect on AVF maturation and survival or is simply a marker of severe comorbidity, poor predialysis care, or uncontrolled uremia cannot be ruled out from the database (Ravani *et al.*, 2004).

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. There has been numerous studies on the correlative factors to the late maturation of fistulae; however, each study has its own limitation, end points and clinical factors consideration. Identifying the risk factors in delaying maturation or fistulae failure will help the clinicians in selecting suitable patients at good timing.

2.1.1 Sociodemographic Factors

Factors which contribute to the primary failure of fistulas include demographic factors such as age, obesity, non-white ethic group, female sex , history of diabetes or peripheral vascular disease (Lok, 2006; Huijbregts, 2008). The association between increasing age and greater risk for fistula maturity is consistent with the underlying need for adequate vessels, which deteriorate with the normal aging process and are damaged by concurrent disease (Feldman *et al.*, 2003). Female has been described as a factor that decreases the likelihood of having an AVF placed as vascular access however, Feldman *et al.*, 2003 stated that they did not find an association between sex and AVF maturation. Similar finding by Charmaine *et al.*,2006 stated that the study did not find female gender to be associated with failure to mature. Many studies have given conflicting reports on the impact of these factors on the success of the AVF. Thus, further analysis of the factor would need to be advocated.

2.1.2 Diabetic Mellitus

CKD patients often have factors other than kidney failure that may affect their vasculature, and it is possible that individual patients' blood vessels may respond differently to the same hemodynamic stresses. This could be attributed to heterogeneity in the vascular wall biology, including endothelial function and clinical factors such as age, diabetes and stages of CKD (Fitts *et al.*, 2014). For example, it is known that diabetes leads to increased arterial stiffness and therefore compromise the ability of the feeding artery to expand following an AVF creation surgery. The pathogenesis of arterial stiffness has been mention specifically related to endothelial dysfunction, indicated by impaired endothelium dependent vasodilation and increased plasma concentrations markers of endothelial function. Among important molecules synthesized by endothelial cells is nitric oxide, which is important endothelium derived mediator. Due to the impairment of vasodilatation, hence compromise the expansibility of the feeding artery to expand following an AVF creation surgery (Fitts *et al.*, 2014).

The nature of the pathogenic link between high ambient glucose concentrations and diabetic complications remains a matter of debate, but clnormal hyperglycaemia is recognized as the primary culprit in the pathogenesis of diabetic complications. There is evidence showed that hyperglycaemia induces repeated acute changes in intracellular metabolism (activation of polyol pathway, activation of diacylglycerol-protein kinase C, increased oxidative stress), as well as cumulative long-term changes in the structure and function of macromolecules through formation of advanced glycation end products (AGEs)

(De Vriese *et al.*, 2000). Hence, it is concluded that endothelium-dependent vasodilatation as a marker of endothelial dysfunction, which plays a key role in the pathogenesis of diabetic vascular disease (De Vriese *et al.*, 2000). This is well explained by the diagram as below.

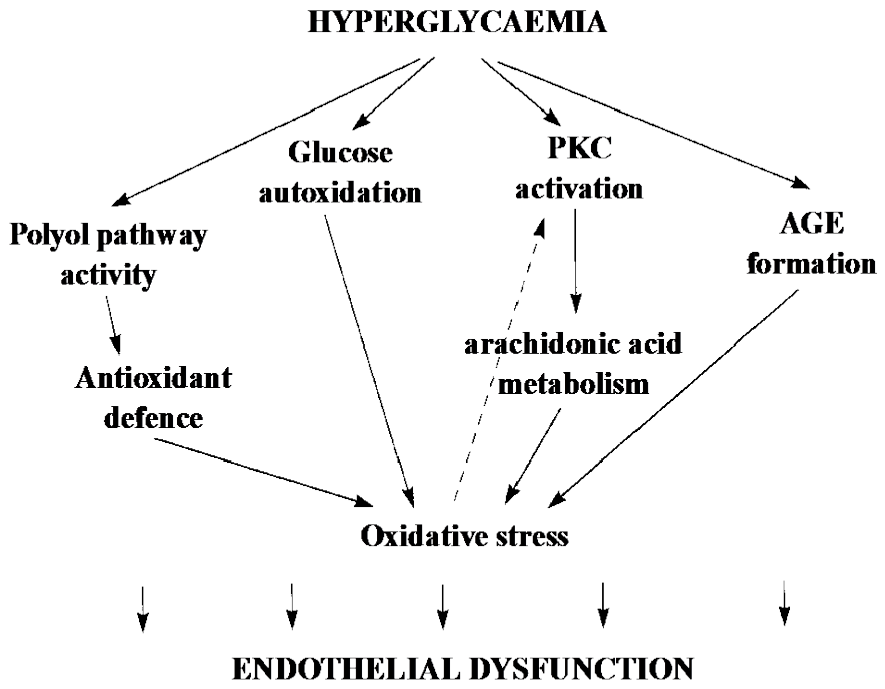


Figure 2 Outline and interactions of hyperglycaemia-induced metabolic pathways potentially involved in the pathophysiology of endothelial dysfunction.

(British journal of pharmacology)

It is known that blood vessels from diabetic and non diabetic CKD patients may react to the same arteriovenous flow differently. However, Lok *et al.*, 2006 stated that the study did not find diabetes a predictor of failure to mature. Indeed, diabetes is associated with suboptimal veins leading to poor fistula adequacy has been described in the study (Lok

et al., 2006). The presence of several comorbidities were associated with failure of the AVF to mature; these included history of hypertension, myocardial infarction, or coronary artery bypass surgery; cerebrovascular events; and thrombophlebitis or pulmonary embolism (Feldman *et al.*, 2003).

2.1.3 Hypertension And Atherosclerosis

Abnormalities of the endothelium underlie a number of human diseases and appear to be central to the pathogenesis of atherosclerosis. Changes in endothelial function and morphology are also cardinal features of hypertension. When there is an increase in blood pressure, it causes ongoing adaptive responses in the microvasculature. There have been observed that, increase vascular smooth muscle cell growth is another common feature in the pathogenesis of both atherosclerosis and hypertension. The growth of vascular smooth muscle is controlled to an important extent by the endothelium. The normal endothelium appears to exhibit an inhibitory influence on vascular smooth muscle cell growth. When there is either atherosclerosis or hypertension, dysfunctional endothelium happened and may contribute to or permit vascular smooth cell growth, which contributes to narrowing of the lumen. Hence, influencing the vasculature dilatation as part of the cardinal phenomena in fistula maturity.

There is evidence suggest that atherosclerosis and hypertension each may enhance the oxidative stress of the arterial wall (Alexander, 1995). It has also been suggested that

superoxide anions might trigger the development of hypertension in some experimental models, presumably by inactivating endothelium-derived nitric oxide and thus mitigating this important vasodilator mechanism. Thus, hypertension facilitates the development and progression of atherosclerosis as such it oxidatively stresses or injures the endothelium, resulting in activation of redox-sensitive mechanisms that recruit mononuclear leukocytes into the arterial wall (Alexander, 1995).

Another effect of hypertension on the arterial wall was shown as the increased wall stress resulting in arterial stiffening. Arterial stiffening occurs due to the loss of compliance of the vascular wall. This is linked to a disequilibrium of its two prominent scaffolding proteins that are collagen and elastin, with the overproduction of abnormal collagen and diminished quantities of elastin. The principal pathophysiological consequence of arterial stiffness is decreased arterial distensibility. Endothelial function affects arterial stiffness through influencing vascular smooth muscle cell tone. The dilator effects of endothelium are under the control of the activity of the endothelial nitric oxide synthase. Nitric oxide plays a crucial role in vascular protection because it inhibits proliferation and migration of VSMC, expression of adhesion molecules, and platelet aggregation (Channon *et al.*, 2000). Thus, the lack of availability of nitric oxide is a major factor in the occurrence of atherosclerosis.

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

In order for as 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). Thus, for an AVF to mature, it needs sufficient flow through the fistula to support hemodialysis and preventing thrombosis. 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).

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. 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 thill were significantly associated with premature failure of autogenous AVF (Najmi *et al.*, 2012).

The fistula with the best outcome is the lower arm radiocephalic (RCF); however this access 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 upper arm brachiocephalic fistula (BCF). This type of fistula is being placed with increased frequency because of the high failure rate of RCF. The third recommended fistula is the brachiobasilic fistula (BBF), which usually involves a two step surgical procedure and may be difficult to cannulate given the medial location of the basilic vein.

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.

2.1.5 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 and 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. Clnormal, 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 clnormal 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 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. Vascular calcification, induced by calcium and phosphate excess and uraemia, is a major risk factor and is independently associated with cardiovascular events and death. This 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).

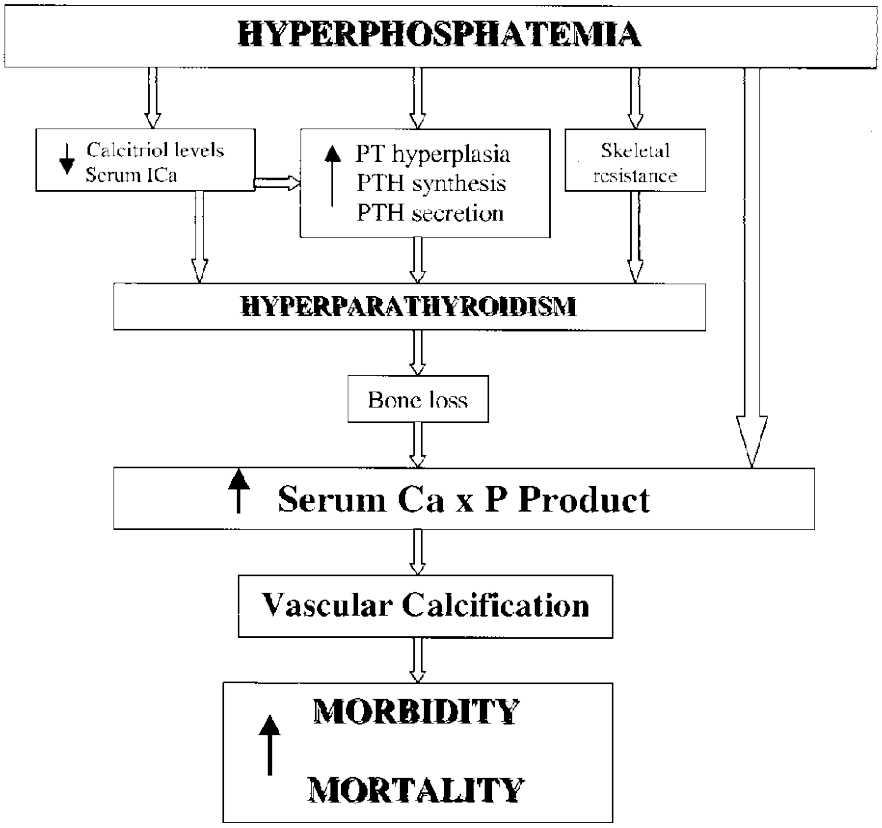


Figure 3: Impact of hyperphosphatemia on vacular calcification

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.

2.1.6 Uremia

Many studies have shown the involvement of uremic toxins and endothelial dysfunction in several aspects of uremic atherosclerosis. Endothelial dysfunction is especially significant in end stage renal disease (ESRD) population due to the negative impacts of uremia and oxidative stress on the endothelium (Hammes, 2011). Indeed, endothelium-dependent vasodilation has been found to be markedly impaired in chronic hemodialysis patients (Hammes, 2011). Vascular dysfunction due to chronic uremia in CKD patients can be delineated into four categories, reviewed by Brunet P *et al.,* 2011 stated that (a) increased rate of atherosclerosis, (b) increased arterial stiffness, (c) increased formation of vascular calcifications, and (d) impaired vascular repair and increased neointimal hyperplasia formation. Uremia is associated with a range of elevated toxins, including guanidines, advance glycation end products (AGE), p-cresyl sulfate, platelet diadenosine polyphosphates, and indoxyl sulfate (Brunet *et al.*, 2011). These toxins are associated with increases in arterial stiffness, calcification, impaired vessel wound healing responses, and neointimal hyperplasia (Brunet *et al.*, 2011). Increased asymmetric dimethyarginine (ADMA) and AGE leads to a decrease in nitric oxide bioavalibility, leading to impaired dilatory function of arteries, as well as impaired signaling for nitric oxide-related processes such as wound healing (Fitts *et al.*, 2014). Thus, blood vessels from CKD patients may react to the same arterioveneous flow differently, depending on the endothelial damages by uremia.

Arterial vascular histologic changes and remodeling occur in response to increasing hemodynamic alterations and increasing uremia from progressive CKD. The arterial vascular pathology associated with CKD and ESRD is commonly characterized by the presence of significant intimal and medial calcification (London, 2003). In addition to CKD and ESRD, calcification has also been associated with increasing age, hypertension, diabetes, dyslipidemia, and smoking (Guérin *et al.*, 2000). These changes to the vasculature from uremia and complications of advanced CKD may potentially effect the vascular access maturity and development of future stenosis. Thus, there has been a tremendous interest in developing therapies to reduce, minimize, and treat vascular development and complications, which may also have implications to vascular access creation and functional use.

2.2 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 caliber 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 600ml/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).