The role of pulse wave velocity as a marker of severity of coronary artery disease (CAD) in male patients in a single centre study

By

Dr.Ahmed Yahya ALArhabi

Thesis submitted in fulfillment of the requirement for the degree of

Master of Science

Acknowledgements

First, all the thanks belong to Allah whom I believed in, for the guidance and help in all of my life.

I wish to express my gratefulness and thanks to my main supervisor **Dr. Mohd Sapawi Mohammed** and my co-supervisors **Prof. Zurkurnai Yusof** and **Dr. Suhairi Ibrahim** for their kind supervision, valuable opinions and gaudiness, generous advices, excellent comments and continuous encouragement.

I would like to express my gratefulness and thanks to **Dr. Kamarul Imran Musa**, for his great help in biostatistics.

Thanks to all my lecturers, colleagues and staff members at Medical Department for their valuable effort to overcome limitation.

Most of all, my warm and my special thanks to my father Yahya ALArhabi, my mother Zahara Mohammed, my wife Dr.Belges ALTahami, my kids Aya, Mohammed and Aws, my brothers (Dr.Mohammed, Dr.Anwar and Abdulrahman) and my sisters (Samerah, Amerah, Belqes and Amenah) for their overwhelming encouragement and support.

LIST OF CONTENT

List of tables

Page

List of Figures

LIST OF ABBREVIATIONS

BMI	Body mass index
BP	Blood pressure
CABG	Coronary artery bypass grafting
CAD	Coronary artery disease
CRP	C-reactive protein
D	Distance
DBP	Diastolic blood pressure
ECG	Electrocardiography
FBS	Fasting blood sugar
GXT	Graded-exercise stress test
HDL	High density lipoprotein
HR	Heart rate
HUSM	Hospital Universiti Sains Malaysia
ICL	Interventional cardiology lab
IGT	Impaired glucose tolerance
LDL	Low-density lipoprotein
MI	Myocardial infarction
MRI	Magnetic resonance imaging
MVD	Multiple vessel disease
PAI-1	Plasminogen activator inhibitor-1
PP	Pulse pressure

PTCA Percutaneous transluminal coronary angioplasty

PWV Pulse wave velocity

SBP Systolic blood pressure

SVD Single vessel disease

TD Time delay

T1DM Type 1 diabetes mellitus

T2DM Type 2 diabetes mellitus

TG Triglyceride

TVD Two vessel disease

PERANAN KELAJUAN GELOMBANG DENYUTAN SEBAGAI PENANDA KEPARAHAN PENYAKIT ARTERI KORONARI(PAK) DI KALANGAN PESAKIT LELAKI DALAM KAJIAN TUNGGAL BERPUSAT

ABSTRAK

Penyakit arteri koronari merupakan salah satu penyakit yang biasa ditemui diseluruh dunia hari ini. Ia melibatkan 6.9 juta kematian setiap tahun dan merupakan punca utama kematian pramatang di Negara-negara membangun. Terdapat banyak faktor risiko yang berkait dengan peningkatan risiko PAK seperti merokok, umur, DM, hiperkolesterolemia dan tekanan darah tinggi. Diagnosis awal dan pencegahan PAK telah menggiatkan pencarian satu kaedah boleh harap 'noninvasive' untuk pengesanannya. Salah satu ujian noninvasive untuk diagnosa PAK ialah pengesanan kekerasan arteri dengan mengukur kelajuan gelombang denyutan menggunakan mesin sphygmoCor®.

Kajian ini bertujuan untuk menentukan peranan kelajuan gelombang denyutan sebagai penanda kekerasan PAK dan juga menetukan perkaitan antara PWV (pulse wave velocity) dan faktor risiko kardiovaskular lain. Satu kajian silang yang melibatkan 92 orang pesakit yang menjalani angiografi koronari di ICL HUSM di jalankan untuk penilaian disyaki berpenyakit arteri koronari. Ketegangan arteri di nilai melalui kelajuan gelombang denyutan karotid-femoral menggunakan mesin sphygmoCor®.

Keputusan kajian ini menunjukkan PWV pesakit dengan PAK lebih tinggi berbanding pesakit tanpa PAK (11.3 ± 0.9 vs 8.14 ± 1.25 m/s, $p<0.05$). Apabila kekerasan PAK dinyatakan sebagai satu-, dua-, dan penyakit pelbagai saluran atau normal, terdapat perkaitan bererti antara kekerasan PAK dan PWV ($p<0.05$), faktor risiko bebas bererti PAK adalah umur dan jumlah kolesterol ($p>0.05$) dan berkait secara bebas dengan PAK. PWV berbeza secara bererti dengan kategori kekerasan PAK yang berbeza walaupun umur dan jumlah kolesterol mereka di kawal.

Kesimpulannya, kekerasan arteri yang di ukur melalui PWV adalah faktor risiko bebas dan pelengkap kardiovaskular. Pengukuran kekerasan aortik boleh dijadikan sebagai penanda kerosakan organ sistem arterial, menunjukkan pertambahan risiko untuk komplikasi kardiovaskular dan membantu mengenalpasti pesakit yang berisiko tinggi untuk PAK yang mungkin bermanfaat dari diagnostik yang lebih agresif dan strategi terapeutik.

THE ROLE OF PULSE WAVE VELOCITY AS A MARKER OF SEVERITY OF CORONARY ARTERY DISEASE (CAD) IN MALE PATIENTS IN A SINGLE CENTRE STUDY

ABSTRACT

Coronary artery disease (CAD) is one of the most common diseases in the world today, causing 6.9 million deaths worldwide each year, and is the leading cause of premature death in developed countries. There are many risk factors associated with increased risk of CAD such as smoking, age, DM, hypercholesterolemia and hypertension. Early diagnosis and prevention of CAD have stimulated a search for reliable noninvasive methods of detection. One of the noninvasive test to diagnose CAD is the detection of arterial stiffness by measuring pulse wave velocity using the sphygmoCor® machine. This technique is valid, reproducible and widely applied.

The aims of this study was to determine the role of pulse wave velocity (PWV) as a marker of the severity of CAD as well as to determine the association between PWV with other cardiovascular risk factors. A cross sectional study with 92 patients undergoing coronary angiography in ICL HUSM for the assessment of suspected coronary artery disease was carried out. Arterial stiffness was assessed through left carotid – right femoral pulse wave velocity using automated SphygmoCor® machine.

The results of this study showed that PWV was higher in patients with CAD than those without CAD (11.13 ± 0.91 vs 8.14 ± 1.25 m/s, $p < 0.001$). When the severity of CAD was expressed as single-, two- and multiple-vessel diseases, there was significant association between the severity of CAD and PWV ($p < 0.05$). The significant independent risk factors of CAD were age and total cholesterol ($P < 0.05$) whereas smoking, BMI, SBP, DBP and DM were not significant ($P > 0.05$). PWV differed significantly with different categorical severity of CAD even when their age and total cholesterol were controlled ($P < 0.05$).

In conclusion, PWV is an independent and complementary cardiovascular risk factor. Measuring aortic stiffness should serve as marker of end organ damage regarding the arterial system, indicating an increased risk for cardiovascular complications, and helps to identify patients at high risk of CAD who may benefit from more aggressive diagnostic as well as therapeutic strategies.

CHAPTER 1 - INTRODUCTION

1.1 Incidence of coronary artery disease.

Coronary artery disease (CAD) is a disease of the coronary arteries, most commonly caused by atherosclerosis (Jamrozik, 2001). Atherosclerosis is the thickening or hardening of the artery walls, formed by a combination of damage to the endothelial lining, the deposition and accumulation of cholesterol, and the development of atherosclerotic plaques within the walls, which advance in size gradually over time and narrow the vessel lumen (Rogers and Sharp, 1997; Tegos et al, 2001 and Mathur, 2002). This process develops over many years, eventually causing the complete obstruction of coronary blood flow if left untreated (Davidson, 2000, Mathur, 2002 and Fox, 2002). Other non-coronary manifestations of atherosclerosis include stroke, peripheral vascular disease and aortic aneurysm.

CAD is one of the most common diseases in the world today, causing 6.9 million deaths worldwide each year, and is the leading cause of premature death in developed countries (Mathur, 2002 and Australian Institute of Health and Welfare, 2003). In developing countries such as Malaysia, CAD may not be the number one cause of death at the moment. However, it has been estimated that by year 2020, there will be tremendous changes in disease pattern and demography that CAD will be the main cause of death in these countries (Murray et al, 1997). Over the past three to four decades, there has been a phenomenal progress in the understanding of this disease due to the considerable rapport between basic scientists, clinicians, epidemiologists, communities as well as governments in an effort to overcome this modern scourge of the human race. This has led to remarkable successes in not only treating the disease but also preventing it altogether or controlling its natural history (Marmot, 1985). However, the battle is not yet over and for some countries, it is perhaps just beginning (Braunwald, 1997; Yusoff, 2000).

Mortality statistics of Peninsula Malaysia for the period 1950-1989 have been studied in relation to cardiovascular diseases, with particular emphasis on coronary artery disease as an important cause of death. It was observed that among six major disease groups reviewed, cardiovascular diseases which occupied third place as a cause of death in 1950 emerged as the number one killer during the 1970s and has remained so since then (with exception in 1980) (Khoo, 1995).

The United Kingdom (and in particular Scotland and Northern Ireland) has one of the highest death rates in the world from CAD, while rates in Japan and the Mediterranean countries of Europe; such as Greece was low (Tunstall et al, 2000 and Lawlor et al, 2001). The incidence of CAD is also alarmingly high. Each year, an estimated 700,000 Americans will have a new coronary attack. In addition, about 500,000 Americans will have a recurrent attack. Among those who had myocardial infarction, 25 percent of men and 38 percent of women are expected to die within one year.

In spite of the unique fact that 50 percent of Indians are life long vegetarians, they have the highest incidence of coronary artery disease in the world. Presently in India about 25 million (estimated) people are suffering from heart disease. In England, Asian men and women are 40 percent more likely to die of heart disease compared to the local population. In the USA, Indians are 4 times more likely to die of heart disease compared to the American counterparts and 10 times more likely to die of this disease compared to the Japanese (Narinder and Saini, 2006).

1.2 The risk factors of CAD.

Numerous coronary risk factors have been identified, some modifiable, others not. No doubt many more risk factors will be identified in the coming years as the current milieu of coronary risk factors cannot entirely account for the occurrence of CAD. Their eradication in specific community is necessary as it can prevent the onslaught of the disease, or their control can reduce the consequence of the disease on individual patients. Identification of coronary risk factors cannot be a finished business. Yet in Malaysia, perhaps caught on the onslaught of the disease, too much attention is given to treating (sometimes with minimal scientific basis) and too little to prevention. This is ´´fire-fighting´´ with no strategic planning. It is in this setting that the unwavering efforts by the National Heart Foundation of Malaysia through its Heart Week programmes deserve applause. These programmes serve to arouse the awareness and perhaps interest in the population on their health, in particular in relation to heart disease (Yusoff, 2000).

It has been well documented that diabetes mellitus, smoking, hypertension and dyslipidemia are major risk factors for CAD. However epidemiologic and experimental data suggest that activation of the renin-angiotensin-aldosterone system also has an important role in increasing the risk of cardiovascular events (Lonn et al, 1994). The established major risk factors amenable to changes are smoking, high blood pressure, high serum cholesterol and diet (Stamler, 1992).

Table 1.1 Risk factors for coronary artery disease (CAD) (Barker, 2003)

Fixed factors	Exposure	Composite markers of risk
Age Gender Family history Race of origin Genotype	Smoking Physical activity Diet Psychosocial stress In utero experience Infection Climatic factors (e.g high altitude)	Serum lipids Blood pressure Obesity Insulin resistance Hormonal status Homocysteine

1.2.1 Age:

Age is the strongest risk indicator for CAD incidence and mortality (Lioyd-Jones et al, 1999). Compared to men aged 40, 50 year-old-men have five times the risk, 60 years-old-men have 15 times the risk, and 70 years-old-men have over 40 times the risk of dying from CAD. With aging there is a gradual but progressive accumulation of coronary plaques. This accounts for the increasing risk of CAD with advancing age (Scott, 1999). With aging, the aorta and predominantly elastic central arteries dilate; develop thicker walls and stiffer arteries. These changes are progressive from adolescence. However, changes in peripheral muscular arteries are far less marked compared to aorta (O'Rourke, 1994). Stiffening and dilatation of the aorta and elastic arteries with age in human is due to breakdown in the arrangement of musculo-elastic fascicles in the media of the vessels wall (O'Rourke, 1987). These changes occur independently of atherosclerosis and are seen in the absence of significant atherosclerosis (Avolio et al, 1983).

The primary changes appear to be in the elastin fibers and lamellae. The vessel walls thicken with age, mainly due to intima hyperplasia. Elastic arteries in older subjects show the same number of musculo-elastic fascicles, but elastin fibers are thinned and fractured. There is an increase in collagen and other fibers together with increase in intercellular matrix (Virmani et al, 1991). In older subjects, areas of cystic and medial necrosis are frequently seen in the arterial wall, with complete breakdown in the structural arrangement compared to the young (Eagle and Sanctis, 1992).

1.2.2 Gender:

Rates of death attributed to CAD in men are consistently three to four times higher than those in women across countries with differing background level of disease (Mathur and Gajanayake, 1998). As the disease is commoner in men the differences in absolute risk are much greater for men than for women. Men are more likely to smoke than women, but difference in smoking and other established coronary risk factor levels do not appear to fully explain the observed excess of CAD in men. The lower risk of CAD among women has understandably led to studies of sex hormones as potential protective effect for CAD. It has often been stated that women are only protected against CAD before the menopause, and that their risk progressively increases towards that of men after the menopause (Hulley, 1998).

1.2.3 Family history and genetic factors:

A family history of CAD in first-degree relatives is associated with an increased risk of CAD over and above that produced by a shared environment (Bennett, 1996). The family history of CAD is a well-known risk factor in atherosclerosis, and therefore CAD

development; a family history of familial hypercholesteremia increases the risk.

Familial hypercholesteremia is an inherited disorder, which causes a series of mutations in LDL receptors so that they no longer function, causing an excessive amount of LDL cholesterol to be present in the circulation that leads to atherosclerosis and increase risk of CAD (Jairath, 1999).

1.2.4 Diet:

Certain regional dietary patterns are associated with low risk of CAD, for example the Mediterranean diet, which contains more fish, fresh fruit, fresh vegetables, and olive oil than the English diet (Willett, 1994). One small, randomized trial allocated people with a recent myocardial infarction to receive either advice to eat a Mediterranean diet (more bread, more vegetables, more fruit, more fish, and less meat) and to replace butter and cream with rapeseed margarine, or to usual dietary advice. After 27 months the trial was stopped early because there was a marked reduction in the relative risk of death in those given advice to eat Mediterranean diet. Of particular interest is the observation that these dietary changes did not alter blood cholesterol levels, implying that the protective effect was not mediated through an effect on cholesterol (Egger, 1998). Moreover, cohort studies consistently reported a protective association between increased intake of dietary fibers from cereals and reduced rates of CAD (Keys et al, 1986).

1.2.5 Smoking:

Since the results of prospective epidemiological studies in the 1950s it has been clear that heavy cigarette smokers have an approximately two-fold increase in risk of CAD incidence and death, which is reduced by giving up the use of cigarettes, the risk returning to close to

that of non-smokers after about 10 years (Prescoh, 1998 and Edwavdis, 2000). Smoking accounts for some of the international variation in CAD rates, but it is noticeable that some countries with a high prevalence of smoking, such as Japan, have low CAD rates (National Physical Activity Survey, 1999). This is borne out by data from the MONICA study that found that smoking alone (when included in models that adjust for cholesterol and blood pressure) accounts for only 5 percent of the variance in rates of death attributed to CAD across countries (Peterson et al, 1999). It is estimated that exposure to second hand smoke (passive smoking) causes almost 40000 deaths from heart disease each year in the United State (Elantz and Parmly, 1995), and increases the risk of coronary disease and coronary death by approximately 20% (Wells, 1994). Smoking is associated with adverse effects on serum lipids which include elevation of triglyceride (TG) and reduction of high density lipoprotein (HDL), formation of proatherogenic oxidized particles. Also it leads to activation of sympathetic nervous system, enhanced prothrombotic state and endothelial dysfunction (James, 2001).

In large study of middle-aged men in the United States, smokers in the highest quintiles for serum total cholesterol and systolic blood pressure were around 20 times as likely to die from CAD over the next 11.6 years as non-smokers in the lowest quintiles of serum cholesterol and blood pressure (Tunstall, 1999).

1.2.6 Obesity:

For nearly 100 years insurance data have demonstrated that very obese individual have an increase risk of death, including death from heart disease. Obesity is at least partially related to higher CAD risk because it is in turn associated with higher blood pressure and an unfavourable blood lipid profile (high low density lipoprotein (LDL) cholesterol and

lower high density lipoprotein (HDL) cholesterol). Low levels of physical activity and a high caloric intake to physical activity ratio are particular implicated in the rising prevalence of obesity (National Physical Activity Survey, 1999). The distribution of body fat may also play a role in development of CAD, with abdominal adiposity posing a substantially greater risk in both women and men (Wells AJ, 1994). Weight reduction programmes that include a structured exercise component should be considered in primary prevention for all obese (BMI>30kg/m^2) patients and in most over weight (BMI>25 kg/m^2) patients with a history of CAD (Michael, 2001).

1.2.7 Insulin resistance and diabetes mellitus:

A spectrum of metabolic disorder running from frank diabetes to minor level of glucose intolerance is of relevance when considering CAD risk. Examining fasting glucose levels or glucose levels after standardized intake (e.g. in glucose tolerance test) demonstrates this, with some what arbitrary cut-offs being used for "impaired glucose tolerance (IGT)" and type 2 diabetes mellitus (T2DM) (Law et al, 1994). This is not the case with type 1 diabetes mellitus (T1DM), where in most instances people either have or have not the disease, but this is a considerably rare condition that contributes substantially less to the population levels of CAD than T2DM and IGT (Prospective diabetes study group, 1998).

A cluster of physiological risk factors for CAD have been grouped into the insulin resistance syndrome (also known as the metabolic syndrome or syndrome X). These involve resistance to insulin-stimulated glucose uptake, high circulating levels of insulin, high triglycerides levels, low HDL cholesterol level, and elevated blood pressure (Robert, 1995).

The most important environmental factor influencing diabetes risk and level of glucose intolerance is obesity, in particular central obesity. Physical activity also appears to protect against T2DM and IGT (National Physical Activity Survey, 1998).

Unlike other risk factors such as circulating blood cholesterol or blood pressure, fasting glucose does not show a continuous association with CAD risk in prospective epidemiological studies. Only 5 to 10 per cent of the population with high fasting glucose levels are associated with risk of CAD in studies in the United States and United Kingdom. Thus, the use of fasting glucose as a mean of predicting risk in individual is limited (Jarrett, 1996).

1.2.8 Physical activity:

In the early days of the CAD epidemic in industrialized countries it was thought that exercise may predispose to CAD through cardiac strain. However, research starting in the mid-twenty century suggested that occupational physical activity was associated with reduced risk of CAD. With the increasing sedentary nature of many occupations this has become a less important risk factor for CAD and attention has shifted to physical activity in leisure time (Berlin and Colditz, 1990).

Physical activity results in increased exercise capacity and physical fitness, which may lead to many health benefits. Individuals who are more physically active appear to have lower rates of all-cause mortality, probably due to a decrease in chronic diseases, including CAD. This low rate may result from an improvement in cardiovascular risk factors, as it helps in control of blood cholesterol, diabetes and obesity, as well as help to lower blood pressure, enhanced fibrinolysis, improved endothelial function and decreased sympathetic tone (Lowensteyn et al, 2000).

1.2.9 Stress:

Stress is widely considered by the general public to be an important cause of CAD. Indeed, several surveys of lay beliefs have shown that it is one of the most widely recognized risk factors for the disease. People with little control over their work (e.g. shift workers in a factory) are; almost by definition; in less favorable social situation than those with greater control over their work (e.g. managers or senior academics) (Harding, 1995).

1.2.10 Cholesterol:

Circulating blood cholesterol is strongly and positively associated with CAD (Rosenson, 1998 and Michael, 2001). A series of large-scale, randomized, controlled trials of blood cholesterol reduction demonstrate that this process is reversible and risk reduction of the magnitude predicted from the observational data can be produced through lowering blood cholesterol (Rosenson, 1998). It has been shown that lowering serum cholesterol by 10 percent reduces the risk of CAD death by 15 percent (Guld et al, 1998). Treatment for more than 5 years yields a 25 percent reduction in CAD events, thus, long term compliance is important for successful intervention (Law, 1994).

One issue not directly relevant to CAD that led to controversy regarding cholesterol reduction was the suspicion that low circulating blood cholesterol caused an increased risk of morbidity and mortality from non-coronary causes, including cancer, psychiatric disease, and gastrointestinal and respiratory diseases. Observational studies tended to report inverse association between blood cholesterol and these conditions, though it was clear that these associations could be generated through early stages of ill-health or adverse health-related behaviours leading to lower circulating cholesterol levels. Finding from randomized

controlled trials of cholesterol reduction were initially ambiguous, in that there was evidence of elevation of non-coronary morbidity and mortality in some studies. This now appear to reflect specific adverse effects of certain cholesterol lowering drugs, in particular the fibrates. More recent studies in which circulating cholesterol levels were reduced more profoundly than in earlier studies, through the use of statins, suggest there are no detrimental effects of cholesterol lowering itself (Graham, 1997 and Farnier, 1998).

Based on the evidence provided by recent clinical studies, the benefits provided by statins cannot be fully explained by their cholesterol lowering effects alone, and that other factors must be involved (Nissen, 2004). The pleiotropic effects of statins include antithrombotic actions, an increase in fibrinolysis, anti-inflammatory effects, and a decrease in smooth muscle cell proliferation and migration (Pierre-Paul and Gahtan, 2003).

Familial hypercholesterolaemia is an important condition illustrating the impact of raised serum lipids on the incidence of coronary artery disease; the monozygotes tend to get artery disease early with myocardial infarctions in their teens or early twenties (Brown et al, 1986). A number of mutations of the gene encoding for the LDL receptor proteins have been identified (Hobbs et al, 1992).

1.2.11 Blood pressure (BP):

There is a strong, consistent dose-response relationship between increased casual blood pressure measured in middle age and increased risk of CAD. The effects on arterial stiffness: the first effect is functional and reversible, the second effect is structural and irreversible (Nicholas and O'Rourke, 1990). The first affects all arteries (both elastic and

muscular), while the second, as with aging, has its greatest effect on the aorta and elastic arteries. The central arteries pulsate greatly and are principally responsible for cushioning function of the arterial tree. Long standing hypertension accelerates arterial degeneration. The aging changes occur prematurely in hypertensive subjects and are attributable to the same process. High strains on the arterial wall results in fracture of elastin fibers and lamella occurring at an earlier age than in normotensive subjects. The aorta and elastic arteries of patients with chronic hypertension are stiffer than in normotensive controls, even when measured at the same distending pressure (Ting et al, 1990). Systolic blood pressure(SBP) is at least as powerful a coronary risk factor as the diastolic blood pressure(DBP) in increasing risk of CAD and isolated systolic hypertension is now established as a major hazard for CAD and stroke (Wilking, 1988). The excess coronary risk associated with hypertension is primarily in subgroups with other risk factors or underlying target organ damage. These patients benefit the most from antihypertensive therapy. An enhanced risk for cardiovascular events also appears to be associated with increase in pulse pressure (Wilson, 2001).

In observational studies a 10 mmHg increase in diastolic blood pressure is associated with a 37 per cent increase in the risk of CAD. Large, randomized trials of pharmacological blood pressure reduction in middle age confirmed that blood pressure reduction reduce subsequent risk of CAD (Collins, 1990). Several observational studies have also shown that increased blood pressure in early adult life, suggesting that risk trajectory may be set early. Blood pressure is continuously distributed in population and increase in blood pressure across the range of blood pressure measures are associated with increased risk of CAD (Rose, 1992).

Blood pressure is determined both by environmental factors, such as increased sodium intake (increase blood pressure); increased alcohol intake (increase blood pressure); increased fruit and vegetables intake (lower blood pressure), and genetic factors. While individual blood pressure response to environmental factors may be influenced by genetic factors, migrant studies illustrate the substantial and relatively rapidly acting effect of environmental factors. In one study of migrants from a rural community in Western Kenya to urban Nairobi, mean diastolic blood pressure increased by around 6-10 mmHg from that measured previously (Powles, 1996).

1.2.12 Haemostatic factors:

Over the last 20 years a number of cohort studies have examined the association between haemostatic factors and CAD. There is a consistent, independent association between increased fibrinogen levels and increased risk of CAD (Heinrich, 1995). The subsequent risk of CAD comparing those in the highest third with those in the lowest third of the fibrinogen distribution at base line is increased by around 80 per cent (Sweetman, 1996). Some prospective studies have reported increased coronary risk with factors VII and VIII but the data are less extensive and less consistent (Scarabin, 1998). Other studies have reported association with tissue-type plasminogen activator (t-PA) and with plasminogen activator inhibitor-1 (PAI-1); while platelet function tests do not appear to predict subsequent coronary risk (Naito, 1990). Smokers have higher fibrinogen levels than non-smokers and this may in part explain their excess coronary risk. Fibrinogen levels are also associated with higher levels of other acute phase reactants suggesting that chronic inflammatory or infective processes may increase fibrinogen levels and thus risk of symptomatic CAD (Rosenson, 1993). Increase fibrinogen level and high C-reactive protein

(CRP) are associated independently with a variety of cardiovascular end points in unhealthy and apparently healthy patients. Fibrinogen is involved directly in coagulation, and CRP is a sensitive marker of inflammation and tissue damage. In patients with stable angina, fibrinogen and CRP levels are predictors of cardiac events. A CRP level of at least 3 mg per L (28.6 nmol per L) predicts more ischemic episodes and the need for revascularization procedures; fibrinogen also has prognostic value in this circumstance (Sadovesky, 2004). CRP has prognostic utility in patients with acute coronary syndromes and is a strong independent predictor of future coronary events in apparently healthy subjects (Rifai and Ridker, 2001).

1.2.13 Homocysteine:

Homocystinuria refers to a group of rare inborn errors of metabolism resulting in high levels of circulating homocysteine (10µmol per liter) and urinary homocysteine. A characteristic feature in patients with this condition is premature vascular disease. If homocystinuria is untreated, about 50 percent of patients have thromboembolic events, and mortality is about 20 percent before the age of 30 years (Mudd et al, 1985).

Observations in patients with homocystinuria led to the idea that homocysteine may be involved in the pathogenesis of atherosclerosis and prompted a large number of epidemiologic studies of the relation between moderately elevated Homocysteine levels and vascular disease (Verhoef and Stampfer, 1995 and Brattström, 1996).

Elevated plasma homocysteine is a risk factor for CAD, but the prognostic value of homocysteine levels in patients with established CAD has not been defined (Glueck et al, 1995 and Soinio et al 2004).

1.3 Confirmatory tests to diagnose CAD:

The emphasis on early diagnosis and prevention of ischemic heart disease has stimulated a search for reliable noninvasive methods of detection (Epstein, 1971; Moore, 1971 and Friedberg, 1973). Risk factor screening and resting electrocardiography (ECGs) are useful in epidemiologic and mass screening programmes but are not diagnostically helpful in the asymptomatic subject. Although exercise electrocardiography is widely used as a noninvasive procedure to diagnose CAD, the large proportion of false-positive and false-negative results precludes its use as a standard screening device in the asymptomatic person (Borer et al, 1975; Redwood et al, 1976 and Froelicher et al, 1977). Clinical or laboratory markers that might identify those with asymptomatic coronary artery disease would be useful. Previous reports have established the positive relationship between the presence of coronary calcification on fluoroscopy and angiographically demonstrated CAD in symptomatic patients (Oliver, 1964).

Exercise stress testing, exercise Thallium 201 imaging, and coronary angiography are established methods of evaluating patients with suspected CAD. In addition to providing diagnostic information, these tests have been shown to yield important prognostic information (Ladenheim et al, 1986 and Kaul et al, 1988).

The standard electrocardiographic treadmill exercise test is an established clinical procedure for detecting and evaluating patients with coronary artery disease (Fortuin and Weiss, 1977). As a diagnostic tool, however, the procedure is limited by the somewhat low sensitivity and specificity of electrocardiographic ST-segment depression as a marker of ischemia. Thus, when the procedure is used in a population with a low prevalence of disease, a very high false-positive rate is inevitably observed (Hartley, 1975).

1.3.1 Non-invasive tests:

1.3.1.1 Electrocardiography (ECG):

The electrocardiogram (ECG) is a graphic recording of the electrical potentials generated by the heart. The signals detected by means of metal electrodes attached to the extremities and chest wall and are then amplified and recorded by the electrocardiograph. The basic ECG waveform consists of three deflections termed P wave, QRS complex and T wave. The P wave is the surface electrocardiographic manifestation of atrial depolarization. The QRS complex is the surface electrocardiographic manifestation of ventricular depolarization. The S-T segment and T wave represent ventricular myocardial repolarization. The atrial repolarization is indicated by the Ta wave, which is small, asymmetrical negative wave following the P wave, usually obscured by the QRS complex which occurs at the same time (Rowland, 1991).

The clinical utility of the ECG derives from its immediate availability as a non-invasive, inexpensive, and highly versatile test. In addition to its use in detecting arrhythmias, conduction disturbance, and myocardial ischemia, electrocardiography may reveal other findings related to life-threatening metabolic disturbances (e.g., hyperkalemia) or increased susceptibility to sudden cardiac death (e.g., QT prolongation syndromes). An abnormal electrocardiogram increases the suspicion of significant CAD, but a normal result does not exclude it (Mintz et al, 2001).

1.3.1.2 Exercise ECG:

The present-day use of the exercise stress electrocardiogram in the diagnosis of CAD (in the form of the graded-exercise stress test-GXT) has evolved as a result of numerous observations and development (Fletcher et al, 1995). Although the exercise ECG may be used for several purposes, it is commonest in the diagnosis and assessment of CAD. In this respect, however it is extremely important at the outset to recognize that the test has a significant false-negative rate, even in population with an appreciable prevalence of CAD, and that the false-negative rate may be unacceptably high in populations with a low prevalence. The test is therefore of very limited value in screening low-risk, asymptomatic subjects. Most subjects who have undergone exercise stress testing as a screening procedure and who subsequently experience sudden cardiac death are found in retrospect to have had a normal exercise test result (Ellestad et al, 1996.).

A meta-analysis of 147 consecutive studies involving a total of 24074 patients who had undergone both exercise stress testing and coronary angiography revealed sensitivity ranging from 23 to 100 per cent (mean 68) and specificity ranging from 17 to 100 per cent (mean 77). In patients with multi-vessel coronary disease the sensitivity ranged from 40 to 100 per cent (mean 81) and the specificity from 17 to 100 (mean 66). For patients with single-vessel disease a positive GXT is most likely for lesions in the left anterior descending artery. Patients with lesions in the circumflex artery are least likely to give a positive result while those with lesion in the right coronary artery occupying intermediate position (Chaitman, 1997).

Inevitably, a GXT carries a risk, but multiple studies have shown the risk to be remarkably low. In 1971 a survey of 73 medical centers summarized the risks in relation to approximately 170000 stress tests. A total of 16 deaths were reported (Mortality rate 0.01 per cent), and 0.04 per cent required admission within 24 hours because of arrhythmia or prolonged chest pain. The risk is greater when the test is conducted after ischemic events (Ellestad et al, 1996 and Chaitman, 1997).

1.3.2 Invasive tests:

1.3.2.1 Stress Echocardiography:

Stress Echocardiography can be used to detect occult CAD and predict cardiac risk, whilst the administration of contrast agents may allow visualization of myocardial perfusion. Imaging can be performed either during or immediately after exercise, but more commonly an intravenous infusion of dobutamine is used to mimic the cardiac response to exercise. Development of reversible systolic regional wall motion abnormalities suggests CAD and stress echo is used increasingly as a diagnostic test in patients with chest pain (Oh, 1999 and Flachskampf et al, 2001).

1.3.2.2 Myocardial perfusion imaging:

Myocardial perfusion imaging is undertaken using an injection of a radiopharmaceutical agent that is taken up by the myocardium in proportion to the myocardial blood flow at the time of the injection and which remains in the myocardial cells during the period of the imaging. Studies are performed both at rest and under stress, in order to evaluate differing regional perfusion in these two states. The three compounds mainly used in current clinical

practice are Thallium201, technetium-99m-Cardiolite (sestamibi-MIBI), and technetium-99m-Myoview (tetrofosmin) (Travin et al, 1999).

In patients with known CAD the main indications include:

1. Evaluating the functional significance of coronary stenosis detected by angiography.
2. Assessment of the most significant functional stenosis in patients with multiple coronary lesions;
3. Evaluation of post infarction patients to establish the size of the infarct and the presence or absence of ischemia in other areas of the myocardium.

It is also of value in the investigation of restenosis after revascularization with angioplasty or coronary artery bypass grafting (Rigo, 1998).

1.3.2.3 Coronary artery angiography:

Coronary arteriography or angiography is presently the single most essential application of cardiac catheterization. The vast majority of patients presenting for cardiac catheterization have CAD. Angiography of the coronary arteries performed during cardiac catheterization is essential for patients in whom revascularization is indicated. Coronary angiography is indicated for patients having chronic stable angina that persist in spite of reasonable efforts at pharmacological therapy. It is also indicated for patients whose survival would be improved, regardless of symptoms. Such patients are those with severe stenosis of the main left coronary artery and those with severe two-and three-vessels CAD in combination with impaired left ventricular function. Depending upon the availability of emergency revascularization, patients having acute myocardial infarction may be best served by immediate catheterization. Finally, catheterization is sometimes indicated for obtaining a definitive diagnosis when non-invasive testing has yielded equivocal or inconsistent results (Baim et al, 2000).

1.4 Arterial stiffness:

The blood vessels represent the largest structure in the body. It consists of a complex network of branching elastic tubes, which at its arterial origin receives blood in spurts from the heart and transmits it downstream to the arterioles, capillaries, venules and veins before returning to the heart.

The mechanical properties of the artery are dependant on its structure. Arteries are divided histologically into three layers; the innermost layer is tunica intima, intermediate layer is tunica media and the outermost layer tunica adventitia. The tunica intima consists of endothelial cell layer, sub-endothelial connective tissue layer and the elastic tissue layer (internal elastic membrane). The tunica media is composed of smooth muscle fibers and variable amount of collagen and elastic tissue.

The mechanical properties of arteries depend on the tunica media which consist of elastic fibers, collagen fibers and smooth muscles. At low level of pressure within the artery, the mechanical load is borne predominantly by the elastic fibers that are readily stretchable. As the pressure increase, the collagen fibers are progressively recruited and arterial wall stiffness increase. Therefore, at high pressure, the collagen fibers are fully recruited and the artery behaves as though it was composed of collagen alone (Glagov et al, 1992).

The mechanical properties of elastic and collagen fibers are modified by smooth muscle in the arterial wall. Muscle contraction and increase in tone transfer stress from elastic to the collagen fibers, making the arterial wall stiffer, whereas decrease in muscular tone unloads

the collagen fibers and transfers stress back to the elastic fibers so that the artery appears to be more complaint and less stiff (Gow, 1980). The endothelium plays an important physiologic modulator of arterial smooth muscle tone. Release of endothelial vasoconstrictor and dilator substances provide an important local control mechanism that is modulated by reflex and hormonal influences that change with age and disease.

The arterial system has two functions: first is to act as a conduit, transmitting blood to tissue according to need; second is to act as a cushion, smoothing the pulsation imposed by intermittent cardiac ejection so that flow through the tissues occurs in a steady continuous stream (O'Rourke, 1982). Disorder of the cushioning function like arterial stiffness has the effect on the heart upstream.

Arterial stiffness is a part of endothelial dysfunction and therefore it explained the various associations and represents a valuable predicator of cardiovascular disease (Weber et al, 2003). There are several independent risk factors associated with arterial stiffness; these include male gender, hypertension, diabetes mellitus, hypercholesterolemia, increasing age, CAD, atherosclerosis, renal failure and cerebro-vascular disease (Nicole et al, 2001). Arterial stiffness is also correlated with atherosclerosis, probably through the effects of cyclic stress on arterial wall thickening. There are several possible explanations for the observed association between arterial stiffness and atherosclerosis that can be hypothesized. One possibility is that presence of atherosclerosis leads to stiffening of the arteries. An alternative possibility is that increased arterial stiffness leads to vessel wall damage and atherosclerosis. A third possibility is that both mechanisms apply and that atherosclerosis is not a consequence of arterial stiffness but in advance stages also increase arterial stiffness

(Nicole et al, 2001). In subjects with and without cardiovascular disease, arterial stiffness correlates with diastolic blood pressure, heart rate, height and gender.

Arterial stiffness is a cause of premature return of reflected waves in late systole, increasing central pulse pressure (PP) and the load on the ventricle, reducing ejection fraction, and increasing myocardial oxygen demand. Arterial stiffness is associated with left ventricular hypertrophy, a known risk for coronary events, in normotensive and hypertensive patients. Stiffening of the arterial tree leads to an increased systolic blood pressure and simultaneously a decreased diastolic blood pressure (London and Guerin, 1999). The increased systolic blood pressure has a negative effect on the heart due to increased workload, while a reduced diastolic blood pressure may limit coronary perfusion, therefore will lead to myocardial ischemia. The effect may explain the association between arterial stiffness and myocardial infarction (MI), as observed in cross-sectional studies (Benetos et al, 1997; Millar, 1999). Increased arterial stiffness is also a risk factor for stroke (Domanski et al, 1999; Somes et al, 1999).

Arterial stiffness may predict CAD beyond classical risk factors (Benetos et al, 1997; Millar, 1999). Aortic stiffness was determined from carotid-femoral pulse wave velocity at baseline in 1045 hypertensive. The risk assessment of CAD was made by calculating the Framingham risk score according to the categories of gender, age, blood pressure, cholesterol, diabetes, and smoking. Framingham score significantly predicted the occurrence of coronary and all cardiovascular events in this population ($P<0.01$ and $P<0.0001$, respectively). In a multivariate analysis, pulse wave velocity remained significantly associated with the occurrence of coronary event after adjustment either of

Framingham score (for 3.5 m/s: relative risk, 1.34; 95% CI, 1.01 to 1.79; *P*=0.039) or classic risk factors (for 3.5 m/s: relative risk, 1.39; 95% CI, 1.08 to 1.79; *P*=0.01) (Pierre et al, 2002).

There are several different methods of assessing arterial stiffness, such as pulse pressure, pulse wave velocity (PWV), waveform analysis, ultrasound derived indices and magnetic resonance imaging (MRI) derived indices.

1.5 Role of pulse wave velocity:

The pulse wave velocity is the speed at which the forward pressure wave is transmitted from the aorta through the vascular tree. It is calculated by measuring the time taken for the arterial waveform to pass between two points a measured distance apart, and involves taking readings from the two sites simultaneously, or gating separate recordings to a fixed point in the cardiac cycle, usually the R wave of the ECG. Various different methods have been used, both invasive and non-invasive, and can be applied to either flow or pressure waves.

Problems with this technique include the inaccessibility of the central arteries, necessitating compromise by using the nearest superficial arteries. There can also be some difficulty in estimating the actual arterial distance between recording sites using only surface measurements. The pulse wave velocity becomes less accurate if the recording points are very close together, and the technique is, therefore, limited to use on the larger arteries. PWV is affected by respiratory variability. This is because respiration modifies the intra-thoracic pressure, the vessel wall tension and arterial blood pressure. For this reason, the