PRESENCE OF MICROCALCIFICATION IN THE ARTERIAL WALL AND ITS RELATIONSHIP TO THE MATURATION OF THE ARTERIOVENOUS FISTULA IN PATIENTS WITH CHRONIC KIDNEY DISEASE STAGE 4 AND 5.

DR TANG WENG JUN

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ABSTRACT

Introduction: A functioning and reliable arteriovenous fistula is a lifeline for individuals suffering from chronic kidney disease who require regular haemodialysis treatment. The success and failure to arteriovenous maturation have been frequently related to patient and surgeon factors. The goal of this study is to evaluate the impact of pre-existing artery wall abnormality on arteriovenous outcome. Specifically, to assess the association between the formation of microcalcification in the intima-media of the arterial wall and arteriovenous fistula maturation.

Method: 138 participants with stage IV and V chronic kidney disease were included in this prospective observational study. Preoperative vascular mapping using an ultrasound was performed by the operating surgeon to evaluate the condition and size of the vessels to fulfil the inclusion criteria. Intraoperatively, the vessel size was measured again prior to anastomosis under magnified view. A specimen from the arterial wall of 3-4 mm in diameter was obtained from the arterotomy for histopathology assessment. Specimens were stained with Hematoxylin and Eosin, Verhoeff's Van Gieson and Von Kossa stains. A pathologist who was oblivious to the patients′ clinical information, examined the specimen for microcalcification in the intima-media of the arterial wall and the media thickness. Arteriovenous maturation was assessed at 6 weeks, post-operatively, with the guidance of a duplex ultrasound.

Results: From the total of 138 participants, 110 participants (79.7%) had matured arteriovenous fistula in 6 weeks. The mean size of the artery measured intraoperatively was 3.82 ± 1.33 mm and the vein was 4.05 ± 1.20 mm. Microcalcification in the arterial tunica media which was hypothesised to be the cause of the arteriovenous fistula failure was

insignificant, with a *P*-value of 0.115. Despite having atherosclerosis in the artery, 83.3% of the arteriovenous fistula matured.

Conclusion: Microcalcification and atherosclerosis are frequently seen in the arteries of chronic kidney disease patients, but they do not explain arteriovenous fistula non-maturation.

ABSTRAK

Pengenalan: “Arteriovenous fistula” yang berfungsi and bertahan lama adalah penting untuk pesakit yang menghidapi penyakit buah pinggang yang memerlukan rawatan hemodialisis. Punca kejayaan dan kegagalan “arteriovenous fistula” untuk matang bergantung kepada keadaan pesakit dan teknik pembedahan. Matlamat kajian ini adalah untuk menganalisis hubungan antara kewujudan “microcalcification” pada dinding intima-media arteri dan kematangan “arteriovenous fistula”.

Kaedah: 138 peserta yang menghidapi penyakit buah pinggang kronik tahap IV dan V telah dimasukkan dalam kajian ini. Sebelum pembedahan dimulakan, pakar bedah akan menganalisis saluran darah dengan menggunakan ultrasound. Ini adalah untuk menilai keadaan dan saiz saluran darah untuk memastikan kriteria dipenuhi. Semasa pembedahan, saiz saluran darah diukur terlebih dahulu sebelum melakukan “anastomosis”. Spesimen daripada dinding arteri bersaiz 3-4mm diperolehi daripada “arteriotomy” untuk kajian histopatologi. Spesimen dianalisis dengan menggunakan hematoxylin dan eosin, Verhoeff Van Gieson dan Von Kossa. Maklumat peserta tidak didedahkan kepada pakar patologi yang bertanggungjawab untuk mengkaji spesimen. Specimen dikaji untuk melihat “microcalcification” dalam dinding intima-media arteri dan ketebalan dinding media. Kematangan “arteriovenous fistula” dinilai selepas 6 minggu dari tarikh pembedahan dengan bantuan “duplex ultrasound”.

Keputusan: Daripada jumlah 138 peserta, 110 peserta (79.7%) berjaya memiliki “arteriovenous fistula yang matang dalam 6 minggu. Saiz “mean” arteri yang diukur semasa pembedahan adalah 3.82mm ± 1.33 dan “vein” adalah 4.05mm ± 1.20. “Microcalcification” dalam dinding media arteri yang dijangkakan antara salah satu punca kegagalan “arteriovenous

fistula" tidak ketara dengan P-value 0.115. 83.3% "Arteriovenous fistula" berjaya matang walaupun dengan kehadiran "atherosclerosis".

Kesimpulan: Kehadiran "microcalcification" dan "atherosclerosis" dalam arteri pesakit yang menghidapi penyakit buah pinggang tidak menjejaskan kematangan "arteriovenous fistula".

1. Introduction

1 INTRODUCTION

A functioning and reliable arteriovenous fistula is a lifeline for every chronic kidney disease patient on regular haemodialysis treatment. The factors that determine the success or failure of the arteriovenous fistula maturation depend on the patient and surgeon. The patient's factors comprise of the size, type and condition of the chosen vein and artery; the preoperative and postoperative care; and their comorbidities. The surgeon's factors mainly focus on the experience, skills and methods in creating an excellent and functional arteriovenous fistula.

Based on previous studies, it was discovered that 20-50% of arteriovenous fistula had failed to mature (1, 2). This failure rate is alarming. A vein is the ultimate determinant used to assess the outcome of the arteriovenous fistula based on its size and rate of blood flow within it. Therefore, failure of maturation of the arteriovenous fistula is always associated with poor dilatation or stenosis of the veins (3, 4).

However, some studies had discovered that the artery used for anastomosis plays a crucial role in the failure of the arteriovenous fistula. The artery is important in providing sufficient blood flow through the fistula circuit. Blood flow has to be increased from 25-50 ml/min to at least 600 ml/min (5). Based on Pouseuille's law of $Q = \pi Pr^4/8\eta l$, the artery must be able to adequately dilate to allow the increase of blood flow required for maturation of the fistula. There are a few intrinsic and extrinsic factors contributing to the poor dilatation of the artery, which include the histopathology condition of the artery and the atherosclerotic plaque constricting the lumen. In a study by Kim et al., it was shown that there were correlations between the radial artery wall intima-media thickness and intima hyperplasia with the failure rate of the arteriovenous fistula (6, 7).

Moreover, arterial calcification that develop in the intima or media wall of the artery further contributes to the failure rate of the arteriovenous fistula (8, 9). Intimal calcification is mainly due to atherosclerotic plaque which may lead to ischaemia-related occlusion (10).

Media calcification (also known as Monckeberg's sclerosis) is when the media wall of the artery is calcified independently of atherosclerosis, and it was reported to be found in 14-23% of hemodialysis patients (8, 11, 12). Arteries with media calcification will be stiffer, causing them to poorly dilate and, thereby, affecting the maturation of the fistula (13). In the findings of Allon et al., a high degree of arterial microcalcification in nonmaturing fistula was discovered, however, there were no connections between medial fibrosis and arteriovenous fistula outcomes (14).

1.1 LITERATURE REVIEW

1.1.1 Chronic kidney disease

Chronic kidney disease can be defined as either functional or structural abnormalities of the kidney for more than 3 months or glomerular filtration rate (GFR) of less than 60mL/min/1.73m^2 (15). Pathologic abnormalities of a damaged kidney could be detected in the composition of blood, urine test and imaging studies such as ultrasonographic or computer tomography of the kidney. Chronic kidney disease is staged from 1 to 5 based on the National Kidney Foundation (Table 1) (15).

Table 1. Stages of chronic kidney disease (15)

STAGE	**DESCRIPTION**	**GFR (mL/min/1.73m^2)**
1	Kidney functioning normally but urine and blood tests are showing kidney disease.	$\geq$ 90
2	Mild reduction in kidney function	60-89

3A 3B	Moderate reduction in kidney function	45-59 30-44
4	Severe reduction in kidney function	15-29
5	Established renal failure	< 15

(Adapted from the National Kidney Foundation, KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for 2006 Updates. Am J Kidney Dis. 2006;48(suppl 1):S1-S322.)

At stage 4 when GFR is less than 29mL/min/1.73m^2, patients are to be educated on the options of kidney replacement therapies such as peritoneal dialysis, hemodialysis and kidney transplant (15, 16). If the patient opts for hemodialysis treatment, the chosen upper limb vessels should be preserved from needle puncture and placement of intravenous catheters (peripheral or central) (15). This is to ensure an ideal vascular access is available for arteriovenous fistula creation before the initiation of hemodialysis therapy. Ideally, a functional permanent access should be ready prior to the initiation of the first hemodialysis treatment.

Patients with chronic kidney disease stage V usually require kidney replacement therapies. Temporary catheter inserted at the subclavian or femoral vein for hemodialysis allows immediate use while waiting for a functional permanent access (15, 17). Long-term use of a catheter via the subclavian and femoral veins is not encouraged due to high morbidity and complications.

1.1.2 Epidemiology

More than 2 million people worldwide are diagnosed with chronic kidney disease stage V who require dialysis therapy or kidney replacement to survive (18). Despite the advancements in the health care system, it was reported that the requirement for dialysis

increases 6-12% annually worldwide (18, 19). Chronic kidney disease was ranked 18th (annual death rate of 16.3 per 100 000) in the 2010 Global Burden of Disease (18).

In Malaysia, from 1993 to 2012, the number of new dialysis patients has shown a drastic increase from 358 in 1993 to at least 5830 in 2012. In 2012, the total number of patients receiving dialysis was 28 590, and the majority had opted for hemodialysis which brings the number to 26 067 (20). Diabetes mellitus and hypertension remain as the main causes of primary renal disease, accounting for 58% and 11%, respectively, of new dialysis patients in 2012 (20).

1.1.3 Arteriovenous fistula and maturation

A functioning and reliable arteriovenous fistula is precious to the chronic kidney disease patient who requires regular hemodialysis treatment. The types of available vascular access are autogenous arteriovenous fistula, prosthetic arteriovenous graft and central venous catheter insertion. Autogenous arteriovenous access has shown to be more superior compared to prosthetic graft and central venous catheter in view of the duration of usage and patient's morbidity and mortality (21, 22).

After the arteriovenous fistula has been created, the fistula needs to be matured before it can be used for hemodialysis. The maturation process involves a complex vascular remodeling procedure which will cause the vessels to dilate and subsequently increase the blood flow rate in the feeding artery and draining vein (5). There will be structural changes in the vessel walls to maintain the high flow rate and the pressure of blood flowing through the vessels. Maturation of the fistula is classified by KDOQI in which it requires the fistula to be 6 mm in diameter with discernible margins, 6 mm or less in depth, 6 cm straight segment for cannulation, as well as, 600 mL/min of blood flow; and it should be matured by 6 weeks post-operatively (15).

1.1.4 Vascular understanding and its importance

Hemodialysis is one of the renal replacement therapy which is widely used these days. Good quality and reliable vascular access is needed for adequate hemodialysis to last for a few years. Therefore, understanding the arterial hemodynamics and vein caliber of the upper limb is important. Choosing the site of arteriovenous fistula in pre-fistula surgery planning is of great significance in attaining excellent vascular access for hemodialysis; thus, reducing the morbidity of the high failure rate of maturation.

The anatomy of the upper extremity varies from person to person. It has been described in words and graphically drawn in many anatomy books by different writers throughout the years. The walls of the arteries and veins consist of 3 layers, which are: (1) tunica adventitia – connective tissue with collagen fibers, (2) tunica media – circularly arranged smooth muscle and elastic fibers and (3) tunica intima – specialized simple squamous epithelium called endothelium (23). The basement membrane, a subendothelial layer composed of connective tissue supports the endothelium. Differences between the artery and vein are: the artery has a thicker tunica media to support higher blood pressure which requires it to dilate, stretch and recoil when necessary; while the vein has thinner walls and large lumen to accommodate a larger volume of blood where its tunica adventitia is the thickest (23).

The artery plays the main role in maturation of the arteriovenous fistula due to its ability to dilate and subsequently accommodate 10-25 fold of increase in blood flow from the heart (24). Increase of blood flow in the artery depends on the quality of the vessel wall and the capacity to dilate to increase the lumen size (24). The artery has a larger diameter at the proximal part of the upper limb and becomes smaller as it goes distally. In creating an arteriovenous fistula, it will be from the distal forearm to the proximal arm and non-dominant to dominant hand. Arteries that are usually used in arteriovenous fistula are the radial and brachial arteries, followed by the ulnar and axillary arteries. Radial artery has a narrower

diameter compared to the brachial artery; therefore, the blood flow in the radial artery depends on the length and quality of the vessel wall for adequate dilatation. The artery is located in the deep fascia, together with deep veins and nerves forming the neurovascular bundle (24).

Venous return of the upper limb can be divided into deep and superficial veins. The superficial veins are located at or below the investing layer of the superficial fascia while the deep veins are situated in the deep fascia accompanying the artery and nerves. Cephalic vein is more commonly used over basilic vein. Cephalic vein travels in the anterolateral aspect of the upper limb as a superficial vein. Basilic vein is a superficial vein in the forearm but as it travels to the arm, it becomes a deep vein accompanying the brachial artery (24).

Common sites for arteriovenous fistula creation include the radiocephalic fistula at the wrist, brachiocephalic fistula at the cubital fossa, brachiobasilic fistula at the cubital fossa and ulnabasilic fistula at the wrist. If a brachiobasilic fistula is created, it may need to be superficialized at the arm during the same or different setting in order to attain less than 6 mm in depth from the skin for the hemodialysis needle access (15).

1.1.5 Vascular calcification

Vascular calcification is frequently present in the artery of the elderly, diabetic and uraemic patients. Deposition of calcium salts can be found in either or both the intima and media of the artery. Several studies have shown that a large proportion of vascular calcification is found to be in the large vessels; namely, the aorta, iliac and femoral arteries (25, 26). However, it does not exclude the presence of calcification in the small vessels which are the peripheral arteries; mainly, the digital arteries (8, 26).

Intimal calcification is mainly due to atherosclerotic plaques, while media calcification is known as Monckeberg's sclerosis (8, 27). Atherosclerotic plaque will narrow the lumen of the artery, leading to a reduced blood flow through the artery which will cause ischaemia and

infarction (28). However, in Monckberg's sclerosis, the size of the artery lumen is not affected, but an increase in stiffness of the artery develops, leading to poor dilatation of the artery and thereby causing left ventricular hypertrophy, coronary hypoperfusion and failure of arteriovenous fistula to mature (5, 9).

The aim of this study is to outline the association between the upper limb artery and the maturity of the arteriovenous fistula. By assessing the vasculature histopathology, it is postulated that the microcalcification and thickness of the media wall in the artery plays a role in arteriovenous fistula failure. In addition, the diameter of the vessels and arteriotomy were measured intraoperatively to obtain the ideal size in attaining a matured arteriovenous fistula.

2. Study Protocol

Microcalcification of the Arterial Wall and Maturation of the Arteriovenous Fistula

Student: Dr Tang Weng Jun

Matric No.: PUM 0103/14

Programme: Master of Surgery (Plastic Surgery)

Co-investigators: 1) Dr Arman Zaharil Mat Saad

2) Prof Madya Dr Md Salzihan Md Salleh

3) Prof Madya Azreen Syazril Adnan

4) Dr Azimah binti Fakri

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1.Introduction

1.1 Chronic kidney disease and arteriovenous creation

At the end of 2004, about 1.7 million people of different nationality were undergoing treatment for end stage renal disease worldwide.(1) About 1.3 million people (77%) were on dialysis treatment and only 413 000 people (23%) received functioning renal transplant.(1)

In Malaysia, the number of new dialysis patients from 1993 to 2012 has showed a drastic increase from 358 in 1993 to at least 5830 in 2012. In 2012, the total number of patients receiving dialysis was 28 590, and majority opted for hemodialysis which brings the number to 26 067.(2) Diabetic mellitus and hypertension remained as the main causes of primary renal disease accounting for 58% and 11% of new dialysis patients respectively in 2012.(2)

Chronic kidney disease is defined as decreased in glomerular filtration rate (GFR) of less than 60mL/min/1.73m^2 for more than 3 months or in layman term, kidney damage. It is staged from stage I to V based on the National Kidney Foundation. Stage IV chronic kidney disease is when the kidney function is only 15-30% (eGFR 15-29ml/min/1.73 m^2).(3) Stage V chronic kidney disease is when the kidney function is less than 15% (eGFR <15ml/min/1.73 m^2) and is also known as end stage renal failure.(3) At stage IV and V, patients may need kidney replacement therapies such as peritoneal dialysis, hemodialysis and kidney transplant.(3, 4) Therefore when a patient reaches stage IV, he or she will be prepared for kidney replacement therapy and those who opted for hemodialysis will need a vascular access for dialysis. The 3 forms of available vascular access are autogenous arteriovenous fistula, prosthetic arteriovenous graft and central venous catheter insertion. However autogenous

arteriovenous access has shown to be more superior as compared to prosthetic graft and central venous catheter in view of duration of usage and patient's morbidity and mortality.(5, 6)

After the arteriovenous fistula has been created, the fistula needs to be matured before it can be used for hemodialysis. The maturation process involves a complex vascular remodeling process which will cause the vessels to dilate and subsequently increases the blood flow rates in the feeding artery and draining vein.(7) There will be structural changes in the vessel walls to maintain the high flow rate and pressure of blood flowing in the vessels. Maturation of the fistula is classified by KDOQI in which it requires the fistula to be 6 mm in diameter with discernable margins, 6 mm or less in depth, 6 cm straight segment for cannulation, 600 mL/min blood flow and should be matured by 6 weeks post operatively.(3)

1.2 Vascular understanding and its importance

Hemodialysis is one of the renal replacement therapy which is widely used these days. Good quality and reliable vascular access is needed for adequate hemodialysis to last for a few years. Therefore, understanding of the arterial hemodynamics and vein caliber of the upper limb is important. Choosing site of arteriovenous fistula in pre-fistula surgery planning is of great significance in attaining excellent vascular access for hemodialysis and thus reducing the morbidity of the high failure rate of maturation.

Anatomy of the upper extremity varies in different person. It has been described in words and graphically drawn in many anatomy books by different writer throughout the years. The walls of the arteries and veins consist of 3 layers which are (1) tunica adventitia – connective tissue with collagen fibers, (2) tunica media – circularly arrange smooth muscle and elastic fibers, (3) tunica intima – specialized simple squamous epithelium called

endothelium.(8) The basement membrane, a subendothelial layer composed of connective tissue supports the endothelium. Differences between artery and vein are artery have thicker tunica media to support the higher blood pressure which requires the artery to dilate, stretch and recoil when necessary while the vein have thinner walls and large lumen to accommodate larger volume of blood where its tunica adventitia is the thickest.(8)

Artery plays a main role in maturation of the arteriovenous fistula due to its ability to dilate and subsequently accommodate 10-25 fold of increase in the blood flow from the heart.(9) Increase of blood flow in artery depends on the quality of the vessel wall and the capacity to dilate to increase the lumen size.(9) Artery has a larger diameter at the proximal part of the upper limb and becomes smaller as it goes distally. In creating an arteriovenous fistula, it is always started from the distal to proximal and non -dominant to dominant hand. Arteries that are usually used in arteriovenous fistula are the radial and brachial artery followed by ulnar and the axillary artery. Radial artery has a narrower diameter as compared to brachial artery, therefore the blood flow in the radial artery depends on the length and quality of the vessel wall for adequate dilatation. Artery is located in the deep fascia together with the deep veins and nerves forming the neurovascular bundle.(9)

Venous return of the upper limb can be divided into deep and superficial veins. The superficial veins are located at or below the investing layer of the superficial fascia while the deep veins are situated in the deep fascia accompanying the artery and nerves. Cephalic vein is more commonly used over basilic vein. Cephalic vein travels anterolateral aspect of forearm as a superficial vein. Basilic vein is a superficial vein in the forearm but as it travels to the arm, it becomes a deep vein accompanying the brachial artery.(9)

Common sides for arteriovenous fistula creation are the radiocephalic fistula at the wrist, brachiocephalic fistula at the cubital fossa, brachiobasilic fistula at the cubital fossa and ulnabasilic fistula at the wrist. If a brachiobasilic fistula is created, it may need to be

superficialized at the arm at the same or different setting so that it will be 6mm or less in depth from the skin for the hemodialysis needle access.(3)

1.3 Literature reviews on causes of poor maturation

Failure of maturation of the arteriovenous fistula is always associated with poor dilatation of the veins or stenosis of veins.(10, 11) In addition, studies have found that the artery used for the anastomosis was shown to have contributed to the failure of the arteriovenous fistula. A few studies have shown that there were correlations between the radial artery wall intima-media thickness and intima hyperplasia with the failure rate of that fistula.(12, 13) Arterial calcification may develop in the intima or media wall of the artery.(14, 15) Intimal calcification is mainly due to atherosclerotic plaques which may lead to ischaemia-related occlusion. (16) Media microcalcification (also known as Monckeberg's sclerosis) is when the media wall of the artery is calcified independently of atherosclerosis and was reported to be found in 14-23% of hemodialysis patients.(14, 17, 18) Microcalcifications are very tiny specks of calcium deposits which can only be seen under the microscope. Arteries with media microcalcification will be stiffer causing it to poorly dilate thereby affecting the maturation of the fistula. (19) A study by Guerin et al found that the calcification in which leads to vessels stiffness and increased in left ventricular after-load does not relate to the vessel size in the vasculature of the end stage of renal patients.(19) Elevated serum calcium and phosphate level are one of the contributors to calcified vessels where deposition of this product in the vascular matrix will induced transformation of the vascular smooth muscle cells.(15)

This study attempts to outline the vasculature histopathology based on the artery on the upper limb used in the anastomosis to see its association with the successful rate in maturity of the arteriovenous fistula.

2. OBJECTIVES

2.1 General objective

- To assess the relationship between the formation and severity of microcalcification in the arterial wall with the serum calcium phosphate level and the maturation of the arteriovenous fistula in chronic kidney disease stage 4 and 5 patients.

2.2 Specific Objectives

1. To evaluate the amount of microcalcification present in the intima media of the arterial wall in chronic kidney disease stage IV and V patients
2. To determine whether the microcalcification in the intima media is associated with early failure of arteriovenous fistula
3. To evaluate the thickness of the intima media in the artery of stage IV and V chronic kidney disease patients
4. To determine the effect of serum calcium phosphate level in the formation of microcalcification in the arterial wall and maturation of arteriovenous fistula.

(Arteriovenous creation and blood taking is done as the usual clinical management for chronic kidney disease patients stage 4 and 5 with exception that the arterial wall is not discarded but sent to the pathology lab for analysis)

2.3 Hypothesis

1. Calcification of the arterial wall leads to poor maturation of the arteriovenous fistula

2. Calcification in intima media is the cause of vessel stiffness which leads to fail maturation
3. Intima media will be thicker in stage IV and V chronic kidney disease patients
4. Calcium phosphate product plays a role in formation of the arterial wall calcification

3. METHODOLOGY

3.1 Study population and sample size calculation

This is an observational prospective cohort study. 132 patients with chronic kidney disease stage IV and V who are scheduled for elective arteriovenous fistula creation from 1st January 2016 – 31st December 2016 under Reconstruction Sciences Unit will be included in the study based on the inclusion and exclusion criteria as below:

Inclusion criteria:

- Patient age 18 years old and above with chronic kidney disease stage IV-V (GFR<29ml/min)
- Vein diameter of > 2 mm and arterial diameter of > 2 mm
- Consented for arteriovenous fistula creation and compliance to follow up

Exclusion criteria:

- Patients unable or refused to give consent
- Prosthetic arteriovenous graft creation

The demographic information of the patients will be collected, which includes: (Appendix 1)

1. Age

2. Gender
3. Body mass index
4. Ethnic group

Sample size calculation

$$n_1 = \kappa n_2$$

$$n_2 = \frac{(z_\alpha + z_{\beta/2})^2 \sigma^2 (1 + 1/\kappa)}{\delta^2} \quad (20)$$

Level of significance (α) = 0.05

Type 1 error (Zα) = 5%

Type 2 error (Zβ) = 20%

Power (1-β) = 0.8

Detectable difference between maturation and failure of AVF (21) = 0.4

Standard deviation (σ) = 0.8

Sample size (n) = 120

Estimated dropout rate = 10%

Final sample size = 132

3.2. Study design

This is an observational prospective cohort study to evaluate the influence of histopathology findings of the arterial wall on the maturation of the arteriovenous fistula in stage IV and V chronic kidney disease patients. This study will be conducted in Hospital Universiti Sains Malaysia (HUSM), Kubang Kerian, Kelantan.

3.3 Procedure

3.3.1 Preparation of participants

Participants who had consented to this study were preoperatively and clinically examined by the same single operating surgeon, and vascular mapping was performed using doppler ultrasound in the Plastic and Reconstructive Surgery Clinic. The site of incision was determined preoperatively based on the clinical and ultrasound findings. Participants who had consented were admitted to the surgical ward a day prior to the surgery. Arteriovenous fistula creations were done under local anaesthesia (by the surgeon) or regional block (by the anaesthetist) in the operation theatre.

3.3.2 Intraoperative

Participant would lie supine with the upper limb which is chosen as the site of fistula creation extended onto the arm board. Placement of incision, marking of chosen artery, as well as the vein were clinically confirmed again by the operating surgeon prior to surgery. Artery and vein were carefully dissected out in a magnified view. A segment of arterial wall was excised under magnified view by cutting out 3-4 mm in diameter of the arterial wall in a circular shape to facilitate an end to side anastomosis of the vein to artery (Figure 1). Sizes of the chosen vessels and arteriotomy were measured intraoperatively in millimetres (mm) before anastomosis. End to side anastomosis with interrupted 8/0 polypropylene was constructed under magnified view. Success of the anastomosis was determined by the presence of thrill on palpation of the vein at about 1-2 cm from the anastomosis site prior to the closure of the skin with nylon 4/0 suture.

3.3.3 Laboratory

The arterial wall specimen was put into formalin and sent to the histopathology laboratory to be processed and analysed. Histopathology of the arterial wall was analysed using a light microscope (Olympus BX51 UL 10x/0.30∞/-/FN26.5) where the clinical data of the patient was not revealed during the analysis to avoid bias.

The specimen was cut into 5µm sections and stained with Hematoxylin and Eosin, Verhoeff's Van Gieson and Von Kossa staining. Verhoeff's Van Gieson staining was performed to delineate the intima by enhancing the elastic lamina which outlines the border between the intima and media layers. Although microcalcification can be seen by using Hematoxylin and Eosin stain, in this study, we added Von Kossa staining to optimise visualisation of the microcalcifications as it is more specific for this mineral (Figure 2). Microcalcification may be present in both intima and media layers of the arterial wall.

3.3.4 Postoperative

Follow-ups of the patient at 2 and 6 weeks were conducted to check for arteriovenous fistula complications and maturity of the fistula. If the fistula is matured at 6 weeks, it is classified as a matured fistula. If the fistula is not matured after 6 weeks, it is classified as a failed fistula. Maturation assessment of the fistula is according to the National Kidney Foundation. KDOQI Clinical Practice Guidelines were shown in Table 1 (15). Outcomes of the fistula were measured by the operating surgeon who is experienced in using a non-invasive ultrasound (SonoSite TITAN® high resolution ultrasound system) provided with an L38 transducer (10-5Mhz linear ultrasound transducer).

Outcomes to be assessed

- Maturation: rule of 6's (Foundation, 2006)
    - 6 mm diameter with discernable margins
    - 6 mm or less in depth
    - 6 cm straight segment for cannulation
    - 600 ml/min blood flow
    - Fistula to be assessed by post operative 6 weeks for maturation
        - Fistula matured within 6-12 weeks post op – matured fistula
        - Fistula not matured after 12 weeks – failed fistula

3.4 Data collection and statistical analysis

Demographic data will be collected as stated in the proforma. Thickness of the intima and calcification is measured in μm. Data analysis will be done using Statistical Package for Social Science (SPSS) software version 22. Data will be presented as mean $\pm$ SD. Statistical significance is sited at $P<0.05$. Independant t-test will be used for analysis.

4. EXPECTED RESULTS / BENEFITS

- To provide early prediction of the success of the fistula creation.
- To overcome precipitation of calcium phosphate in the vessel wall.

- To improve outcome of the arteriovenous fistula after determining the possibility factors which contribute to the failure of maturation and thus reduced the morbidity in chronic kidney disease patients.

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