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Ultrasound measurement of abdominal fat index
for predicting cardiovascular disease

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MSc. In Health Technology & Imaging
THE HONG KONG POLYTECHNIC UNIVERSITY
2010
DECLARATION

The work submitted in this thesis is the result of investigation carried out by the author. The material in this thesis has not been accepted in any substance for any degree, and is not being concurrently submitted in candidature for any degree.

Signed ______________________________

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Michael T.C. Ying

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Abstract of dissertation entitled:

Ultrasound measurement of abdominal fat index for predicting cardiovascular disease

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at The Hong Kong Polytechnic University in December, 2010

ABSTRACT

Haemodialysis patients have a higher risk for different cardiovascular diseases including coronary artery disease and carotid atherosclerosis, which are the leading causes for the morbidity and mortality in these patients. It has been reported that visceral fat accumulation is strongly associated with cardiovascular diseases in the general population. Therefore, increased visceral fat may be a useful indicator of increased risk of cardiovascular diseases. Ultrasound has been found to be useful in the quantification of visceral fat by measuring the subcutaneous (S) and preperitoneal fat thickness (P), and expressing as the abdominal fat index (AFI=P/S). However, a recent study reported that the mesenteric fat thickness had a stronger association with
carotid atherosclerosis when compared to the subcutaneous and preperitoneal fat thickness. Therefore, the purpose of the present study was to establish a new AFI by including the mesenteric fat thickness (M) in the visceral fat quantification. A total of 40 haemodialysis patents were recruited in the present study (27 men; 13 women; mean age = 58.9 years). Each patient had an abdominal ultrasound (US) and computed tomography (CT) examinations. The visceral fat area (V) and subcutaneous fat area (S) were measured in CT, whilst the mesenteric, preperitoneal and subcutaneous fat thicknesses were measured in US. The CT visceral fat assessment was determined by established method (V/S ratio). Each patient also had a cardiac CT examination to evaluate the coronary calcium score. Result showed that in the US visceral fat assessment the AFI calculated by the equation, AFI = (M/S) x (P/S) showed the highest correlation with the V/S ratio from CT (r=0.69, p<0.05) and with the coronary calcium score from CT (r= 0.51, p<0.05). In conclusion, the new AFI = (M/S) x (P/S) may be a useful indicator of the regional fat distribution in the assessment of cardiovascular disease, and may be useful as an alternative method for predicting cardiovascular disease in haemodialysis patients.
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Chapter One

INTRODUCTION

In last two decades, there was a high incidence of coronary artery disease in patients receiving haemodialysis. The progressive coronary atherosclerosis in haemodialysis patients is termed as ‘accelerated cardiovascular disease (Linder et al., 1974). The mortality rate in haemodialysis patient is higher than in the general population (Locatelli et al., 2001). Accelerated cardiovascular disease and increased macrovascular complications are the leading causes for the morbidity and mortality in these patients (Martin et al., 2008).

Coronary calcium scoring of computed tomography is closely correlated with cardiovascular disease and thus is a sensitive marker for detecting atherosclerosis in coronary arteries. It is a quantitative assessment of calcifications detectable by multi-detector computed tomography. Previous studies indicated that coronary calcium scoring can predict cardiac events in asymptomatic patients (Bellasi, et al., 2007, Rumberger et al., 1997).

Obesity is one of the risk factors contributing to the development of atherosclerosis and cardiovascular complications in the general population (Tatsuyuki et al., 2003). The distribution of body fat including abdominal visceral and subcutaneous fat has been reported to be closely related to metabolic disorders and atherosclerosis.
Vague et al. (1956) reported that centralized obesity is more closely associated with atherosclerosis or cardiovascular diseases than peripheral obesity. Matsuzawa et al (1997) also found that the severity of cardiovascular diseases was related to the body fat distribution. More specifically, increased deposition of adipose tissue predominantly in the visceral cavity plays a major role in the development of the metabolic syndrome and cardiovascular disease (Fujioka et al., 1987). Odamaki et al. (1999) found that haemodialysis patients had an excessive visceral fat (VF) accumulation relative to healthy subjects. Thus, excessive VF accumulation in haemodialysis patients may accelerate systemic atherosclerosis, as it does in the general population (Tatsuyuki et al., 2003). Measurement of visceral fat accumulation is therefore important in assessing the susceptibility to cardiovascular disease in haemodialysis patients.

Fujioka et al (1991) reported that the ratio of visceral fat area to subcutaneous fat area (V/S ratio) at the umbilical level, measured by computed tomography, is significantly correlated with the plasma glucose, serum triglyceride, and total cholesterol levels. These findings indicated that specific visceral fat measurement method may be useful to predict the occurrence of cardiovascular disease. Computed tomography has been considered to be an accurate and reliable imaging technique for body fat measurement, particularly for the abdominal adipose tissue (Rossner et al., 1990). However, CT
examination is costly and time-consuming, and involves exposure to ionizing radiation. Therefore, alternative methods have been used to assess fat distribution and to estimate intra-abdominal fat deposition (Kooy et al., 1993). Other studies used ultrasound to measure visceral fat (Armellini et al. 1990; Suzuki et al. 1993). Suzuki et al. (1993) measured the preperitoneal fat thickness (P) and the subcutaneous fat thickness (S) using ultrasound, and defined the abdominal fat index (AFI) as a ratio of preperitoneal fat thickness to the subcutaneous fat thickness (P/S). They found that the AFI is significantly correlated with the V/S ratio as determined by CT (r=0.746, p<0.0001). In addition, it has been reported that the mesenteric fat thickness measured by ultrasound is better correlated with various cardiovascular risk factors when compared to the subcutaneous and preperitoneal fat thickness (Liu et al. 2003). Therefore, inclusion of the mesenteric fat thickness in the calculation of the AFI may allow more accurate evaluation of abdominal fat and predication of cardiovascular diseases.
**Aims**

The aim of the present study is to develop and apply a new abdominal fat index for ultrasound assessment of abdominal fat and for predicting cardiovascular diseases.

**Objectives**

The specific objectives of this study are:

- To develop a new equation for accurate determination of the abdominal fat index by sonographic measurement of the mesenteric, preperitoneal and subcutaneous fat thicknesses.

- To investigate the association between new abdominal fat index and the coronary calcium score measured by computed tomography.

- To investigate the association between the new abdominal fat index and the V/ S ratio measured by computed tomography.
Chapter Two

LITERATURE REVIEW

2.1 Etiology of Cardiovascular disease

2.1.1. Clinical Background of Cardiovascular disease

Cardiovascular disease (CVD) refers to the diseases that affect either the heart or the blood vessel system within a person's body. It is not a single disease but a group of diseases which are related to over 60 cardiac or vascular disorders.

According to the National Center for Complementary and Alternative Medicine, CVD is the top leading cause of death worldwide of men and women, including in the United States. Over 70 million Americans (almost one-fourth of the population) have some forms of CVD, with high blood pressure, heart disease, and stroke being the most common forms. (Wilson et al., 2010) CVD accounts for nearly 40 percent of deaths each year. Coronary artery disease and stroke are the first and third most common causes of death in the United States, respectively (Toth et al., 2007).

According to the statistics from the Department of Health in Hong Kong, CVD is the second leading cause of death in Hong Kong and kills more than 5,000 people a year. Dialysis-dependent renal failure is well recognised to be associated with an excess of vascular death (Parfrey et al., 1999). Thus, a 25-year-old man with renal failure has a
100-fold increased risk of vascular death compared to an age and sex matched control (Parfery et al., 1999). Although the relative risk falls with time, by the age of 80 there is still a 10-fold increased risk of vascular death. It is only a mild exaggeration to state that patients with chronic kidney disease (CKD) rarely die from complications of dialysis, but instead suffer premature death from accelerated vascular disease (Culleton, et al 1999). Little is known about the mechanism by which the CKD increase the risk of CVD. However, the variety of cardiovascular events associated with CKD suggests that there are multiple underlying causes which may include chronic inflammation, erythropoietin deficiency with accompanying anemia, and a generalized metabolic disorder. Evidence clearly shows that CKD is an independent risk factor for CVD.

The pathology of the increased vascular morbidity is multi-factorial, but important factors include arterial calcification, increased arterial stiffness, and LVH (Henry et al., 2002). Coronary artery calcification occurs early in the progression of chronic kidney disease and is strongly associated with cardiovascular death (Goodman et al., 2000). Arterial stiffness increases in both large and small vessels and is associated with premature hypertension. An increased pulse pressure in hypertension is a major risk factor for vascular death (Franklin et al., 1999).
2.1.2 Definition of Atherosclerosis

Arteries are blood vessels that carry blood from the heart to other parts of the body. They are lined by a thin layer of cells called the endothelium. The endothelium works to keep the inside of arteries toned and smooth, which keeps blood flowing (Sanz et al., 2008). Atherosclerosis starts when high blood pressure, smoking, or high cholesterol damages the endothelium (Figure 1) (American Heart Association).

![Figure 1 Development of plaque formation (atherosclerosis)](http://www.mayoclinic.com/health/medical/IM00642)

With the endothelium of an artery being damaged, cholesterol plaque formation begins (Figure 2), and the white blood cells stream in to digest the cholesterol. Over years, the accumulating mess of cholesterol and cells become a plaque in the arterial wall. As the process of atherosclerosis continues, the plaque gets bigger. A big enough plaque can create a blockage of the artery (Sanz et al., 2008).
According to the National Heart, Lung and Blood Institute, plaques from atherosclerosis can also behave in different ways as following:

(i) They can stay within the artery wall when they grow to a certain size and stops. These plaques may never cause any symptoms, as there is no blockage of normal flow.

(ii) They can grow in a slow, controlled way into the path of blood flow and eventually, cause significant blockages with pain on exertion site, e.g. chest or legs.

(iii) In the worst case scenario, the plaques can suddenly rupture, allowing blood to clot inside an artery, causing stroke or heart attack.

Although atherosclerosis is often considered a heart problem, it can also affect arteries in other body regions. The atherosclerotic plaques cause three main kinds of cardiovascular disease (American Heart Association, 2010):
1. Coronary artery disease: Stable plaques in the heart's arteries cause chest pain on exertion. Sudden plaque rupture and clotting causes heart muscle to die. This is a heart attack, or myocardial infarction.

2. Cerebrovascular disease and carotid stenosis: Ruptured plaques in the cerebro-arteries cause strokes, with the potential for permanent brain damage. Temporary blockages in an artery can also cause transient ischemic attacks (TIAs), which are warning signs of stroke; however, there is no brain injury. Plaque deposits can roughen and deform the artery wall, causing blood clots to form and blocking blood flow to the brain (Figure 3A). Plaque deposits can also rupture and break away, traveling downstream to lodge in a smaller artery and block blood flow to the brain (Figure 3B).

3. Peripheral artery disease: Narrowing in the arteries of the legs caused by plaque. Peripheral artery disease causes poor circulation. This causes pain on walking and poor wound healing. Severe disease may lead to amputations.
2.1.3 Definition of Coronary Arteries Disease

Coronary artery disease is the most common form of heart disease. According to National Lung, Heart, and Blood Institute, about 7 million Americans suffer from coronary heart disease and 500,000 Americans die from the disease a year.

Coronary arteries are importance branches of the aorta. They maintain blood flow to the heart muscle; provide nutrients and oxygen to maintain the heart function. When the aorta or its major branches, including the coronary arteries, developed atherosclerosis in the vessel wall, it causes narrowing of the blood vessels (National Lung, Heart, and Blood Institute). CAD is the result of severe narrowing of the coronary arteries, causing insufficient blood supply to the heart muscles.

Narrowed coronary arteries reduced the blood supply to the heart muscles, which causes chest pain, shortness of breath or other CAD symptoms. Complete occlusion of the coronary arteries causes damage to the heart muscles (Toth et al., 2007), which leads to permanent ischemic injury to the heart muscles or myocardial infarction. Without prompt treatment patient may die of heart failure or abnormal heart rhythm (National Lung, Heart, and Blood Institute).

In CAD, fatty deposits and cell-proliferation build-up are found in the coronary arteries (Figure 4).
Once the inner wall of an artery is damaged due to high blood pressure, hardening of endothelium, or high cholesterol damage, fatty deposits (plaques) made of cholesterol and other cellular waste products tend to accumulate at the site of injury in a process called atherosclerosis (Morrow et al., 2007). If the surface of these plaques breaks or ruptures, platelets will clump at the site to try to repair the artery. This clump of platelets can block the artery, leading to a heart attack (Toth et al., 2007). A blood clot can also be detached from the plaque and travel to other parts of the body causing partial or complete blockage of blood flow to another organ.

In many patients, the first sign of CAD may already be myocardial infarction or sudden death, with no preceding chest pain as a warning (National Lung, Heart, and
Blood institute). It is important to perform screening tests to detect signs of CAD before serious medical events occur, and the tests should be designed to detect plaque before a coronary artery becomes completely blocked. Screening tests are of particular importance for patients with risk factors for CAD. These risk factors include a family history of CAD at relatively young ages, an abnormal serum cholesterol profile, cigarette smoking, elevated blood pressure (hypertension), obesity, chronic kidney disease and diabetes mellitus (Toth et al., 2007).

2.1.4 Common initial screening tests for coronary artery disease

*Electrocardiogram (ECG)*

ECG is a common initial investigation of coronary artery disease. An electrocardiogram records electrical signals as they travel through the heart (Hall et al., 2010). The test for detecting CAD include resting and exercise ECGs, which can provide evidence of previous silent myocardial infarctions and silent or inducible myocardial ischemia (Yanowitz et al., 2008). Several resting ECG findings (ST depression, T-wave inversion, Q waves, and left axis deviation) increase the likelihood of coronary atherosclerosis and of future coronary events (Yanowitz et al., 2008).
**Echocardiogram.**

An echocardiogram uses ultrasound to produce images of heart. During the scans, the pumping activity of cardiac muscle can be investigated. Regions of the heart that move weakly may indicate damages of the heart muscle due to previous heart attack or limited blood supply, and is a useful indicator of coronary artery disease (Sharma, 2010).

**Blood test (Cholesterol test).**

Cholesterol cannot dissolve in the blood. It has to be transported to and from the cells by carriers called lipoproteins. Low-density lipoprotein, or LDL, is known as “bad” cholesterol. High-density lipoprotein, or HDL, is known as “good” cholesterol. These two types of lipids, along with triglycerides, make up total cholesterol count, which can be determined through a blood test (National Lung, Heart, and Blood Institute). A cholesterol test, measuring the fats (lipids) in blood, can indicate risk of having a heart attack or other heart disease. The test typically includes measurements of:

1. **Total cholesterol**

The normal level of total cholesterol should be below 200 milligrams per deciliter (mg/dL), or below 5.2 millimoles/liter (mmol/L) (National Lung, Heart, and Blood Institute).
Low-density lipoprotein (LDL) cholesterol

LDL may cause the accumulation of fatty deposits within arteries, which reduces blood flow. Fatty deposition in the arteries can cause atherosclerotic plaque which can narrow the arteries and make them less flexible. This condition is known as atherosclerosis. If a plaque forms and blocks a narrowed artery, heart attack or stroke may result due to lack of blood supply. A plaque is made up of two major parts including a lipid core and an overlying fibrous cap in which having more of a fibrous cap and less of a lipid core can keep the plaque stable and prevents it from breaking off (National Lung, Heart, and Blood Institute). However, in case of the plaque with more lipid component and less of a fibrous tissue, it is more likely to rupture. This may cause the formation of clot which can further impede the blood flow, leading to a heart attack or stroke (National Lung, Heart, and Blood Institute).

The normal LDL cholesterol level should be less than 130 mg/dL or 3.4 mmol/L (National Lung, Heart, and Blood Institute).

**Computed tomography (CT)**

Coronary calcium scoring of computed tomography is closely correlated with cardiovascular disease and thus is a sensitive marker of detecting atherosclerotic plaques in coronary artery. It is a quantitative assessment of calcification within the
coronary arteries. Not only for the symptomatic patients, previous studies have indicated that the coronary calcium scoring can predict cardiac events in asymptomatic patients (Bellasi, et al., 2007, Rumberger et al., 1997).

Besides, computed tomography angiogram (CTA) is also a useful imaging method for the diagnosis of heart diseases, and is becoming a popular diagnostic tool for patients who have a moderate risk of artery stenosis (Gerber et al., 2008). According to the Gerber (2009) MDCT had a higher success rate (100% versus 88%, P<0.001) and enabled identification of more segments (963 versus 726, P<0.001) than did magnetic resonance coronary angiography. The examination procedure of CTA is less invasive than conventional angiograms, and thus has a shorter patient recovery time.

*Positron emission tomography (PET) scan*

A positron emission tomography (PET) scan is an imaging tool that reveals tissue and organ function, by administering a small amount of radioactive agent into the body (Driver et al., 2009).

Noninvasive quantification of absolute myocardial blood flow by PET allows the detection of abnormal vasodilatory response to intravenous adenosine in patients with family history of CAD and high-risk lipid profiles. Early assessment of alterations of vascular reactivity to adenosine in relation to high-risk lipid profiles in asymptomatic
patient may allow early detection of preclinical atherosclerosis and may initiate modification and/or elimination of risk factors that may slow, retard, or even reverse the progression of CAD (Dayanikli F et al., 1994).

**Coronary angiography**

The "gold standard" for the evaluation of coronary artery disease remains the coronary angiogram. Coronary angiography can be used to identify the location and severity of coronary artery disease. During a coronary angiographic examination, a small catheter (a thin hollow tube with a diameter of 2-3 mm) is inserted through the skin into an artery usually in either the groin or the arm. Guided with the fluoroscopy, the catheter is then advanced to the opening of the coronary arteries, and a small amount of radiographic contrast is injected into the coronary arteries (Gerber et al., 2009). However, the catheter has to be inserted into artery through a patient's groin which is an invasive procedure and may pose some risk for complications, e.g. arterio-dissection. Besides, those catheters used in coronary angiogram are quite costly.
2.2 Clinical Evaluation of Abdominal Fat

2.2.1 Anthropometric Measurement

Clinical anthropometric measurements such as waist circumference (WC), waist to hip ratio (WHR) and Body mass index (BMI), are simple and convenient methods to assess regional adiposity. Some of these surrogate markers are well correlated with the adiposity measurements using magnetic resonance imaging (MRI) and CT (Lapidus et al., 1984; Despres et al., 1989). It has been reported that increased regional adiposity increased the risk of cardiovascular disease (Lapidus et al., 1984; Despres et al., 1989).

BMI is calculated as the body weight divided by the height squared. Waist circumference is measured in orthostatic position at the midpoint between the lateral iliac crest and lowest rib, and hip circumference is measured at the level of the greater trochanter of femur. Because of the simple procedure, low cost and acceptable accuracy in assessing regional adiposity, anthropometric measurements have been used for initial risk assessment of cardiovascular disease (Bray et al., 1988; Han et al., 1995).

Hsieh et al., (1995) reported that the waist/height ratio may be a better predictor of multiple coronary disease risk factors such as blood pressure, fasting plasma glucose, haemoglobin A, TG, TC and HDL-C than BMI or the waist/Hip ratio. In Fenando’s
study (2003), waist circumference showed the best correlation with CT-determined visceral fat area among different anthropometric measurement methods ($r=0.55$, $p < 0.001$). Although anthropometric methods have been used for risk assessment of cardiovascular disease, they are not sensitive enough for accurate assessment, particularly in non-obese individuals (Hsieh et al., 1995). Various other medical imaging methods have been found to be useful in the assessment of visceral fat, which aids the risk assessment of cardiovascular disease.

2.2.2 Computed Tomography

Vague et al., (1956) reported that the centralized obesity is more closely associated with cardiovascular disease. On the other hands, Fujioka et al., (1987) also reported that the ratio of visceral fat area to subcutaneous fat area (V/S ratio) at the umbilical level (L4 level), estimated by computed tomography, is significantly correlated with the plasma glucose, serum triglyceride, and total cholesterol levels and is a useful indicator for predicting cardiovascular disease.

Computed tomography used to be the standard method in the quantification of abdominal adiposity (Rossner et al., 1990). There are two types of abdominal obesity quantification: visceral fat obesity characterized by a marked accumulation of fat in the abdominal cavity; and subcutaneous fat obesity characterized by fat accumulation
mainly in the subcutaneous tissue (Tokunaga, 1983). In CT scans, abdominal obesity is usually quantified by measuring the visceral fat area (VFA) and subcutaneous fat area (SFA) (Rossner et al., 1990). Some of the previous reports suggested that the SFA was better than VFA in correlation with the insulin resistances which is one of factors causing atherosclerosis in the general population (Abate et al., 1996; Kelly et al., 2000).

Although computed tomography has been considered the most accurate, and reproducible technique of body fat measurement (Rossner et al., 1990), particularly abdominal adipose tissue, CT scans are costly, time-consuming and involve exposure to ionizing radiation (Kooy et al., 1993).

2.2.3 Ultrasonography

The use of ultrasonography in the assessment of intra-abdominal fat was initiated by Armellini et al. (1990), and the visceral and subcutaneous fats were measured. In ultrasound examinations, the subcutaneous fat thickness was defined as the distance between the skin and external surface of the rectus abdominal muscle, and the visceral fat thickness was defined as the distance between the internal surface of the same muscle and the anterior wall of the aorta (Figure 5) (Armellini et al., 1990). Armellini et al. (1990) reported that the intra-abdominal thickness measured by ultrasonography
was well correlated with the visceral fat area measured by CT ($r = 0.669$, $p < 0.001$),

Fenando et al (2003) applied the same ultrasound scanning protocol as described by Armellini et al. (1990) on the measurement of the visceral and subcutaneous fat thickness, and found that there was a moderate correlation ($r = 0.47$, $p$ less than 0.001) between ultrasound and CT on measuring the visceral/subcutaneous fat ratio.

\[ \text{AFI} = \frac{P_{\text{max}}}{S_{\text{min}}} \]

Besides of the measurement of visceral fat, ultrasound was also used to measure the preperitoneal and subcutaneous fat thickness (Suzuki R et al., 1993). The ultrasonographically determined abdominal fat index (AFI), which is defined as the ratio of the maximum thickness of preperitoneal fat thickness ($P_{\text{max}}$) to the minimum thickness of subcutaneous fat thickness ($S_{\text{min}}$) (Figure 6), was closely correlated with
the visceral to subcutaneous fat thickness ratio \( r = 0.746, p < 0.0001 \) (Suzuki et al., 1993). Suzuki et al. (1993) also noted that ultrasonographically determined AFI in non-obese men is highly correlated to the occurrence of carotid atherosclerosis.

![Ultrasound of the distribution of fat in the abdominal wall of the upper median abdomen. (Suzuki et al. 1993)](image)

Kawamoto et al. (2002) reported that the preperitoneal and subcutaneous fat thickness measured by ultrasound and expressed as AFI can reflect the body fat distribution accurately, and be useful in evaluating metabolism disorders and atherosclerosis. They suggested that AFI determined by ultrasound was correlated to the systolic blood pressure, diastolic blood pressure and triglycerides (TG), and was an independent risk factor for carotid atherosclerosis.

Figure 6 Ultrasound of the distribution of fat in the abdominal wall of the upper median abdomen. (Suzuki et al. 1993)

Kawamoto et al. (2002) reported that the preperitoneal and subcutaneous fat thickness measured by ultrasound and expressed as AFI can reflect the body fat distribution accurately, and be useful in evaluating metabolism disorders and atherosclerosis. They suggested that AFI determined by ultrasound was correlated to the systolic blood pressure, diastolic blood pressure and triglycerides (TG), and was an independent risk factor for carotid atherosclerosis.
Leite et al. (2002) applied the intra-abdominal thickness measured by ultrasonography (IATU) and correlated with cardiovascular risk factors. IATU was defined as the distance between the internal surface of abdominal muscles and posterior wall of the aorta measured by ultrasound. They found that an IATU greater than 9 cm predicted high risk for CVD [OR = 5.55 (95% CI, 2.32 to 13.28)] in men and greater than 8 cm in women [OR = 3.27 (95% CI, 1.63 to 6.56)].

Kawasaki et al. (2008) applied a novel method for the measurement of abdominal fat which contained the component of para- and perirenal fat thickness. The thickness of the para- and perirenal fats was measured from the inner side of the abdominal musculature to the surface of the kidney on ultrasound images and designated ultrasound measure (UM). The average of the UM value on both sides was defined as ultrasound fat thickness (UFT) (Figure 7). There was a high correlation (r=0.75; p>0.0001) between the UFT and the CT intra-abdominal fat area (Kawasaki et al., 2008).

The equation of UFT is as followed:

\[
UFT = \frac{(UM \ in \ right \ side + UM \ in \ left \ side)}{2}
\]

where UM defined as component of para- and perirenal fat thickness
In addition, Liu et al. (2005) measured the mesenteric, preperitoneal and subcutaneous fat thicknesses, and compared their association with carotid atherosclerosis using carotid IMT as a surrogate marker. They found that the mesenteric fat layers showed significant association with the carotid IMT which is independent of preperitoneal and subcutaneous fat thickness. Liu et al. (2005) suggested that measurement of mesenteric fat thickness is a useful indicator of the regional fat distribution in the assessment of cardiovascular risk. In Liu’s study, there are some limitations. Firstly, only the value of individual fat thickness in the assessment of cardiovascular risk was assessed, but the value of a combination of those three fat thicknesses in the assessment of cardiovascular risk was not evaluated. Besides, the carotid IMT was used as a surrogate marker rather than direct assessment of the coronary artery in Liu’s
study.

Figure 8 Ultrasonogram of mesenteric leaves. The mesenteric appeared elongated structures with high reflecting peritoneal surfaces (arrows). The maximum mesenteric thickness on the image was measured with the calipers (+) (Liu et al., 2005)

To the best of our knowledge, literature is devoid of any comprehensive study to investigate the value of different abdominal fat thicknesses measured by ultrasound in the prediction of cardiovascular disease. Therefore, the present study aims to develop and apply a new AFI by measuring the three abdominal fat layers (i.e. mesenteric, preperitoneal and subcutaneous fat thicknesses) using ultrasonography for predicting the risk of cardiovascular disease.
Chapter Three

MATERIALS AND METHODS

3.1 Study Design

A prospective study of sonographic measurement of different abdominal fat thickness in haemodialysis patient was undertaken. B-mode ultrasound examination was performed to measure three different abdominal fat thicknesses: mesenteric fat thickness, preperitoneal fat thickness and subcutaneous fat thickness. The ultrasound findings were compared to the ratio of visceral fat area to subcutaneous fat area (V/S ratio) and the coronary calcium scoring obtained from the computed tomography, in order to investigate the correlation between different abdominal fat thickness and calcium score or V/S ratio from CT in predicting cardiovascular disease.

3.2 Study Population

A total of 40 patients who were classified as stage 5D of chronic renal disease in the Department of Medicine of Queen Mary Hospital were recruited from January 2010 to August 2010. All recruited patients were maintained on regular haemodialysis three times a week for 4-4.5 hours/day using hollow-fibre dialysers with a bicarbonate-buffered dialysate. All recruited patients had performed a cardiac computed tomography examination for another research project. The findings of the
CT scan were retrospectively reviewed for comparison with the findings obtained in the ultrasound examination of the present study. Both ultrasound and computed tomography examinations performed for each patient were within a maximum time interval of 1 month between the two examinations. Informed consent (Appendix I) was obtained from all recruited patients before the study.

Human Subjects Ethics approval of this prospective study had been sought from the Institutional Review Board of the University of Hong Kong/ Hospital Authority Hong Kong West Cluster, and the Human Subjects Ethics Subcommittee of the Hong Kong Polytechnic University.

3.3 Equipment

*Ultrasound examination (prospective)*

All ultrasound examinations were conducted using a Philips UH22 ultrasound unit in conjunction with a 5-12 MHz linear transducer and a 1-5 MHz curvilinear transducer (Philips Medical System, Bothell WA, U.S.A.).
Multi-slice Computed Tomography (Retrospective)

Protocols of coronary calcium scoring and abdominal fat areas evaluation, including plain thorax and L4-5 level of abdomen, were performed using a 16-slice multi-detector CT scanner (General Electric LightSpeed VCT XT, USA).

Figure 10 shown the 16-slice multi-detector CT scanner (General Electric LightSpeed)

3.4 Computed Tomography

3.4.1. Coronary calcium Scoring (CT)

In the thoracic CT examination, all scans were obtained with 120 kV, 300 mAs, and 0.5s tube rotation speed, with prospective ECG triggering at 45% and 75% of the R-R interval. For the scanning of the heart, scans were obtained from the level of carina to the base of the heart. Images were acquired using conventional axial scanning, with 8 x 2.5mm slices acquired at one time. The total number of slices acquired was about 48 for one patient for the heart. The image acquisition requires approximately 10-second breath hold to completely interrogate the heart.
CT scanning data was electronically transmitted to a workstation, and was analyzed using the Sure Plaque software (Advantage Windows, Version 4.2, GE Healthcare). In the image analysis, the Agatston scores of the plaque were determined from the CT images that encompassed the entire coronary arteries. According to the Agatston’s method (Agatston et al., 1990), the image pixels greater than 130 Hounsfield units (HU) were classified as plaques in the coronary arteries. Depending on the peak density of the plaque, an area of at least 0.52 mm$^2$ (i.e. 2 pixels) was multiplied by one of the following cofactors: a factor of one for peak densities ranged 130-199 HU, a factor of two for 200-299 HU, a factor of three for 300-399 HU and a factor of 4 for peak densities greater than 400 HU. The total coronary artery calcium score was calculated as the sum of the individual lesion scores in all coronary arteries (Agatston et al., 1990).

3.4.2. Abdominal Fat Area (CT)

Abdominal CT scan was performed between the level of the 4$^{th}$ and 5$^{th}$ lumbar vertebrae with the patient in supine position, and a total of three slices of 5mm in thickness are taken during suspended inspiration. The subcutaneous fat area and visceral fat area of each patient were determined from the image at the level of the umbilicus which is equivalent to L4 level. Subcutaneous fat is defined as the
extra-peritoneal fat between the skin and muscles, with attenuation ranging from -140 to -40HU. The intra-peritoneal fat with the same image density as the subcutaneous fat tissue is defined as visceral fat. The subcutaneous fat area (SFA) (Figure 11A) and visceral fat area (VFA) (Figure 11B) were determined by the automatic planimetry performed by the inbuilt software (Agastston et al., 1990).

*Figure 11a shown the definition of subcutaneous fat area (SFA)*

*Figure 11 b show the definition for the visceral fat area (VFA)*
3.4.3 Waist Circumference

In the CT scans of the patient, the waist circumference of the patient was measured at the umbilicus level (L-4 level) using the electronic calipers of the CT image processing workstation.

![Image showing waist circumference measurement](image)

*Figure 12 shows the measurement of waist circumference at the umbilicus (L-4 level)*

3.5 Ultrasound

*Abdominal Fat Thickness (US)*

In the abdominal ultrasound examination, the mesenteric, preperitoneal and subcutaneous fat thicknesses were measured (Liu et al., 2003; Liu et al., 2005). Patients lay supine on the examination table. The mesenteric fat was assessed transversely and longitudinally at the para-umbilical level using the 1-5 MHz curvilinear transducer. The mesenteric fat leaves appeared as elongated structures with highly reflective peritoneal surfaces, and they were divided from each other by the specular echoes corresponding to their peritoneal surfaces (Derchi et al., 1987). The
mesenteries did not demonstrate peristalsis, in contrast to the small bowel loops attached to the distal end (Derch et al., 1987). Not all the mesenteric fat could be visualized on ultrasound as some of them were obscured by bowel gas. The thickness of all ultrasound visible mesenteric leaves were measured directly on the screen using electronic calipers, and the mean of the three thickest mesenteric fat layers (M mean) was calculated and used for the analysis of the study.

Figure 13 show the measurement of mesenteric fat thickness

The preperitoneal and subcutaneous fat thicknesses were measured using a 5-12 MHz linear transducer, because they were superficially located. The transducer was kept perpendicular to the skin surface of the upper abdomen, and longitudinal scans were performed from the xiphoid process to the navel along the linea alba. Patient was asked to hold the breath in arrest inspiration to keep the surface of the liver parallel to
the skin. The thickness of subcutaneous and preperitoneal fat was measured on the screen using electronic calipers. The maximum thickness of preperitoneal fat (Pmax) and the minimum thickness of subcutaneous fat (Smin) was identified and measured. For the Pmax and Smin, three measurements were made and the average value was calculated. In order to avoid the influence of respiratory status or abdominal wall tension, all measurements were obtained with the patient at arrested inspiration and with relaxation in abdomen.

Figure 14 show the measurement of minimum value of subcutaneous fat thickness and maximum value of preperitoneal fat thickness
3.6 Data Analysis

Data of quantitative variables was presented as mean +/- standard deviation and range of the data.

3.6.1 Derivation of new abdominal fat index

Ultrasound measurements of the 40 haemodialysis patients (27 men, 13 women, age range 36 to 81, mean age =58.9) were used to derive the new abdominal fat index equation. In each patient, measurement of the mesenteric fat thickness, preperitoneal fat thickness and subcutaneous fat thickness were included. Previous study found that patients with thickened subcutaneous fat tended to have thicker subcutaneous fat in the upper abdominal wall, and patients with more visceral fat tended to have thickened preperitoneal fat (Suzuki et al., 1993). Therefore, the abdominal fat index (AFI) was previously expressed as (AFI = Pmax / S min), which showed a high correlation (r=0.75, p<0.0001) with the V/S ratio as determined by CT (Suzuki et al., 1993).

The conventional AFI was expressed as AFI = Pmax / S min (Suzuki et al., 1993). As the mesenteric fat thickness was found to have a higher association with the atherosclerosis than preperitoneal fat thickness (Liu et al., 2005), the mesenteric fat thickness was incorporated in the AFI, and three different AFI were investigated in the
present study:

1. Conventional AFI = Maximum preperitoneal Fat thickness / Minimum
   subcutaneous Fat thickness [AFI = Pmax / Smin]

2. Revised AFI-1 (AFI₁) = Mean mesenteric Fat thickness / Minimum
   subcutaneous Fat thickness [AFI = Mmean / Smin]

3. Revised AFI-2 (AFI₂) = AFI * AFI₁ = (Maximum preperitoneal Fat thickness/
   Minimum subcutaneous Fat thickness)*( Mean mesenteric fat thickness/
   Minimum subcutaneous Fat thickness), \{(Pmax / Smin) * (Mmean/ Smin)\}

The correlation of the three AFI with the V/S ratio and waist circumference was
 calculated by Pearson correlation test, whilst the correlation of the three AFI with the
coronal calcium scoring was evaluated by Spearman correlation test (GraphPad
InStat, GraphPad Software Inc., Chicago, IL, US, and Microsoft Excel 2002,
Microsoft Crop., Redmond, WA, USA).
3.6.2 Grading for the coronary calcium score

The coronary calcium scoring from CT was graded according to the method as described by Agatston et al. (1990) (Table 1): Table 1. Grading for the coronary calcium scoring (Agatston et al., 1990).

<table>
<thead>
<tr>
<th>Actual Calcium Score</th>
<th>Potential risk for cardiovascular disease</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-0</td>
<td>No identifiable atherosclerotic plaque</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Very low cardiovascular (CVD) risk</td>
<td></td>
</tr>
<tr>
<td>1-10</td>
<td>Minimal plaque burden</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Low CVD risk</td>
<td></td>
</tr>
<tr>
<td>11-100</td>
<td>Mild plaque burden</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Moderate CVD risk</td>
<td></td>
</tr>
<tr>
<td>101-400</td>
<td>Moderate plaque burden</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>High CVD risk</td>
<td></td>
</tr>
<tr>
<td>Greater than 401</td>
<td>Extensive plaque burden</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Very high CVD risk</td>
<td></td>
</tr>
</tbody>
</table>

P-value of less than 0.05 was considered significant. All statistical analyses were performed with GraphPad InStat, GraphPad Software Inc., Chicago, IL, US,
Chapter Four

RESULTS

In the 40 haemodialysis patient examined, the mean mesenteric fat thickness was 0.81 cm (standard deviation = 0.27 cm; range = 0.33 to 1.49 cm), the mean preperitoneal fat thickness was 1.43 cm (standard deviation = 0.50 cm; range = 0.73 to 2.47 cm), and the mean subcutaneous fat thickness was 0.76 cm (standard deviation = 0.22 cm; range = 0.31 to 1.32 cm).

Abdominal fat index Vs V/S ratio (CT)

Retrospective data of the visceral fat area (V) and subcutaneous fat area (S) from computed tomography were also obtained. The mean visceral fat area was 12162.45 mm$^2$ (standard deviation = 6058.4 mm$^2$; range = 2114 to 27319 mm$^2$), the mean subcutaneous fat area was 14006.9 mm$^2$ (standard deviation = 7556.3 mm$^2$; range = 2715 to 33423 mm$^2$). The mean V/S ratio was 0.88 (standard deviation = 0.38; range = 0.05 to 1.68).

Figures 15 to 17 show the correlation of the three AFI with the V/S ratio. Results showed that the revised AFI-2 (i.e. $\text{AFI} = (\text{Pmax}/\text{Smin}) \times (\text{Mmean}/\text{Smin})$) had a higher correlation coefficient with the V/S ratio (Pearson correlation, $r = 0.69$, $p<0.05$) than
the conventional AFI (i.e. AFI = Pmax / Smin) and the revised AFI-1 (i.e. AFI = Mmean / Smin) (Pearson correlation, r = 0.55 and 0.52 respectively, p < 0.05 for both).

Figure 15 Linear correlation of the V/S ratio obtained from CT and the conventional AFI (AFI = Pmax / Smin). The Pearson correlation coefficient, r = 0.55, p < 0.05)
Figure 16 Linear regression analysis between V/S ratio from CT and the revised AFI-1 (Mmean / Smin). The correlation coefficient, $r = 0.52$, $p < 0.05$.

Figure 17 Linear regression analysis between V/S ratio from CT and the revised AFI-2 ($(P_{max}/S_{min})*(M_{mean}/S_{min})$) in the 40 subjects. The correlation coefficient, $r=0.69$, $p < 0.05$. 

$$AFI= \frac{M_{mean}}{S_{min}}$$

$$AFI= \frac{P_{max}}{S_{min}} * \frac{M_{mean}}{S_{min}}$$
Abdominal Fat index Vs Calcium scoring from CT

In the retrospective data from CT, the mean coronary calcium scoring of the 40 haemodialysis patients was 1830.37 (standard deviation = 2790; range = 0 to 13486). Figures 18 to 20 show the correlation of the three AFI with the coronary calcium scoring. Results showed that the revised AFI-2 had a higher correlation coefficient with the coronary calcium scoring (Spearman correlation, $r = 0.51$, $p<0.05$) than the conventional AFI (Spearman correlation, $r = 0.38$, $p < 0.05$) and the revised AFI-1 (Spearman correlation, $r = 0.48$, $p < 0.05$).

\[
\text{AFI} = \frac{P_{\text{max}}}{S_{\text{min}}}
\]

\[r = 0.38, \ p < 0.05\]

*Figure 18 Linear correlation of the coronary calcium score grade obtained from CT and the conventional AFI ($\text{AFI} = \frac{P_{\text{max}}}{S_{\text{min}}}$). The Spearman correlation coefficient, $r = 0.38$, $p < 0.05$.\]
Figure 19 Non-parametric correlation analysis between calcium score grade from CT and the revised AFI-1 \((AFI = \frac{M \text{ mean}}{S \text{ min}})\) among 40 haemodialysis patients. The Spearman coefficient, \(r = 0.48, p < 0.05\).

Figure 20 Non-parametric correlation analysis between calcium score grade from CT and the revised AFI-2 \(= (P \text{ max}/S \text{ min}) \times (M \text{ mean}/S \text{ min})\) among 40 haemodialysis patients. The Spearman coefficient, \(r = 0.51, p < 0.05\).
**Abdominal Fat index Vs Waist Circumference**

In the retrospective data from CT, the mean waist circumference (WC) of the 40 haemodialysis patients was 79.37 cm (standard deviation = 7.09 cm; range = 63 to 93.5 cm). Figures 21 to 23 show the correlation of the three AFI with the waist circumference of the patients. Results showed that the revised AFI-2 had a higher correlation coefficient with the waist circumference of the patients (Pearson correlation, \( r = 0.45, p < 0.05 \)) than the conventional AFI (Pearson correlation, \( r = 0.32, p < 0.05 \)) and the revised AFI-1 (Pearson correlation, \( r = 0.42, p < 0.05 \)).

\[ \text{AFI} = \frac{P_{\text{max}}}{S_{\text{min}}} \]

\( r = 0.32, p<0.05 \)

*Figure 21 Linear correlation of the waist circumference of the patients and the conventional AFI (AFI = \( P_{\text{max}} / S_{\text{min}} \)). The Pearson correlation coefficient, \( r = 0.32, p<0.05 \)
Figure 22 Non-parametric correlation analysis between Waist Circumference (CT) and the product of abdominal fat index \( AFI = \frac{M\text{ mean}}{S\text{ min}} \) among 40 haemodialysis patients. The correlation coefficient, \( r = 0.42, p<0.05 \)

Figure 23 Non-parametric correlation analysis between Waist Circumference (CT) and the product of abdominal fat index \( AFI = \frac{(P\text{ max}/S\text{ min}) \times (M\text{ mean}/S\text{ min})}{(M\text{ mean}/S\text{ min})} \) among 40 haemodialysis patients. The correlation coefficient, \( r = 0.45, p<0.05 \)
Chapter Five

DISCUSSION

5.1 Correlation between V/S ratio and Abdominal Fat Index

Obesity is a major risk factor for metabolic and cardiovascular diseases. However, not all overweight people carry the same health risk. Central fat has been a high risk factor in cardiovascular disease in general population compared with peripheral fat (Tatsuyuki et al., 2003). Abdominal fat can be subdivided into visceral and subcutaneous fat. Central fat, especially central visceral fat, is strongly associated with different diseases, such as type 2 diabetes and cardiovascular disease (Despres et al., 2001). The use of ultrasonography in the assessment of intra-abdominal fat was initially proposed by Armellini et al. (1990), and the visceral and subcutaneous fats were evaluated. The abdominal fat index (AFI) had been established by Suzuki et al. in 1993, and they found that an AFI which expressed as the ratio of the maximum thickness of preperitoneal fat to the minimum thickness of subcutaneous fat was mostly correlated with the V/S ratio obtained from CT (r=0.746, p<0.0001). Based on the result from Suziki et al. (1993), Kawamoto et al. (2002) also demonstrated that the preperitoneal and subcutaneous fat thickness measured by ultrasound and expressed as AFI can reflect the body fat distribution more accurate and be useful in evaluating
disorders of metabolism and atherosclerosis. In the present study, there was a moderate correlation between the conventional AFI and the V/S ratio \((r = 0.55, p < 0.05)\) which was lower than that reported in Suzuki et al. (1993). The lower correlation may be due to different setting of the study. When comparing with Suzuki’s study, the image quality for either CT or US images should be upgraded as more advanced technology is used in present study.

Liu et al. (2005) compared the mesenteric fat thickness with the preperitoneal and subcutaneous fat thickness on the association with atherosclerosis using carotid IMT as a surrogate marker. They reported that the mesenteric fat layers measured by ultrasound showed the highest association with the carotid IMT which was independent of the preperitoneal and subcutaneous fat thickness. They suggested that measurement of mesenteric fat thickness is a useful indicator of the regional fat distribution in the assessment of cardiovascular risks (Liu et al., 2005). In the present study, we modified the conventional AFI by incorporating the mesenteric fat thickness in the equation, and devised two new AFI. When the mesenteric fat thickness replaced the preperitoneal fat thickness in the AFI evaluation (i.e. AFI-1), the correlation between the AFI and V/S ratio \((r = 0.52, p < 0.05)\) was similar to that between the conventional AFI and V/S ratio \((r = 0.55, p < 0.05)\). However, when the mesenteric fat thickness was added in the conventional AFI, the correlation with V/S ration was
markedly improved ($r = 0.69$, $p < 0.05$). This significant improvement may be due to the different characteristic of mesenteric fat when comparing with preperitoneal and subcutaneous fat. Mesenteric fat is a specific type of portal adipose tissue while preperitoneal and subcutaneous fat depots are regarded as non-portal adipose tissue. Portal adipose tissue has a higher lipolytic activity and lower response to anti-lipolytic effect of insulin than non-portal adipose tissue, resulting in high rate of Free Fat Acid (FFA) production (Despres et al., 1997). The high concentration of FFA in portal circulation further inhibits the hepatic clearance of insulin, resulting in hyperinsulinaemia, insulin resistance, and in predisposed individuals, diabetes mellitus, hypertension and dyslipidaemia (Defronzo et al., 1991), all of these can lead to atherosclerosis (Yamamoto et al., 1997).

Besides, mesenteric fat is also a kind of visceral fat. Inclusion of the mesenteric fat in the equation of AFI could better represent the visceral fat content and thus result in a higher correlation with the V/S ratio.

### 5.2 Correlation between coronary calcium Score and Abdominal Fat Index

According to Miguel et al. (2010), coronary calcium score showed high accuracy for the diagnosis of $> 50\%$ and $> 70\%$ coronary artery stenosis, with the area under the ROC curve of 0.75 and 0.70 respectively. Coronary calcium score proved to have a
good diagnostic and prognostic performance for cardiovascular events evaluation in chronic renal failure (CRF) patients (Miguel et al., 2010). In the present study, the AFI-2 showed a high correlation with the coronary calcium score ($r = 0.51$, $p<0.05$), whilst the AFI-1 and original AFI had lower correlation with the coronary calcium score ($r = 0.48$ and $0.38$ respectively, $p < 0.05$ for both). Similar to the situation when comparing the correlation between different AFI with V/S ratio, a higher correlation with coronary calcium score have been noted with the inclusion of mesenteric fat in the equation of AFI-1 and AFI-2. The inclusion of mesenteric fat into the origin AFI could better represent the visceral fat content as mesenteric fat is also a kind of visceral fat.

5.3 Correlation between Waist Circumference and Abdominal Fat index

Waist circumference has been the most commonly used anthropometric parameter for the evaluation of abdominal obesity (Berker et al., 2010). Convenience and cost-effectiveness of this method have resulted in its inclusion in several guidelines for determining cardiovascular risk, particularly for metabolic syndrome. It has been reported that the WC had a moderate to high correlation with the intraperitoneal adipose tissue mass (ATM) and posterior subcutaneous abdominal ATM ($r = 0.669$ and $0.856$ respectively) (Chan et al., 2002). In contrast to the result in Chan et al.
(2002), the present study found a lower correlation between WC and AFI \((r = 0.32 \text{ to } 0.45)\). The relative lower correlation of WC and AFI, when compared to the correlation of WC and ATM, may be due to different method used for measuring WC in present study. We obtained the WC for the subject by selecting the CT abdominal image at L-4 level and measuring the WC automatically by the build-in software. However, the usual practical of measurement of WC between men and women is different. According to Friedl (2004), the military-adopted sex-specific abdominal girth measurement sites, with men measured at the level of the navel and women measured at the thinnest point between the ribs and the suprailiac crest. This WC measurement site can be any of multiple possibilities between the lowest rib margin and the top of the iliac crest.

Nevertheless, among the three AFI studied in the present study, the AFI-2 \((r = 0.45)\) had a higher correlation with WC than the conventional AFI \((r = 0.32)\) and AFI-1 \((r = 0.42)\). The higher correlation of AFI-2 with the WC may be related to the fact that the addition of mesenteric fat with the preperitoneal fat could be represent the visceral fat content more completely.
5.4 Limitations and further studies

5.4.1 Ultrasound measurement of abdominal fat thickness

Of the 40 patients included in the present study, the ultrasound examination for the evaluation of different abdominal fat thickness was performed by the same sonographer, and thus the inter-operator variability of the measurement was eliminated. However, due to the limited time for data collection, repeated ultrasound examinations were not arranged and intra-operator variability of the abdominal fat measurement was not evaluated.

During the abdominal ultrasound examination, compression by the transducer was maintained to minimal, though the effect of transducer compression on ultrasound measurement of abdominal fat thickness is still unclear. In 2 cases, the presence of excessive bowel gas hindered the visualization of the mesenteric fat layers. Instead of applying compression on the abdomen to squeeze the bowel gas away from the ultrasound field of view, the patients were asked to walk around for a while and then come back for the re-scan. Although the ultrasound measurements could be obtained with this technique, the examination time of the patient was increased. Therefore, further studies are needed to evaluate whether common ultrasound scanning techniques such as transducer compression and change of patient position affect the measurement of abdominal fat thickness.
5.4.2 Coronary calcium scoring

Coronary calcium score obtained from coronary CT provides an accurate and reproducible method for the quantitative assessment of total plaque and calcified plaque areas. However, the method is less accurate for the quantification of non-calcified plaque area and lipid core size, which is ascribed to the limited spatial and contrast resolution of the images. With the present technique, the detection of vulnerable plaques by multi-detector computed tomography (MDCT) remains uncertain (Knollmann et al., 2008). The MDCT detected total plaque area ($r = 0.73, p < 0.0001$) and calcified plaque area ($r = 0.83, p < 0.0001$) correlated well with the histopathology finding, whereas of the correlation of non-calcified plaque area ($r = 0.53, p < 0.0001$) and lipid core size ($r = 0.43, p < 0.0001$) with the histopathology finding was lower. Moreover, MDCT may overestimate the total plaque area and calcified plaque area, whilst it may underestimate the non-calcified plaque area and lipid core size (Knollmann et al., 2008).

Several limitations are also inherent in contrast-enhanced 16-MDCT itself, including restrictions of its spatial and temporal resolution (Leber et al., 2004, Kuettner et al. 2004, Ropers et al., 2003). Partial volume effects caused by large coronary calcifications and lumen contrast enhancement are frequently considered to be false-positive and false-negative results. Achenbach et al. (2004) reported a sensitivity
of 78% and a specificity of 87% for the detection of noncalcified plaque in coronary artery segments by 16-MDCT. Leber et al. (2006) found a sensitivity of 84% and a specificity of 91% in the detection of proximal coronary plaques by 64-MDCT.

The accuracy of CT for identifying non-calcified plaque components such as lipid-rich (soft) or fibrotic components remains low (Schroeder et al., 2004, Nikolaou et al., 2004).

Non-calcified plaque may be more unstable and prone to rupture than calcified plaque. Recent studies have shown that CT can identify non-calcified plaques in the coronary arteries in vivo (Budoff et al., 2005). Moreover, the CT features of non-calcified and calcified plaques correlate well with the histopathologic stages of atherosclerosis (Becker et al., 2003).

5.4.3 Measurement of waist circumference

In the routine clinical practice, the measurement of waist circumference is taken with a measuring tape at the point midway between the costal margin and iliac crest, with the patient standing and breathing normally. However, in the present study, the waist circumference of the patients was obtained retrospectively from the abdominal CT image at L4 level.
5.4.4 Study Sampling

The quality and quantity of the samples is crucial to the research result. The sample size of the present study was small (n = 40). Larger sample size has smaller standard errors in the result (Bartlett et al., 2001), and has more power which defined as the probability of retaining the alternative hypothesis when the alternative hypothesis is actually true in the population (Portney et al., 2009). Further studies with a larger sample size are suggested.

On the other hands, choosing a correct sample with appropriate exclusion criteria is important for any research study as the results that are obtained must be generalized to the target population for which the test is being development (Portney et al., 2009). Ideally, since our study is focused on the abdominal fat tissue, subject with history of family dyslipidaemia, medical disorders or drugs known of affecting the lipid metabolism should be excluded. In our study, the samples were selected from another study in which the haemodialysis patients have already performed a CT scan for evaluation of calcium score and abdominal fat thickness. Due to difficulties in subject recruitment, we exclude the aforementioned selection criteria in designing the present study.

Only the haemodialysis patients at stage 5D of chronic renal disease were included in the present study. Therefore, the result of the study may only apply for this kind of
patients. Further study to evaluate the effectiveness of the new AFI in predicting the cardiovascular risk in the general population is suggested.
Chapter Six

CONCLUSION

In conclusion, ultrasound measurement of mesenteric, preperitoneal and subcutaneous fat thickness is feasible in end-stage chronic renal failure patients with haemodialysis. The new abdominal fat index (AFI2 = (Pmax / Smin) * (Mmean/ Smin) with the addition of mesenteric fat thickness as an important component is higher association with the V/S ratio (r = 0.69, p < 0.05), coronary calcium score (r = 0.51, p < 0.05) and waist circumference (r = 0.45, p < 0.05) than the conventional AFI in the population of end-stage chronic renal failure patients. Previous studies reported that the V/S ratio, coronary calcium score and waist circumference are useful in predicting cardiovascular disease.

Therefore, the new abdominal fat index, $AFI = \{(Pmax/Smin)\times(Mmean/Smin)\}$ developed in the present study may be helpful in assessing cardiovascular risk in haemodialysis patients.
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一項針對中國慢性腎病病人患有心血管病的病學普查

研究項目標題：
利用超聲波量度腹部脂肪厚度比對過往之電腦掃描影像以評估心血管病變發生之可能性

病人同意書

研究人員已對我說明此項研究的目的、檢查內容、方法及後果，並且解釋此項研究不會影響我現時所接受的治療。我完全明白這份研究通知書，並且同意進行此項研究及會遵守一切有關指示。在整個研究期間，我可以隨時提出終止參與此項研究，同時也不會因此而影響本人的權利和治療。

研究人員已對我說明，對於這個研究內所有有關病人的個人資料都會確保絕對保密。

病人簽署：______________  研究人員簽署：______________

病人姓名：______________  研究人員姓名：______________

日期：__________________
Research for Cardiovascular Disease in Haemodialysis Patient

TITLE OF RESEARCH PROJECT:
Ultrasound Measurement of Visceral Fat Thickness for Predicting Cardiovascular Disease

CONSENT TO PARTICIPATE IN RESEARCH

The procedure as set out in the attached information sheet has been fully explained. I understand the benefit and risks involved. My participation in the project is voluntary.

I acknowledge that I have the right to question any part of the procedure and can withdraw at any time without penalty of any kind.

I hereby consent to participate in the captioned research conducted by Department of Radiology, Queen Mary Hospital and The Hong Kong Polytechnic University.

Name of participant _______________   Name of researcher __________________

Signature of participant ____________   Signature of researcher________________

Date    ________________