A STUDY ON THE EFFECT OF ORAL HYPOGLYCAEMIC AGENTS ON ARTERIAL STIFFNESS AMONG MALAY PATIENTS WITH TYPE 2 DIABETES MELLITUS.

By

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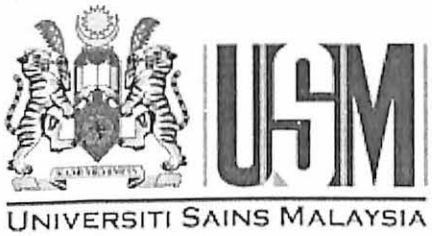

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ABBREVIATIONS

ACE	Angiotensin converting enzyme
ANCOVA	Analysis of covariance
AI	Augmentation index
ADA	American Diabetes Association
BMI	Body mass index
BP	Blood pressure
CI	Confidence interval
CHD	Coronary heart disease
CRF	Clinical report form
DBP	Diastolic blood pressure
ESRF	End stage renal failure
HDL	High density lipoprotein
HUSM	Hospital Universiti Sains Malaysia
IDDM	Insulin dependent Diabetes Mellitus
IMT	Intima media thickness
KRK	Klinik rawatan keluarga
LDL	Low density lipoprotein
MRI	Magnetic resonance imaging
NIDDM	Non Insulin Dependent Diabetes Mellitus.

PWA	Pulse wave analysis
PP	Pulse pressure
PWV	Pulse wave velocity
SBP	Systolic blood pressure
SD	Standard deviation
SPSS	Statistical package for social science

ABSTRAK

Objektif: Tujuan kajian ini dijalankan adalah untuk melihat perbezaan keanjalan salur darah arteri yang signifikan diantara penghidap diabetes dan tanpa diabetes melalui pengukuran indeks augmentasi dan juga melihat kesan diantara dua kumpulan ubat anti-diabetik, (monoterapi sulfonylurea dan kombinasi sulfonylurea dengan metformin) terhadap keanjalan salur darah arteri.

Metodologi: Ini adalah kajian kes-kontrol yang dijalankan di klinik rawatan keluarga dan klinik diabetik, HUSM daripada May 2004 hingga May 2005. Seramai seratus dua subjek pengidap diabetes dan 102 subjek tanpa diabetes yang berpadanan umur dan jantina telah dipilih selepas penerangan mengenai protocol kajian dan mendapat kebenaran secara verbal. Indeks augmentasi diukur dengan menggunakan alat "Sphygmocor® " dan segala pengukuran dilakukan oleh penyelidik selepas kajian validasi. Bacaan indeks augmentasi ini kemudiannya dianalisa.

Keputusan: Purata indeks augmentasi bagi pengidap diabetes adalah lebih tinggi dan signifikan daripada subjek tanpa diabetes (140.32 ± 12.0% Vs 128.77 ± 10.69%, $P < 0.0001$). Walau bagaimanapun, tiada perbezaan indeks augmentasi yang signifikan diantara dua kumpulan anti-diabetik, monoterapi sulfonylurea dan kombinasi sulfonylurea dengan metformin(140.51 ± 11.42 Vs 140.14 ± 12.86, 95% CI: -4.40. 5.15, $p = 0.877$).

Kesimpulan: Pesakit diabetes mempunyai salur darah arteri yang kurang anjal berbanding dengan subjek tanpa diabetes yang berpadanan umur dan jantina, ini menerangkan menerangkan mengapa diabetes mellitus berkaitan dengan peningkatan risiko kardiovaskular. Kajian ini juga menunjukkan bahawa dua kumpulan anti-diabetik tidak mempunyai kesan yang berbeza keatas keanjalan salur darah arteri.

ABSTRACT

A STUDY ON THE EFFECT OF ORAL HYPOGLYCAEMIC AGENTS ON ARTERIAL STIFFNESS IN MALAY PATIENTS WITH TYPE 2 DIABETES MELLITUS.

Objective : The purpose of this study is to see whether there was any significant difference in arterial stiffness (as measured by augmentation index) between diabetic and non diabetic subjects and to see whether there was any significant difference between two different oral hypoglycaemic agents(OHA) regimens, (sulphonylurea monotherapy and metformin in combination with sulphonylurea therapy) on arterial stiffness.

Methods : This was a case control study conducted in the Klinik rawatan keluarga(KRK) and Diabetic clinic, HUSM from May 2004 till May 2005. Hundred and two diabetic subjects and hundred and two age- and sex-matched non-diabetic control subjects were recruited after obtaining verbal consent following explanation of study protocol. Augmentation index (AI) was measured using the Sphygmocor apparatus and all measurements were performed by the researcher after an earlier validation study. These mean augmentation index measurements were then analyzed.

Result : The mean of AI of diabetic subjects was significantly higher than non diabetic subjects (140.32 ± 12.0% Vs 128.77 ± 10.69%, $P < 0.0001$). However, there

was no significant difference in mean AI between two different OHA regimen groups in diabetic subjects (140.51 ± 11.42 Vs 140.14 ± 12.86, 95% CI: -4.40, 5.15, p = 0.877).

Conclusion: Diabetic patients have increased arterial stiffness compared with age- and sex-matched non diabetic subjects, which may partly explain why diabetes mellitus are associated with increased cardiovascular risk. This study also showed that two different groups of oral hypoglycaemic agents have no effect in relation to arterial stiffness.

CHAPTER ONE: INTRODUCTION

Insulin resistance and type 2 Diabetes are major causes of morbidity and mortality in the industrialized world. It has been estimated that the prevalence of type 2 diabetes mellitus will increase from the present 160 million to 215 million in 2010 (Amos *et al.*, 1997). Type 2 diabetes mellitus increases the risk of cardiovascular disease 2 to 4 fold as compared to non-diabetic subjects (Kannel WB and McGee DL, 1987). Of the patients diagnosed with myocardial infarction about 20% have previously known type 2 diabetes (Tenerz *et al.*, 2001).

In Malaysia, from the second national Health and Morbidity Survey 1996, the prevalence of diabetes in Malaysia was found to be 8.2%, and about 10% suffered of stroke and cardiovascular diseases associated with their diabetic condition. Their survey also reported that the prevalence of diabetes is higher in Indians (11.5%) compared to other races. However, they found no significant difference in gender.

The most common cause of disability and death among subjects with non-insulin dependent diabetes mellitus (NIDDM) is macro vascular disease. In the United States, 11% of diabetic men and 6% of diabetic women 45 to 64 years of age reported having had a heart attack (Barret-Connor E and Orchard T, 1984). These percentages are 2.5 times higher in men and 4.0 times higher in women than in non-diabetic population. Increased risk of atherosclerosis is found even in prediabetic individuals (Haffner SM

et al., 1990), and in populations at high risk of coronary heart disease (CHD), almost half of middle-aged men and women with NIDDM have symptomatic CHD at the moment their diabetes is diagnosed (Uusitupa M *et al.*, 1985). These findings indicate that atherosclerosis develops gradually during the long latent phase of hyperinsulinaemia and glucose intolerance before the actual onset of NIDDM.

The reasons and mechanisms for the macro vascular disease in subjects with NIDDM are insufficiently known. However, there is evidence that insulin resistance and hyperinsulinaemia play an important role (Laakso M *et al.*, 1991). Insulin resistance is associated with high triglyceride and low high density lipoprotein (HDL) cholesterol concentrations and with an increased tendency to hypertension (Zavaroni J *et al.*, 1989). In addition to its lipid effects, insulin may have an effect on the thickness and structure of the arterial wall. This is shown in the in vitro study that insulin concentrations commonly found in humans can cause proliferation of cultured smooth muscle cells (Pfeifle B and Ditshuneit A, 1981).

In non-diabetic individuals, increased arterial stiffness is an important cause of cardiovascular disease, because arterial stiffness leads to increased systolic pressure and ventricular mass and to decreased diastolic coronary perfusion (Westerhof N and O'Rourke MF, 1995). There is evidence that metabolic alterations in type 2 diabetes and impaired glucose metabolism are associated with increased arterial stiffness. This is supported by Atherosclerosis Risk in Communities Study who found that persons

with NIDDM or borderline glucose intolerance have stiffer arteries than their counterparts with normal glucose tolerance (Salomaa *et al.*, 1995).

In population-based study, they found that Type 2 diabetes mellitus and impaired glucose metabolism are associated with decreased total systemic arterial compliance, increased aortic augmentation index, and decreased carotid-femoral transit time which provides understanding why both type 2 diabetes mellitus and impaired glucose metabolism are associated with increased risk for stroke, heart failure and myocardial infarction (Schram *et al.*, 2004).

The Chennai Urban Population Study also found similar finding where 50 diabetic subjects and 50 age and sex matched non-diabetic control subjects found that the mean augmentation index of diabetic subjects was significantly higher than non-diabetic subjects, which suggest that diabetic patients have increased arterial stiffness (Ravikumar *et al.*, 2002).

In the Japanese study done by Emoto *et al.* (1998) in 60 NIDDM subjects found that arterial wall stiffness as measured using ultrasonic phase-locked echo-tracking system found that the arterial stiffness were significantly higher in NIDDM subjects than in control subjects. The similar finding also found in the study done by Taniwaki *et al.* (1999) in 271 diabetic patients and 285 healthy age-matched control subjects which also demonstrated that aortic pulse wave velocity in Japanese patients with type 2 diabetes were significantly higher than in age-matched control subjects. The same

researcher also found that in the diabetes group, the arterial stiffness were significantly higher in the patients with the II genotype than in those with the DD genotype which suggest that some genetic factors may be associated with stiffening of the arteries. The similar finding also found in the other study. In the Rotterdam study, the insertion/deletion (I/D) polymorphism of the ACE gene was found to increase common carotid stiffness in a cohort study of 3001 adults over aged 54(Mattace-Raso *et al.*, 2004). So, in this study, the researcher focused in the Malay populations.

As arterial stiffness has become established as a cardiovascular risk factor, it has also emerged as a potential target for intervention. Arterial stiffness may become a major primary goal of treatment in particular patients at risk of cardiovascular disease. Drugs may improve the stiffness of the arterial wall through either functional or structural mechanisms (Oliver JJ and Webb DJ, 2003). In a study done by Shimamato H and Shimamoto Y in hypertensive patients, 8 weeks of treatment with lisinopril more effectively reduced pulse wave velocity than nifedipine (Shimamato H and Shimamoto Y, 1995).

In a study done by Tsuji *et al.* (2002) demonstrated that in Otsuka Long-Evans Tokushima fatty rats with type 2 diabetes showed an improvement in aortic wall distensibility after received 15 weeks of pioglitazone. Satoh *et al.*, (2003) also demonstrated that treatment with pioglitazone in patients with type 2 Diabetes mellitus for 3 months resulted in a significant decreased in pulse wave velocity (PWV). This is in agreement with that of Minamikawa *et al.* (1998) and Koshiyama *et al.* (2001) who

reported that intima media thickness (IMT) was significantly reduced in type 2 diabetes patients administered troglitazone or pioglitazone for 3 months.

However, there is lack of clinical data on the effect of traditional oral hypoglycemic agents like metformin or sulfonylurea or combination of both on arterial stiffness in patients with type 2 diabetes mellitus although there is a study shown that treatment with metformin for 12 weeks improved endothelial function (Kieren *et al.*, 2001). The researcher therefore studied the effect of oral hypoglycemic agent on arterial stiffness in type 2 Diabetes mellitus and based on previous studies, this study also took three months as a minimum duration of taking oral hypoglycemic agent. In this study, the arterial stiffness was assessed by measuring augmentation index (AI) at the radial artery.

CHAPTER TWO: LITERATURE REVIEW.

2.1 Overview of arterial stiffness.

Aortic stiffening is as much an important risk factor in cardiovascular morbidity and mortality, as it serves as reliable surrogate marker in clinical endpoints like myocardial and cerebrovascular incidents. Elevated aortic stiffness induces high systolic blood pressure; augmented pulse pressure with increases ventricular after load, reduced subendocardial blood flow and augmented pulsatile stress in the peripheral arteries (Breithaupt-Grogler and Belz GG, 1999).

Arterial stiffness is determined by structural and functional components related to the intrinsic elastic properties of the artery. The endothelium, the elastic tissue within the intima media layer, and smooth muscle contribute to arterial stiffness (Arnett DK *et al.*, 1994).

The relationship between arterial stiffness as measured as pulse wave velocity and the elastic properties of the arterial wall has been extensively studied (Asmar *et al.*, 1995). Despite its non-invasive nature and good sensivity and reproducibility, pulse wave velocity was not applied to clinical practice because its recording and calculation were difficult and time consuming (Asmar *et al.*, 1995). However, recent developments of non invasive method such as applanation tonometry has open a new era to the clinical applications of the analysis of the pulse waveform, amplitude and velocity of the pulse

pressure and its also provides a simple, repeatable and sensitive (Wilkinson *et al.*, 2002). Radial artery pressure waveforms recorded with tonometry have been shown to equal those measured intra-arterially in a large group of normal subjects (Kelly *et al.*, 1989). Several studies have now demonstrated that a single generalized transfer function can be used to accurately determine central from peripheral pressure in normal subjects and in patients with a variety of diseases (Chen *et al.*, 1997, Karamanoglu *et al.*, 1993,O'Rourke and Gallagher 1996).

A number of studies have been done to look in the relationship between arterial stiffness and cardiovascular disease and its shown that arterial stiffness is associated with certain disease such as hypertension, diabetes mellitus, hypercholestrolaemia and end-stage renal failure (Glasser *et al.*, 1997). As changes can be detected before the appearance of clinically apparent vascular disease, arterial stiffness may act either as a marker for the development of future atherosclerotic disease, or may be more directly involved in the process of atherosclerosis (Mackenzie and Wilkinson, 2002).

Assessment of the arterial pulse has always been an important part of clinical examination, and the changes in the character of the pulse indicated the presence of disease. Arterial stiffness may be measured using a variety of different techniques, although at present the majority of measurements are made for experimental and physiological studies rather than in clinical practice. However, it is likely that over the next few year measurement of arterial stiffness will become an increasingly

important part of the process of risk assessment, and may possibly also improve the monitoring of therapy.

2.2. Methods of measuring arterial stiffness.

Measures of arterial stiffness estimate the artery's ability to expand and contract with cardiac pulsation and relaxation. Technologic advancements have provided for direct, non-invasive visualization of arteries with excellent precision, opening the horizon for studies of arterial stiffness in research and clinical practice. While there is currently no gold standard, several measures have been used extensively in a variety of settings.

2.2.1. Pulse pressure.

Pulse pressure is the different between systolic and diastolic blood pressure and is recognized since 1922 by Bramwell and Hill (1922). Pulse pressure is a valuable surrogate marker for arterial stiffness. Both systolic and diastolic blood pressure tends to increase with age (Franklin *et al.,* 1997). However, after the age 50-60 years old, there is no further increase in blood pressure and in some cases it actually declines. So, the pulse pressure widens with increasing age. In the Framingham cohort diastolic blood pressure was the best predictor of CHD in patients < 50 years of age whereas in patients 50-59 years of age systolic and diastolic blood pressure as well as pulse pressure predicted CHD comparably (Franklin *et al.,* 2001). From

60 years of age, pulse pressure was superior to systolic blood pressure in predicting CHD (Franklin *et al.,* 1999). In the Systolic Hypertension in the Elderly Program (SHEP) both stroke and total mortality were related to pulse pressure independent of mean arterial pressure (Domanski *et al.,* 1999). Not only do ageing and hypertension decrease arterial wall elasticity and predispose to CHD, but arteriosclerosis also decreases elasticity and increases pulse pressure (Dart and Kingwell, 2001).

Pulse pressure can be measured with standard sphygmomanometer which is one of the simplest measure of arterial stiffness and easily practicable in the clinical setting. However, pulse pressure alone is inadequate to assess arterial stiffness accurately. This is because, measurements of pulse pressure made in the periphery which do not always accurately reflect the actual central pulse pressure (Pauca *et al.,* 1992).

2.2.2. Pulse wave velocity.

Pulse wave velocity (PWV), the velocity of travel of a pressure wave between two arterial sites, can be measured invasively and non-invasively and can be used as an index of regional vascular stiffness. The speed at which the pulse wave travels through an arterial segment increases with increasing stiffness (Oliver JJ and Webb DJ, 2003). Pressure waveforms can be captured by pressure-sensitive transducers (Asmar *et al.,* 1995), Doppler ultrasonography (Sutton-Tyrell *et al.,* 2001),

applanation tonometry (Wilkinson *et al.,* 1998), photopletsymography (Loukogeorgakis *et al.* 2002) or MRI (Mohiaddin *et al.,* 1993). Pulse wave velocity can be measured between any arterial segments, but it is usually used to measure the wave travel between common carotid and femoral or carotid and radial arteries.

PWV has been shown to increase with various cardiovascular risk factors, including age, hypercholestrolemia, type 2 diabetes and hypertension (Oliver JJ and Webb DJ, 2003). PWV has also been shown to predict all-cause and cardiovascular mortality (Laurent *et al.,* 2001).

The problem with this technique is some difficulty in estimating the actual arterial distance between recording sites by using only surface measurements (Asmar, 1999). The pulse wave velocity becomes less accurate if the recording points are very close together, and therefore, it is limited to use on the larger arteries. However, the technique of PWV is valid and reproducible and it is relatively simple and can learned fairly easily. The alternative method of measuring PWV is by using MRI technique (Mohiaddin *et al.,* 1993). The advantage of MRI is the accurate determination of path length but its use is limited by cost and time.

2.2.3. Augmentation index (AgI).

The augmentation index derived from systolic pulse wave analysis. The augmentation index is the difference between the first and second systolic peaks

expressed as a percentage of the pulse pressure, and can be used as a measure of arterial stiffness (Mackenzie *et al.*, 2002). It can be measured invasively and non-invasively from superficial peripheral arteries by using applanation tonometry. Applanation tonometry is a sensitive pen like instrument and is used to record pressures at the radial or carotid artery, and central blood pressure. The augmentation index (AgI) can be calculated from the aortic pressure waveform derived using a generalized transfer function. A validated generalized transfer function has more than 90% accuracy in generating features of the ascending aortic pressure wave (Chen *et al.*, 1997). The augmentation index (AgI) reflects the degree to which central arterial pressure is augmented by wave reflection (O'Rourke and Gallagher, 1996).

In one study in 66 patients in United States who undergoing diagnostic catheterization, augmentation index from carotid artery tonometry derived augmentation index with an external micro manometer tipped-probe was compared with a micro manometer tipped-catheter and found that there is high correlation between the central augmentation index (AgI) and those estimated from non-invasive carotid tonometry (Chen *et al.*, 1996).

The augmentation index measured from radial artery also correlates with carotid artery intima-media thickness in diabetic and non-diabetic subjects and predicts coronary artery disease independent of other risk factors (Ravikumar *et al.*, 2002).

It also higher in type 2 diabetes subjects compared to non-diabetic subjects (Ravikumar *et al.*, 2002).

So far there is no evidence that the AgI measured from radial artery has prognostic value (Oliver JJ and Webb DJ, 2003), but a high carotid AgI has been shown to be an independent predictor for ischemic threshold in patients with coronary heart disease and of all-cause and cardiovascular mortality in patients with end-stage renal failure (London *et al.*, 2001).

This Pulse Wave Analysis (PWA) system is a simple, non-invasive and validated method of measuring arterial stiffness (O'Rourke and Gallagher, 1996). The tonometer is the size of a pen, the system is easily portable and, therefore, useful in both hospital and clinic settings. This study will use augmentation index measured by SphygmoCor machine as index of arterial stiffness.

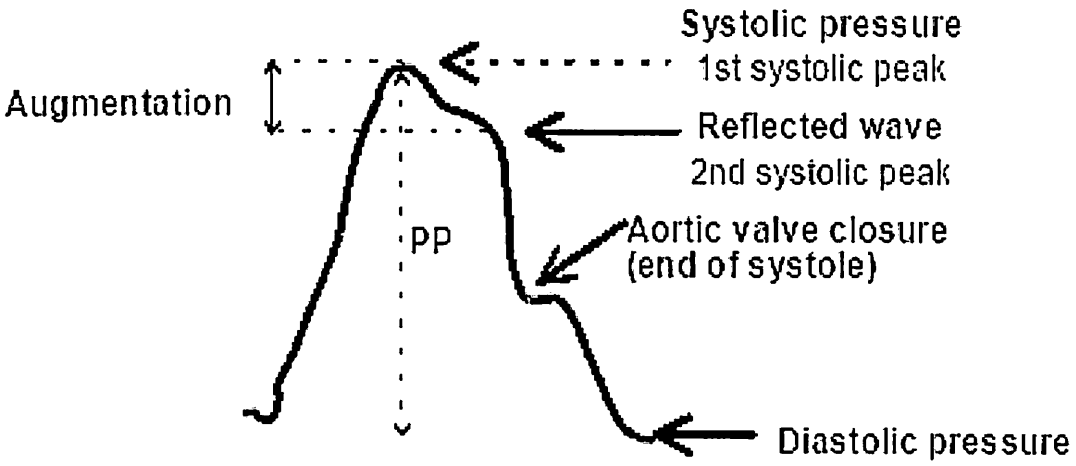

Figure 2.1 Schematic illustration of pulse wave during one cardiac cycle. The augmentation index (AgI) is defined as the ratio between augmentation and pulse pressure (PP).

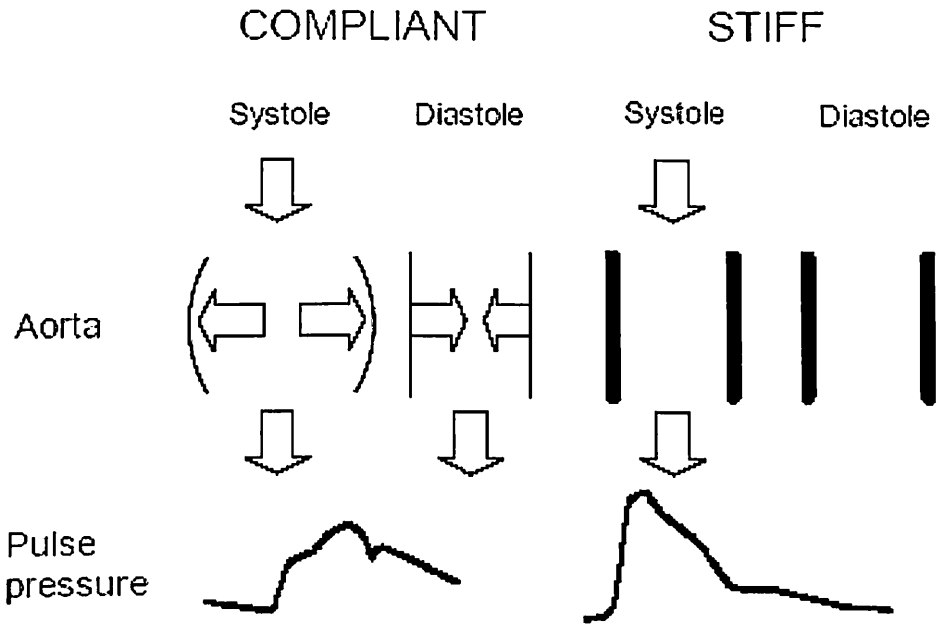

Figure 2.2 Examples of pulse waveforms caused by compliant (on the left) and shift aortas (on the right) at constant stroke volume.

2.2.4. Diastolic pulse contour analysis.

The diastolic part of the pulse wave can be recorded by applanation tonometry and calibrated using a sphygmomanometer (Oliver JJ and Webb DJ, 2003), Information can be received from both large artery compliance referred to as C1 and peripheral artery compliance referred to as C2 (Oliver JJ and Webb DJ, 2003). Compliance calculated from invasive and non-invasive measurements correlate significantly (Cohn *et al.,* 1995). C2 is decreased in hypertensive patients, type 2 diabetes patients, postmenopausal women with CHD, and smokers (Cohn *et al.,* 1995).

The technique does not provide any information on central pressures and augmentation index (MacKenzie *et al.*2002). It depends on diastolic component of the waveform, which tends to be less reliably recorded than the systolic component

2.2.5 MRI derived indices

Magnetic resonance imaging (MRI) technique has been used to measure vascular distensibility and compliance. Most of the human studies have been based on measurements of the aorta. The aortic distensibility is reduced in hypertensive patients (Resnick *et al.,* 1997). Although MRI has the advantage of being non-invasive, it remains expensive, and the availability of scanning facilities is limited.

2.2.6 Vascular ultrasonography

The change in the diameter of an artery at a given distending pressure provides a measure of arterial stiffness (Oliver JJ and Webb DJ, 2003),). Ultrasound and MRI can both be used for measurement of the change in diameter. Mean brachial arterial blood pressure can be combined with applanation tonometry at the site of the carotid artery to measure carotid pulse pressure more accurately (Oliver JJ and Webb DJ, 2003),). The measurement can be made at several different sites such as brachial, radial, and femoral arteries or aorta. Reduced aortic distensibility has been associated with increased age, CHD, hypertension, hypercholestrolemia and smoking (Stefanadis *et al.,* 2000). It also increased in type 2 diabetic patients (Salooma *et al.,* 1995).

2.2.7 Digital volume pulse

Photoplethysmography has been used to record the digital volume pulse. This technique resembles that of pulse oximetry, and measures the transmission of infrared light through the finger, thus detecting changes in flow and producing a volume waveform (Chowienczyk *et al.,* 1999). A problem with this method is that it depends on the temperature changes in the peripheral circulation. A study that compared photoplethysmography of the digital volume pulse with pulse wave velocity, found that pulse wave velocity correlated more closely with the expected influences on vascular compliance (Bortolotto *et al.,* 2000).

2.3. Arterial stiffness and vascular risk.

2.3.1. Age

Arteries become less elastic by ageing. This is caused by an increase in arterial wall thickness secondary to hyperplasia of the intima and by loss of elastin in the media and its replacement by collagen (Davies and Struthers, 2003). Pulse pressure, a surrogate marker of arterial stiffness, increases with age. In the Framingham cohort pulse pressure averaged 42 mmHg before the age of 50 years, 50 mmHg between ages of 50 and 59 years and increased to 62 mmHg after the age of 60 years (Franklin *et al.*, 2001).

In the Framingham Heart Study offspring cohort in 188 men and 333 women who were free of clinical cardiovascular disease, hypertension, diabetes, dyslipidaemia and obesity, found that advancing age was the predominant correlate of higher carotid-femoral pulse wave velocity (Mitchell *et al.* 2004).

Kelly *et al.* determined arterial stiffness from the carotid, femoral and radial arteries using an applanation tonometer, and analyzed arterial pressure waveforms in 1005 normal subjects aged 2 to 91 years and found that aging was associated with an increase in pulse amplitude, steepening of the diastolic decay and a decrease in the pressure of the diastolic wave (Kelly *et al.*, 1989). Stiffening thus explains why diastolic pressures normally decrease and pulse pressure increases during aging.

2.3.2 Obesity

Obesity has reached epidemic levels and carries a risk for cardiovascular disease. According to data from the National Health and Nutrition Examination Survey in US from 1988 to 1994, the prevalence of overweight (BMI > 25 kg/m²) was 11.3% among 6 to 11 year olds, 10.5% among 12 to 19 year olds, and 55% among adults, whereas in 1999 to 2000, corresponding prevalence rates had increased to 15.3%, 15.5%, and 64.5%, respectively (Ogden *et al.,* 2002). Excess weight has been shown to carry a risk for stroke, incident cardiovascular disease, cardiovascular mortality, and all-cause mortality among middle-aged and elderly participants in longitudinal study (Wilson *et al.,* 2002). Excess body fat, abdominal visceral fat, and larger waist circumference have been identified as risk factors for accelerated arterial stiffening in elderly participants (Sutton Tyrell *et al.,* 2001).

In a study done by Wildman *et al.* in 186 young adults and 177 older adults where aortic stiffness was measured by aortic pulse wave velocity found that higher body weight, body mass index, waist and hip circumferences, and waist-hip ratio were strongly correlated with higher pulse wave velocity, independent of age, systolic blood pressure, race, and sex (Wildman *et al.,* 2003).

2.3.3 Gender

In a group of elderly hypertensive patients matched for age, height and mean arterial pressure, there was no difference in systolic blood pressure between men and women, but diastolic blood pressure was lower and pulse pressure and the AgI higher in

women (Gatzka *et al.*, 2001). In the Atherosclerosis Risk in Communities study in a 4701 men and women found that arterial stiffness about twice as high in women as in men. In the Framingham Heart study, the incidence of ultrasonically diagnosed ventricular hypertrophy among subjects with glucose intolerance or diabetes was greater in women than in men (Lougun and Gosling, 1982).

2.3.4 Heart rate

Studies performed in rats have shown that when pacing increases heart rate, carotid and femoral arteries distensibilities are reduced (Mangoni *et al.*, 1996). In paced animal models, increased heart rate was shown to increase stiffness of large elastic arteries while having a variable effect on muscular arteries (Mangoni *et al.*, 1996). These suggest that heart rate might be one of the factors that modulates arterial mechanical properties and thus, potentially participates in their abnormality in several conditions and diseases. However, there is a little evidence exists whether heart rate plays a similar role in humans and the available data have given conflicting results.

In a study reported by Lantelme *et al.*, in 22 subjects with permanent cardiac pacing found that there was a highly significant effect of heart rate on PWV (Lantelme *et al.*, 2002). The similar finding also reported by Giannattasio *et al.*, in 20 outpatients who chronically implanted with a dual-chamber pacemaker because of sick sinus syndrome or an atrio-ventricular block, found that increases in heart rate markedly effect arterial distensibility (Giannattasio *et al.*, 2003). However, Wilkinson *et al.*, showed a negative relationship between augmentation index and heart rate (Wilkinson *et al.*, 2002)

2.3.5 Hyperglycaemia and hyperinsulinaemia.

In the ARIC study, where they used ultrasound for measurements of carotid artery stiffness in a large population survey comprising of 4701 white and black subjects found that 5% subjects had type 2 diabetes. In the entire study group, arterial stiffness increased with increasing concentrations of fasting glucose, independent of race or gender. In all non-diabetic patients, fasting serum insulin was associated with arterial stiffness, again even after adjustment for age, smoking and total cholestrol. This cross-sectional study also found that hyperinsulinaemia and hyperglycaemia synergistically contributed to arterial stiffness, independent of artery wall thickness, in both men and women (Salooma *et al.,* 1995).

In the Strong Heart study, 1810 diabetic and 944 normal American Indians with a mean age of 60 years were studied using an ultrasound technique found that diabetic patients had significantly increased arterial stiffness even after adjustment for age, gender, height, BMI, systolic blood pressure and use of anti-hypertensive drugs (Devereux *et al.,* 2000).

Type 1 diabetic patients have also been shown to have stiffer larger arteries in many studies. Giannattasio *et al* measured arterial stiffness in the abdominal aorta and in radial and common carotid artery using an arterial wall echo-tracking technique in 133 type 1 diabetic patients and in 70 age-matched control subjects and found that in the diabetic patients, regardless of the presence of complications, arterial stiffness was increased at all arterial sites when compared to control subjects (Giannattasio *et al.,* 1999).

In the Hoorn population-based study of 619 individulas and assessed central artery stiffness by measuring total systemic arterial compliance, aortic pressure augmentation index and carotid-femoral transit rime found that after adjustment for sex, age, heart rate, height, body mass index and mean arterial pressure, type 2 diabetes was associated with decreased total systemic arterial compliance, increased aortic augmentation index, and decreased carotid-femoral transit time (Schram *et al.*, 2004).

2.3.6 Hypertension

Blood pressure is a powerful cardiovascular risk factor that acts on the arterial wall and is responsible in part of various cardiovascular even such as cerebrovascular events and ischaemic heart disease. Arterial stiffness and wave reflections have been widely investigated in hypertensive subjects. One of this reason, because from epidemiological studies, systolic blood pressure is more informative as cardiovascular risk factor compared to diastolic blood pressure, particularly in patients older than 50 years of age and it has been shown that pulse pressure is independent marker of cardiovascular risk, mainly for myocardial infarction (Safar , 2001).

Increased arterial stiffness may increase cardiovascular morbidity and mortality because of an elevation of systolic blood pressure (SBP), which raised left ventricular after load, and because of decrease in diastolic blood pressure (DBP), which alters coronary perfusion (Safar, 1989). In a cohort study done by Laurent *et al.* in 1980 essential hypertensive patients who attended the outpatient hypertensive clinic found that the aortic stiffness as measured as PWV is an independent predictor of all-cause

and cardiovascular mortality in patients with essential hypertension (Laurent *et al.*, 1990).

In a longitudinal study done by Demellis and Panaretou in 2512 subjects who assessed the predictive value of aortic stiffness on future hypertension in non-hypertensive subjects with BP< 140/90. In this study, aortic stiffness was determined by echocardiography at baseline and was follow up for 4 years. From this study, they found that there is stepwise increase in hypertension incidence and aortic stiffness significantly associated with the progression to future hypertension (Demellis and Panaretou, 2005). This findings are similar with the previous study by Liao *et al.* , which has shown that lower arterial elasticity (high stiffness) in the common carotid artery is related to the development of hypertension (Liao *et al.*, 1999). This result shows that arterial stiffness may help in the evaluation of the individual risk in hypertensive patients regularly attending the outpatient clinic of a university hospital and helps to identify patients at high risk for hypertension.

2.3.7 Smoking

Smoking is a major risk factor in the development and progression of cardiovascular disease. Smoking may have both short and long term effects on arterial stiffness. In a study which compared the acute and chronic effects of smoking on large-artery properties in 185 healthy young smokers and non-smokers before and for 15 minutes after smoking 1 cigarette (nicotine content, 1.2 mg) found that although brachial blood pressure was not different, the aortic systolic blood pressure and augmentation index were higher in chronic smokers than in non-smokers (Mahmud and Feely, 2003).

Levenson *et al.* studied the relationship between smoking and arterial stiffness in 33 normotensive and 80 hypertensive subjects also found that in both groups, smokers had increased arterial stiffness (Levenson *et al.*, 1987).

In a group of 248 healthy subjects, all 50 years of age, stiffness of the common carotid artery was measured by using ultrasound and showed that smoking was independently associated with arterial stiffness (Jonason *et al.*, 1997). The similar finding also shown in a study by Taniwaki *et al.* , arterial stiffness was measured using aortic PWV measurements in 271 diabetic patients and 285 healthy subjects which demonstrated that smoking was independently associated with increased arterial stiffness (Taniwaki *et al.*, 1999).

2.3.8 Hypercholestrolemia

Data regarding the association between lipid abnormalities and arterial stiffness are controversial. In a study of 62 normotensive and 201 uncomplicated hypertensive subjects with a wide range of total cholestrol concentrations found that arterial stiffness which was measured with ultrasound and by applanation tonometry was not correlated with serum cholestrol (Saba *et al.*, 1999).

In contrast with the study done by Dart *et al.* , who found that there is a significant relationship between serum cholestrol and arterial stiffness in 54 healthy subjects using the ultrasound technique (Dart *et al.* 1991)

Toikka *et al.*, measured compliance of the aorta with MRI in 25 healthy subjects aged 28 to 39 years old and in 10 age-matched subjects with familial hypercholestrolemia found that the aortic compliance was similar between the groups (Toikka *et al.*, 1999)

2.3.9 Anti-hypertensive drugs

Different blood pressure lowering drugs have different effects on central aortic pressures. Short-term studies have shown that various classes of blood pressure-lowering drugs may have profoundly different effects on pulse wave morphology despite similar effects on brachial artery pressures (Chen *et al.* 1995).

In The CAFE study in 2073 participants for up to 4 years follow-up, who examined the impact of 2 different blood pressure (BP) lowering-regimens (atenolol-based regimen and amlodipine based regimen) on derived central aortic pressures, and the results shown that despite similar brachial systolic blood pressure between treatment groups, there were substantial reductions in central aortic pressures with the amlodipine regimen (Williams *et al.*, 2006).

A number of studies have compared the effects on arterial stiffness of ACE inhibitors and calcium channel blocker. In end stage renal failure (ESRF) treatment for 1 year with perindopril or nitrendipine similarly reduced BP and more effectively reduced carotid than brachial pressure. Both similarly reduced PWV and carotid AI, although only perindopril reduced left ventricular hypertrophy (London *et al.*, 1994). There are conflicting data regarding the effects of diuretics on arterial wall stiffness. For example, despite reducing BP, neither indapamide or canrenoate changed PWV

(Laurent *et al.,* 1990). The felodipine more effectively improved brachial artery compliance than hydrochlorothiazide (Asmar *et al.,* 1993).

2.4 Arterial stiffness as a predictor of vascular disease.

In follow-up studies, pulse pressure has been shown to predict cardiovascular disease independent of mean arterial pressure (Domanski *et al.,* 1999). In the Framingham cohort study, pulse pressure was the best predictor in subjects over 60 years suggesting that age-related stiffening of arteries is an important risk factor for coronary heart disease (Franklin *et al.,* 2001).

Recently, the association between stiffness and cardiovascular disease has also been studied using direct measures of arterial stiffness. In cross-sectional studies, PWV correlated with cardiovascular risk factors such as age, gender, systolic blood pressure, diabetes, heart rate, carotid plaques and intima-media thickness (Zureik *et al.,* 2002).

The augmentation index derived from non-invasive radial artery tonometry predicts the presence and severity of coronary artery lesions independent of smoking, total and LDL cholestrol, age, hypertension and diabetes (Weber *et al.,* 2004). Both the AI and PWV predicted ischaemic thresholds in patients with coronary heart disease and the AI measured invasively from the ascending aorta with a fluid-filled system is associated with angiographic changes in coronary arteries (Hayashi *et al.,* 2002).

In over 3000 elderly subjects of the Rotterdam study, aortic stiffness measured by carotid artery distensibility and carotid-femoral PWV measured by ultrasonography