CHAPTER 1
INTRODUCTION

Coronary artery disease (CAD) is the leading cause of death worldwide. It has reached enormous proportions striking more and more at younger subjects and has transformed as the greatest epidemic that mankind has ever-faced. Moreover, people hailing from Indian subcontinent have a higher probability of dying due to CAD. In the past three decades, CAD rates have doubled in rural areas and tripled in urban areas of India (Chopra and Wasir, 1998; WHO, 2009). This alarming trend has to be reversed by intense research into its cause and prevention.

CAD occurs when the arteries that supply blood to the heart muscle, harden and narrow (Bhati, 2011; Heart diseases, 2011). This results in the loss of oxygen and nutrients to myocardial tissue because of diminished coronary blood flow. The reduction in blood flow can lead to acute coronary syndrome.

Atherosclerosis is the usual cause of CAD. In this condition (Figure 1), fatty, fibrous plaques (Figure 2), including calcium deposits, narrow the lumen of the coronary arteries; thereby reducing the volume of blood flowing through them, and leading to myocardial ischemia. Plaque formation (Figure 3) also predisposes to thrombosis, which can provoke myocardial infarction (MI) (Lippincott and Wilkins, 2005). Atherosclerosis usually develops in high-pressure arteries, such as those in the heart, brain, kidneys, and in the aorta, especially at bifurcation points.
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Figure 1: Simplified schema of diversity of lesions in human coronary atherosclerosis (Libby and Theroux, 2005).

Figure 2: Determinants of thrombosis in coronary atherosclerotic plaques. Formation, extent, and duration of coronary thrombi produced by mechanisms such as those outlined in Figure 3 depend on both solid-state factors in plaque itself and fluid-phase determinants in blood (Libby and Theroux, 2005).

Dissecting aneurysms, infectious vasculitis, syphilis, and congenital defects in the coronary vascular system are the uncommon causes of reduced coronary artery blood flow (Lippincott and Wilkins, 2005; Heart diseases, 2011). The American Heart Association model of the coronary tree is described in Figure 4.
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**Figure 3:** Formation and maturation of the young atherosclerotic plaque (Lopez, 2010)

1.1. Prevalence of CAD

1.1.1. Global Scenario

The term 'prevalence' of CAD usually refers to the estimated populations who have CAD at any given time (Rosamond, et al., 2007). According to WHO (2006) 16.7 million people dies of CAD around the globe each year accounting over 29 percent of all deaths globally.

The proportion of deaths due to CAD is projected to rise from 59% in 2002 to 69% in 2030. 20 million people survive CAD related heart attacks and strokes every year around the world; many requiring continuing costly clinical care (WHO, 2006). By 2030, almost 23.6 million people will die from CAD, and projected to remain the single leading cause of death.

According to the Global Burden of Disease Study, the developing countries contributed to 3.5 million of the 6.2 million global deaths from CAD every year. The projections estimate that these countries will account for 7.8 million of the 11.1 million deaths due to CAD in 2020 (WHO - Cardiovascular Disease: Prevention and Control, 2006). WHO (2009) offers 1st position for Turkmenistan (456 deaths per 100,000) and 31st position to India (207.7 deaths per 100,000), but the developed countries are placed at very low rates.

1.1.2. Global Burden and Projection of CAD

Disability-adjusted life years (DALYs) reveal the healthy years of life lost and indicate the total burden of a disease. CAD is responsible for 10% of DALYs lost in low and middle income countries and 18% in high income countries.
CAD is decreasing in many developed countries, but increasing in developing and transitional countries, partly as a result of increasing longevity, urbanization and lifestyle changes. Risk of heart attack can change when people migrate. Japan has a low rate of CAD, but Japanese who have migrated to the USA, found to have a gradually increasing risk, eventually approaching that of people born in the USA. CAD burden is projected to rise from around 47 million DALYs globally in 1990 to 82 million DALYs in 2020 (Global burden of coronary heart disease, 2002).

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Risk</th>
<th>Deaths</th>
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<tr>
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<td>7,185,353</td>
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<tr>
<td>Stroke</td>
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</tr>
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<td>Stomach Cancer</td>
<td>17</td>
<td>602,203</td>
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</table>

**Figure 5**: World ranking of Cause of Deaths (WHO, 2009; www.worldlifeexpectancy.com)

As per WHO (2006) estimate, approximately 3.8 million men and 3.4 million women worldwide die each year from CAD. In 1990, 47% of all CAD-related deaths in developing countries occurred before the age of 70 years, in contrast with only 23% in high income industrialized countries. In an estimation of WHO (2009) on the cause of death in 2009, CAD has been placed in first position with 7,185,353 deaths and Stroke in second position with 5,704,843 deaths among total deaths (6,830,586,985) of all kind of diseases (Figure 5).
1.1.3. Indian Scenario

The prevalence of CAD in India is more than doubled in the past 2 decades. This is so in both rural and urban population, although it is higher in urban population, with a greater prevalence in affluent group. The prevalence of classical CAD risk factors such as high total cholesterol concentration, hypertension, obesity and lifestyle factors such as high saturated fat intake and smoking, tend to be lower in South Asian Indians than Western population (McKeigue, Shah and Marmot, 1991). Earlier studies in UK, USA, Canada, and Trinidad showed that migrant Indians had higher rates of CAD compared to the indigenous population. It is consistently observed that Indians have premature CAD. The median age of first heart attack among Europeans is 59 years, and 60 among Chinese while in Indians it is 50 years; and that their risk for CAD was two to four times higher than the white European population (Enas, Yusuf and Metha, 1992; McKeigue, et al., 1993). India is predicted to bear the greatest CAD burden, according to the estimates from the Global Burden of Disease Study (Murray and Lopez, 1996).

In India, 9.2 million productive years of life were lost in 2000, with an expected increase to 17.9 million years in 2030 (Leeder, Raymond and Greenberg, 2004). SHARE study showed a CAD prevalence of 10.7% among South Asians compared to 4.6% in Europeans (Anand, et al., 2000; Bahl, Prabhakaran and Karthikeyan, 2001). The Chennai Urban Population Study (CUPS) carried out in 1262 individuals, > 20 years of age showed the crude prevalence of CAD to be 11% while the age-adjusted prevalence rate was 9.0% (Mohan, et al., 2001). Remarkably high prevalence of both CAD and cardiovascular risk factors was also
demonstrated in the Jaipur Heart Watch study, on 1800 subjects based on a stratified sampling technique (Gupta, et al., 2002). Few studies carried out to determine the precise cause of death in urban Chennai has revealed that CAD causes about 40% of the death in urban area and 30% in rural area (Mohan, et al. 2001, 2010a and Mohan Venkataraman and Pradeepa 2010b). This still does not account for all of the elevated risk of CAD in Indians compared to Western population. As per the estimations of National Commission on Macroeconomics and Health (NCMH), a government undertaking, there would be approximately 62 million patients with CAD by 2015 in India and among them, 23 million would be patients younger than 40 years of age (Indrayan A, 2005).

1.2. Need for the estimation of risk factors for CAD

Approval of the critical role of risk factors in the development of CAD is one of the most remarkable advances in the understanding of this major disease which is expanding in pandemic proportions. Coronary risk factors such as hypertension, diabetes mellitus, hypercholesterolemia, tobacco consumption and obesity are major health problems in developed as well as in developing economies. Multiple biochemical modifications have been described in diabetes and CAD, and a number of metabolic pathways seem to be implicated in glucose toxicity, with a probable redundancy in their mechanisms (Dutour A, 1997).

1.2.1. Plasma Glucose Levels

Plasma glucose has been shown to have a continuous gradient relationship with CAD both in the diabetic and non diabetic range. Increased plasma glucose
concentration leads to increased glycosylation of proteins, particularly lipoproteins. Glycosylation of low-density lipoprotein-cholesterol (LDL-C) has been shown to enhance its vulnerability to oxidation, which triggers the atherosclerotic processes.

The Bedford Study, Honolulu Heart Study and the Pathological Determinants of Atherosclerosis in Youth Study are some of the studies that have clearly demonstrated the association of hyperglycemia with CAD (Jarrett, Mc Cartteny and Keen, 1982; Donahue, et al., 1987; McGill, et al., 1995). Diminution of CAD events with tight glycemic control by means of insulin was shown in the randomized trial of insulin–glucose infusion followed by subcutaneous insulin treatment in diabetes patients with acute MI (Malmberg, et al., 1995) which indirectly proves the relationship of hyperglycemia with CAD. In the CUPS, prevalence of CAD was seen to augment with increase in fasting plasma glucose levels, even among non-diabetic subjects. The odds ratio (OR) for CAD increased with increase in quartiles of fasting plasma glucose and 2 h post-glucose load, indicating a strong association of plasma glucose levels with CAD, which also reveal that in Indians, as shown in the West, the clock for CAD starts “ticking” even at the impaired glucose tolerance stage itself. It also indicates that the plasma glucose–CAD association is a continuum and that there is no threshold value of risk (Balkau, et al., 1998).

1.2.2. Blood Pressure

The high risk for CAD among hypertensive subjects has been documented in several studies (Barnard, R. J, Martin, D. A., Ugianskis, E. J. and Inkeles, S. B., 1992a, 1992b and Barnard, Jung and Inkeles, 1994). Further, intervention studies
using antihypertensive have shown striking decrease in CAD risk. The overall prevalence of hypertension in the CUPS was 22.1%, of which 8.2% had “known” hypertension. CAD was much more prevalent among hypertensives than normotensives. The CAD risk was even higher among subjects with both diabetes and hypertension (OR 3.13, P= 0.004). Both systolic and diastolic blood pressure showed a strong correlation with CAD on a univariate analysis in the CUPS study (Estacio, et al., 1998; Mohan, et al., 2001; Lindholm, et al., 2002).

1.2.3. Dyslipidemia

Dyslipidemias, which refers to a constellation of abnormalities like high serum cholesterol, serum triglycerides, LDL-C and low high-density lipoprotein-cholesterol (HDL-C), are known to be associated with diabetes. Several intervention studies have clearly shown reduction in CAD mortality through reduction of serum cholesterol and triglyceride levels (Heart Protection Study Collaborative Group 2002). However, the association of isolated hypertriglyceridemia with CAD is still a matter of debate (Gotto, 1998). HDL-C, in contrast to LDL-C, is a protective lipoprotein with anti-atherogenic potential. It is important to note that, in CUPS, subjects with CAD had lipid levels that were much lower than the high-risk category as described by National Cholesterol Education Program guidelines (NCEP, 2001). Indians are known to have much lower HDL-C levels, and hence the total cholesterol/HDL-C and LDL-C/HDL-C ratios are higher in Indians (Mohan, et al., 2001). Previous studies have emphasized the role of small dense LDL-C in atherogenesis and have shown that diabetic subjects have higher levels of small dense LDL-C compared to non-
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diabetic subjects (Kulkarni, et al., 1999). The Chennai Urban Rural Epidemiology Study (CURES) conducted in South Indians showed that small dense LDL-C levels were higher in diabetic patients and even higher in diabetic subjects with CAD (Mohan, et al., 2005).

1.2.4. Metabolic Risk Factors

Type 2 diabetes is a constellation of several metabolic aberrations, and most of these are preceded by insulin resistance. Fasting insulin levels have been found in prospective studies, to be a surrogate marker of insulin resistance (IR) and a predictor of CAD. Most of the cardiovascular risk factors like dyslipidemia, hypertension, obesity, central obesity, and glucose intolerance have been shown to be associated with IR, and a combination of these abnormalities could lead to CAD (Pyorala, et al., 2000; Reaven. 1993). The metabolic cluster appears to explain a major part of the pathogenesis of CAD. In CUPS, the CAD risk increased with increase in the number of metabolic abnormalities (Mohan, et al., 2001). The same study also assessed the prevalence of IRS (Insulin receptor substrate) using the European Group of Insulin Resistance (EGIR) criteria and found that IRS was present in 11.2% of urban South Indians. It is to be noted, however, that this figure of 11.2% was based on the higher cutoff points that the EGIR recommends for dyslipidemia, i.e., serum triglyceride levels more than 200 mg/dl and/or serum cholesterol levels greater than 200 mg/dl. Clustering of these metabolic parameters was evident even among young individuals. This clustering effect suggested that body fat, diet, physical inactivity, and stress are important contributory factors for high prevalence of metabolic syndrome in Indians, and the phrase “cardio-
metabolic syndrome” is used for this entity (Joshi, 2004; Misra and Vikram, 2004; Misra, et al., 1999).

Asian Indians have higher prevalence of hyperinsulinemia, IR and other components of metabolic syndrome. Obesity, particularly abdominal obesity, is considered to contribute to the increased IR in Indians. Though Indians have low rates of generalized obesity, the prevalence of abdominal obesity is higher when compared to other ethnic groups. Further, for any given degree of obesity, Indians also have higher body fat than other ethnic groups and for any given body mass index, the waist-to-hip ratio was higher among Indians. Moreover for any given body fat, Indians have higher IR in contrast with other ethnic groups (Ramachandran, et al., 1997; Chandalia, et al., 1999; Banerji, et al., 1999).

1.2.5. Lipoprotein (a)

Lipoprotein (a) [Lp(a)], an atherothrombogenic moiety, is a complex of apolipoprotein (a) [Apo(a)] and LDL-C, which is determined genetically (Velmurugan, et al., 2003; Mohan, et al., 2001). It can competitively inhibit plasminogen activity, leading to impaired fibrinolysis. Lp(a) has also been implicated in enhanced oxidation and foam cell formation. Smaller the Apo (a), higher the Lp(a) levels and the risk for CAD. Lp(a) levels above 20 mg/dl are reported to be associated with a high risk of CAD (Von Eckardstein, et al., 2001).

1.2.6. Inflammatory Markers

There is ever-increasing evidence that inflammatory processes and specific immune mechanisms are implicated in atherogenesis, and inflammatory markers
are reported to be higher among subjects with IR and diabetes (Jialal and Devaraj, 2001). Inflammation is considered to be a component of insulin resistance syndrome (Festa, et al., 2000), and this, at least moderately, explains the high risk for CAD among diabetic subjects. Inflammatory changes could take place in close proximity to the rupture of the plaque, causing instability in the fibrous tissue in the plaque. Studies on pro-inflammatory markers have revealed that cytokines like tumor necrosis factor α (TNF-α), C-reactive protein (CRP), and interleukin-6 are strongly associated with CAD. CRP also had a strong association with cardiovascular risk factors like obesity, IR and lipids (Chambers, et al., 2001). Asian Indians were shown to have elevated CRP levels, suggesting that pro-inflammatory factors may contribute to increased risk for diabetes and CAD. Asian Indian children were also shown to have 104% higher levels of CRP compared to Europeans. Few studies illustrate that CRP associated significantly with body fat (Cook, et al., 2000; Chandalia, et al., 2003; Mohan, et al., 2005). However, the data related to the association of inflammatory markers in an angiographically proven CAD with and without type 2 DM in a larger South Indian population is lacking.

1.3. Need for the study

CAD rates in India are rising (Gupta and Gupta, 1996) and projected statistics show that it will be the leading cause of mortality by 2015 (Bulatao and Stephens, 1992). These findings are not completely explained by the prevalence of classical CAD risk factors, all of which tend to be lower in South Asian Indian than Western population (McKeigue, Shah and Marmot, 1991, McKeigue, et al., 1993).
A cost-effective preventive stratagem is the need of the hour with a focus on reducing risk factors both in the individual and in the population at large. However, a pivotal factor that hinders the development of such preventive measures in developing countries like India is the scarce amount of (8%) published data on cardiovascular diseases research available from these countries (Mackay and Mensah, 2004). A great deal of the data on risk factors for CAD has been obtained from studies carried out in Western population. It is widely assumed that the involvement of these risk factors with the development of CAD in other populations needs to be ascertained, and there is a hypothesis that the disparity might range from the frequency of presence of conventional risk factors with CAD to their total non-existence or insignificance in these populations; the established risk factors like advanced age, male gender, diabetes, family history of premature CAD, hypertension, dyslipidemia are largely absent in approximately half of those who develop cardiovascular diseases globally (Braunwald E, 1997). Thus improvement in risk assessment is, therefore, dependent on identification of additional factors to identify more effectively those at risk who might benefit from aggressive preventive health measures. Consensus panels assembled by the National Heart, Lung, and Blood Institute and the Centers for Disease Control and Prevention have concluded that population-based data on the risk factors are urgently needed (Centers for Disease Control, 2002).

There is now considerable substantiation from epidemiological and treatment studies supporting novel risk factors like apolipoproteins; higher Apo B and lower Apo AI levels (higher Apo B / Apo AI ratio) are proved to be highly significant
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risk markers of future coronary risk (Van Lennep, et al., 2000; Liu, et al., 2005; Jiang, et al., 2004; Walldius, et al., 2001; Walldius and Jungner, 2004, 2006; Sharp, et al., 2000; Francis and Frolich, 2001; Nissen, et al., 2003; Schlitt, et al., 2005). The cholesterol balance as determined by the Apo B/Apo AI ratio has repetitively been shown to be a better marker than lipids, lipoproteins and lipid ratios in the Western population. The results indicate that the Apo B/ Apo AI ratio is a simple, strong and accurate risk factor for CAD; the lower the Apo B/ Apo AI ratio, the lower is the risk (Pischon, et al., 2005).

Ridker et al., (1999) suggested that the genesis of atherosclerotic plaque is dependent on the interplay of cellular components of the immune system like cytokines, adhesion molecules, lipids, platelets, and endothelial cells. In this regard the role of inflammatory markers like CRP, cytokines like interleukin-6 and adhesion molecules like VCAM, ICAM are significant factors in CAD risk (de Lemos, Hennekens and Ridker, 2000). Several prospective clinical case control studies in Europeans have identified CRP as a strong, independent risk factor for CAD (Kuller, et al., 1996; Tracy, et al., 1997; Ridker, et al. 1998; Koenig, et al., 1999). Basic research studies have revealed that inflammatory markers are high among subjects with IR (Temelkova-Kurktschiev, et al., 2002).

However there are no angiographically verified case control hospital based studies in South Indians with respect to these novel risk factors and thus offering an obviously fertile field for cardiovascular research. In digest, the prevalence of conventional CAD risk factors tends to be lower in Indian than Western
An investigation into the relationship of insulin and other related biochemical parameters with coronary artery disease in a South Indian population, and fail to explain the higher rates of disease. Evaluation of non-conventional risk factors like apolipoproteins possess higher predictive potential for CAD in Western population; there is a paucity of data in South Indian population exploring on multiple conventional and such non-conventional risk factors for CAD, involving a larger sample size and adopting modern methodologies. In analysis of previous studies, the researchers found that in some studies in India the data were inadequate in size of population, duration of study period and methodologies, and the existing information cannot be used for national data projection. They felt that there was an urgent call for well-designed studies within all parts of India in view of the differences in race, culture, lifestyle, diet, stress and strain and the whole host of other factors likely to affect the occurrence of CAD (Bhatia, 1995; Mohan and Deepa, 2004; WHO, 2005; Sharma and Ganguly, 2005). These reasons encouraged and prompted to examine in the present case-control hospital based study, the association of all these multiple conventional and non-conventional risk factors in the same subset of South Indian population with a larger sample size in an angiographically proven CAD and non-CAD subjects.

1.4. Aim of the Study

To investigate the association of insulin, proinsulin, hs-CRP, Lp(a), apolipoproteins, sodium and calcium pump activities with CAD.
1.4.1. Research objectives

- To evaluate the association of biochemical parameters like serum insulin, proinsulin, hs-CRP, Lp(a), apolipoproteins B and A1 in subjects with and without CAD confirmed by angiography.
- To study the association of sodium and calcium pump activity with CAD.
- To determine the prevalence of metabolic syndrome in the study population.

1.5. Research methodology

This study recruited patients with and without CAD admitted in the Cardiac Care Centre of Sri Ramachandra Medical Center (SRMC) and Hospital (a tertiary care teaching hospital), affiliated to Sri Ramachandra University, Chennai, after obtaining the approval of the Institutional Ethical Committee and the consent of the study population.

1.5.1. Study design

Case control study was typically used for the analysis of biochemical parameters associated with CAD. The subjects (n=125) with CAD, confirmed by coronary angiogram were cases. The subjects (n=125) with absence of CAD confirmed by coronary angiogram were controls. Ever since, this is the very first hospital based study with a good sample size and comprising of controls which has been proved for the absence of CAD by angiogram.
Sample size calculation:

Sample size was calculated based on a pilot study of 15 CAD and 15 non-CAD patients. The estimated sample size of 90 per group were considered to be sufficient to detect the difference of 0.1mg/dl in hs-CRP levels between two groups, with >80% power and 5% level of significance (PS sample size calculations version 2.1.3.1).

1.5.2. Analytical criteria

Inclusion criteria:

- Age range of subjects: 35-65 years
- Sex: Male and Female
- Clinical suspicion of CAD: Multiple coronary risk factors and/or typical or atypical angina pectoris and/or positive exercise electrocardiography/myocardial perfusion imaging.

Exclusion criteria:

- Patients with past history of CAD (in the last 60 days) or revascularization procedures or heart transplantation
- Patients with congestive heart failure, significant valvular heart disease, severe hepatic dysfunction, renal failure or renal transplantation
- Patients with known diseases associated with disorganized glucose metabolism such as cushing’s syndrome, acromegaly, pheochromocytoma, chronic pancreatitis, pancreatectomy and clinical thyroid disease.
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- Pregnant women
- Patients with recent surgery (within 60 days)
- Patients with recent high dose corticosteroid therapy
- Patients with other autoimmune diseases like SLE, RA or polyglandular autoimmune syndromes
- Patients with malignancy
- Patients with coagulation disorders
- Patients tested positive for HIV and hepatitis B virus.

1.5.3. Data analysis

A detailed medical history elicitation including demographic data (age, sex, weight (kg), height (cm), body mass index (weight in kg / height in m^2), waist hip ratio and waist circumference), information regarding the chief complaints at the time of admission, history of present illness, past medical history, past medication history, dietary habits, personal history (smoking, alcohol consumption and tobacco chewing), occupational history (sedentary/non-sedentary), physical activity, family history of hypertension, diabetes mellitus, hyperlipidemia and ischemic heart disease (< 55 years if males and < 65 years if females) was collected and recorded in a patient data collection form.

1.5.4. Methods adopted

Patients admitted in coronary care unit with chest pain were subjected for evaluation of cardiac status and underwent the following routine investigations; BP, ECG, treadmill test, ECHO cardiogram and biochemical investigations in
fasting condition prior to coronary angiogram. The routine biochemical investigations analysed in the Central Laboratory, SRMC, included complete blood count (by automated cell counter method), blood sugar levels (enzymatic end point method), renal profile (BUN, creatinine (by photometry)), serum electrolyte levels (sodium, potassium, chloride, bicarbonate (by ion selective electrode method)), lipid profile (serum cholesterol, HDL, TGL, LDL (enzymatic photometry method)), liver function tests (total and direct bilirubin, SGPT, SGOT, total protein, albumin, globulin and A/G Ratio and alkaline phosphatase level (by photometric method)), urine analysis (color, pH, sugar, ketone bodies, blood and epithelial cells (by dipstick analysis)) and cardiac biomarkers (CPK, CPK-MB, LDH (by kinetic photometric method) and Troponin T (by Immunoassay)).

Biochemical parameters such as complete blood counts, serum blood sugar, lipid, renal and liver profiles and cardiac markers namely CPK, CPK-MB, LDH were determined using fully automated analyser Dimension - RXL (Seimens, USA). Troponin-T levels were estimated using Roche (USA), Glycated haemoglobin was estimated by high pressure liquid chromatography using D-10 Bio-Rad, (Bio-Rad Laboratories Inc, USA).

Blood pressure was recorded in the sitting position in the right arm, to the nearest 1mm Hg, using the mercury sphygmomanometer. Two readings were taken five minutes apart and mean of the two was taken as the blood pressure. Waist circumference (cms) was measured using a non-stretchable fibre measuring tape. The subjects were asked to stand erect in a relaxed position with both feet together.
on a flat surface; one layer of clothing was allowed. Waist girth was measured as the smallest horizontal girth between the costal margins and iliac crests at minimal respiration. Hip circumference (cms) was taken as the greatest circumference at the level of greater trochanters (the widest portion of the hip) on both the sides. Waist hip ratio was calculated by dividing waist circumference by hip circumference.

Following definitions were used in the diagnosis: diabetes mellitus, fasting glucose > 126 mg/dl or usage of anti-diabetic drugs; obesity, body mass index > 30 kg/m²; hypertension, blood pressure > 140/90 mmHg on two separate examinations or usage of anti-hypertensive agents; metabolic dyslipidemia, a fasting TG level > 150 mg/dl and/or a TC > 200mg/dl and/or LDL-C level > 100 mg/dl and/or HDL-C level < 40 mg/dl in men and < 50mg/dl in women. Metabolic syndrome was defined as having at least three of the following conditions: TG level > 150mg/dl; HDL-C level < 40 mg/dl in men and < 50mg/dl in women as per ATP III Guidelines (NCEP, 2001). Abdominal obesity was estimated by using Asia Pacific WHO guidelines as waist circumference ≥ 90 cms for males and ≥ 80 cms for females (WHO, 2000).

Subjects signed a written informed consent before the commencement of the study. Then the subjects were screened for the presence of CAD through coronary angiogram, performed through radial artery or femoral artery using omnipaque 350mg and analyzed using a computer assisted analysis system (Seimens, Germany). Cardiac catheterization was performed with standard Judkins technique using 5fr/6fr (1fr=0.33mm). All procedures strictly adhered to the cath-
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Lab standard routines. The coronary angiograms were evaluated by experienced interventional cardiologists blinded to the patients clinical and laboratory data.

CAD was defined as the presence of stenosis ≥ 50% of the diameter in one or more coronary arteries. All subjects were classified into two groups according to coronary angiographic findings: those who had no significantly stenosed vessel as Controls and those who had one or more significantly stenosed vessels as Cases. The Cases were subdivided into three groups according to number of stenosed vessels: single vessel disease (SVD), double vessel disease (DVD), or triple vessel disease (TVD).

![Gensini score methodology](image)

**Figure 6:** Illustration of Gensini score methodology to measure of the extent of myocardial ischemia

Even though there are different methods to evaluate the angiographic severity are in existence, Gensini scoring system was adopted in the current study as it deals with both the severity and the location of the stenosis. Moreover, it is
more effective and gives numeric objective data when compared to other systems. It was computed by assigning the severity score to each coronary stenosis, according to the degree of luminal narrowing and its geographic importance of the extent of myocardial ischemia (Figure 6 a, b). Reduction in the diameter of the lumen, and the roentgenographic appearance of concentric lesions as well as eccentric plaques were evaluated. The reduction of 25%, 50%, 75%, 90%, 99%, and complete occlusion values were given scores of 1, 2, 4, 8, 16, and 32 respectively. To each principal vascular segment a multiplier, according to the functional significance of the myocardial area supplied by this segment was assigned: the left main coronary artery × 5; the proximal segment of the left anterior descending coronary artery (LAD) × 2.5; the proximal segment of the circumflex artery × 2.5; the mid segment of the LAD × 1.5; the right coronary artery, the distal segment of the LAD, the posterolateral artery, and the obtuse marginal artery ×1; and others × 0.5 (Gensini G,1983).

To execute the study specific investigations, fasting blood samples (5ml) were collected from the study population; kept aside for 30 minutes and then subjected to centrifugation at 1500 rpm for ten minutes for serum separation. The hemolysate was separated later. The serum (divided into six aliquots) and hemolysate were then frozen at -40°C using deep freezer (Thermoscientifics, USA), until the assays were performed. Serum was used for the estimations of insulin, proinsulin, hs-CRP, Lp (a), apolipoprotein A I and apolipoprotein B levels. Hemolysate was used for estimating the membrane bound Na⁺K⁺ATPase and
Ca\textsuperscript{2+}ATPase activities. Biochemical analytical methods employed for the above parameters were as follows:

- Serum concentrations of insulin and proinsulin were estimated adopting enzyme linked immunosorbant assay techniques using UBI Magiwell insulin (Mountainview, CA, USA) and DRG Proinsulin ELISA kits (DRG, International, Inc., USA) respectively.

- Proinsulin/Insulin ratio was determined in each patient. IR was calculated using the Homeostasis assessment (HOMA-IR) model using the formula: Fasting insulin (\(\mu\text{IU/ml}\)) x fasting glucose (mmol/litre) / 22.5.

- Serum concentrations of hs-CRP were measured using Daiichi kit (Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan) by immunoturbidimetric method using semi auto analyser (Star 21 plus, Rapid Diagnostics, USA).

- Serum concentrations of apolipoprotein A I and apolipoprotein B were estimated using Daiichi kit (Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan) by immuno-turbidimetric method using semi auto analyser (Star 21 plus, Rapid Diagnostics, USA).

- Serum concentrations of Lp(a) were estimated using Lp(a) latex Daiichi kit (Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan) by immuno-turbidimetric method using semi auto analyser (Star 21 plus, Rapid Diagnostics, USA).

- Na\textsuperscript{+}K\textsuperscript{+}ATPase and Ca\textsuperscript{2+}ATPase activities were determined by UV spectrophotometry at 640nm using spectrophotometer (Perkin Elmer, USA).
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Statistical analysis:

Descriptive statistics were used to summarize the clinical findings and risk factors for CAD. Student’s t test, Chi square and ANOVA were used to get the statistical significance. Pearson’s correlation analysis was done to associate the study specific risk factors of interest with other cardiovascular risk factors. The multiple logistic regression analysis unadjusted and by stepwise addition method, was used to estimate the association of individual risk factor with CAD. ROC analysis was done to calculate the sensitivity and specificity of the marker for CAD. All analyses were done using Windows based SPSS statistical package (Version 15) and a value of P<0.05 was taken as significant.
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An investigation into the relationship of insulin and other related biochemical parameters with coronary artery disease in a South Indian population

References


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