EVALUATION OF THE ROLE OF MATRIX METALLOPROTEINASES AND OXIDATIVE STRESS IN THE PATHOGENESIS AND COURSE OF ACUTE MYOCARDIAL INFARCTION

THESIS SUBMITTED TO THE UNIVERSITY OF CALICUT FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY IN BIOCHEMISTRY
(FACULTY OF MEDICINE)

BY

Mr. SREEKANTH.K.S.

AMALA CANCER HOSPITAL AND RESEARCH CENTRE,
AMALA NAGAR,
THRISSUR- 680 553
Kerala, India.

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CERTIFICATE

Certified that this thesis entitled **"Evaluation of the role of matrix metalloproteinases and oxidative stress in the pathogenesis and course of acute myocardial infarction"** is a bonafied record of research work carried out by **Mr.Sreekanth.K.S** under my guidance and supervision in Amala Cancer Hospital and Research Centre, Thrissur and the same has not been submitted for any other degree, title or associateship.

Amala Nagar
Date: 18-10-2003

Supervising Teacher
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Professor of Biochemistry.

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DECLARATION

I hereby declare that this thesis entitled **"Evaluation of the role of matrix metalloproteinases and oxidative stress in the pathogenesis and course of acute myocardial infarction"** is a bonafied record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any other degree, diploma, associateship or other similar title, of any other university or society.

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Date: 18-10-2003

Sreekanth.K.S.
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DEDICATED TO MY BELOVED PARENTS

"For new experiments and discoveries do usually owe their first rise only to lucky guesses... By observing the errors and defects of a first experiment, we are sometimes carried to such fundamental experiments, as lead to a large series of many other useful experiments and important discoveries"

-Stephen Hales

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CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

INTRODUCTION

Acute myocardial infarction (AMI) continues to be a major public health problem in the industrialized world, despite impressive strides made in its diagnosis and management. Although the death rate from AMI has declined by about 30 percent over the last decade, its development is still a fatal event in approximately one-third of patients. About 50 percent of the deaths associated with AMI occur within one hour of the event and are attributable to arrhythmias, most often ventricular fibrillation. Because AMI may strike an individual during the most productive years, it can have profound deleterious psycho-social and economic ramifications.

Almost all myocardial infarctions result from coronary atherosclerosis, generally with superimposed coronary thrombosis. The most important mechanism underlying the sudden AMI is erosion or uneven thinning and rupture of fibrous cap. The risk of plaque erosion or rupture appears to depend critically on the cellular and extra cellular composition of the plaque. Several pathologic mechanisms are involved in the process of plaque rupture, including inflammation, rheologic factors, circumferential wall stress and vasoconstriction as well as destabilizing changes in fibrous plaque.

A progressive understanding of the underlying pathophysiologic events leading to coronary diseases has also been forthcoming over the past two decades. Pathologic, angiographic and angioscopic observations have allowed formulation of the concept that erosion, fissuring or rupture of a vulnerable atherosclerotic plaque is the initiating mechanism of coronary occlusion, resulting in coronary spasm, intraplaque hemorrhage, occlusive luminal thrombosis and AMI. Additional studies have suggested that plaque erosion or rupture more frequently occurs in lipid-laden plaques with the endothelial cap weakened by internal metalloproteinase activity derived primarly from macrophages. When the plaque ruptures, elements in the blood stream are exposed to plaque matrix elements, including collagen and the intensely thrombogenic lipid core with its associated macrophage derived tissue factor. The result is stimulation of platelet adhesion, activation and aggregation. Secretion of vasoconstrictive and thrombogenic mediators; thrombin generation and fibrin formation, causing vasospasm and the formation of a platelet and fibrin rich

thrombus. The result is reduction (non ST elevation) or interruption (ST elevation) of coronary blood flow, with rapid onset of myocardial cell dysfunction and death.

Matrix metalloproteinases are critical for vascular remodelling by regulating degradation of extracellular matrix. Recent reports show that 72 kDa and 92 kDa type collagenases [matrix metalloproteinase-2 (MMP-2), and matrix metalloproteinase-9 (MMP-9) respectively] act specifically on the basement membrane and partially degraded collagen, play a pathogenic role in the development of atherosclerotic plaques and plaque repture. It has been shown that MMP-2 is constitutively expressed in vascular smooth muscle cells and infiltrating macrophages. Mast cells in rupture prone areas of human coronary atheromas contain tumour necrosis factor-α, a powerful pro-inflammatory cytokine that is able to stimulate the production of MMP-9 by macrophages. Degradation of fibrous cap may result from elaboration of metalloproteinases such as collagenases, elastases and stromelysins. Activated T-lymphocytes may stimulate metalloproteinase production by macrophages in the lesions, which promotes plaque instability and further implicates an immune response. Matrix metalloproteinases may thus, play a role in plaque rupture.

A variety of critical biological molecules, including DNA, cellular proteins and membrane lipids are subjected to oxidative damage. Reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$) have been implicated in the pathophysiology of various states, including ischemia, re-perfusion injury, haemorrhagic shock, atherosclerosis, heart failure and acute hypertension. Several mechanisms are responsible for the protection of the vascular cells from potential cytologic damage caused by free radicals, the main being scavenger enzymes of ROS - superoxide dismutase, catalase, glutathione reductase and gluthathione peroxidase. It has now become apparent that antioxidants may favourably influence coronary artery disease through alternative mechanisms, including improvement of endothelial function, inhibition of platelet aggregability and a decrease in the risk of plaque rupture.

Indians constitute around $1/6^{th}$ of humanity and have a much higher rate of coronary heart disease than other ethnic groups in the world. The World Bank estimates that the death rate from coronary heart disease will increase dramatically in the Indian sub-continent and is expected to contribute to loss of more quality-adjusted life years over the next 20 years. Although there is a high prevalence of coronary artery disease and causative risk factors, not

much data is available linking the oxidative stress and the levels of matix metalloproteinases and the incidence of myocardial infarction in Indian population. Hence, it is considered worthwhile to study the role of scavenger enzymes of oxidative stress and the levels of matrix metalloproteinases in patients admitted with AMI. This study is undertaken with a view to assess the role these parameters as a risk predictor of AMI and also as a guide in the management of patients after myocardial infarction.

1.0.REVIEW OF LITERATURE

1.1.Acute Myocardial Infarction

Acute coronary syndromes (ACS) are a diagnostic and pathophysiologic continuum ranging from unstable angina (UA) to Q- wave myocardial infarction (MI). Acute Myocardial Infarction (AMI) is defined as "gross necrosis of the myocardium as a result of interruption of the blood supply to the area and it is almost always caused by atherosclerosis of the coronary arteries upon which coronary thrombosis is always superimposed. Acute Myocardial Infarction (AMI) occurs during the period when circulation to a region is obstructed and necrosis is occurring. It is characterized by a number of clinical symptoms, such as pallor, perspiration, nausea, shortness of breath and dizziness. A precursor state of AMI is myocardial ischemia, in which obstruction of a coronary artery leads to severe oxygen deprivation of the myocardium prior to necrosis. Angina may be pronounced during myocardial ischemia.

On gross inspection, AMI may be divided into two major types: transmural infarcts, in which myocardial necrosis involves the full thickness (or nearly full thickness) of the ventricular wall, and subendocardial (nontransmural) infarcts, in which the necrosis involves the subendocardium, the intramural myocardium, or both without extending all the way through the ventricular wall to the epicardium (Fallon, 1996). An occlusive coronary thrombosis appears to be far more common when the infarction is transmural and localized to the distribution of a single coronary artery (Freifeld *et al*, 1983). Nontransmural infarction, however, frequently occurs in the presence of severely narrowed but still patent coronary arteries. Studies suggest that between 40% and 50% of non-fatal AMIs are unrecognized (known as silent MI) by the patient and are discovered only by subsequent routine ECG or postmortem examinations (Boden, 1992). Unrecognized or silent infarction occurs more commonly in patients without angina, and is more common in patients with diabetes and hypertension.

1.1.1.Precipitating factors

In as many as one-half of patients with AMI, a precipitating factor or prodromal symptoms can be identified (Braunwald, 1996). Although adequate control studies have not been carried out, evidence suggests that usually heavy exercise (particularly in fatigued or emotionally stressed patients) may play a role in precipitating AMI. Such infarctions could

be the result of marked increase in myocardial oxygen consumption in the presence of severe coronary arterial narrowing. It has been suggested that unusually heavy exertion or mental stress such as that caused by anger (Mittleman *et al*, 1995 and Muller *et al*, 1996) may trigger plaque disruption, leading to AMI (Waxman and Muller, 1996). Accelerating angina and rest angina, two patterns of unstable angina, may culminate as AMI and even trauma can also precipitate an AMI.

1.1.2.Clinical History and Differential Diagnosis

Despite recent advances in the laboratory detection of AMI, the history remains of substantial value in establishing a diagnosis (Braunwald, 1997). The prodrome is usually characterized by chest discomfort, resembling classic angina pectoris, but it occurs at rest or with less activity than usual and can therefore be classified as unstable angina. The pain of AMI is variable in intensity; in most patients it is severe and in some instances intolerable. The pain is prolonged, usually lasting for more than 30 min and frequently for a number of hours. In some patients, particularly elderly, AMI is manifested clinically not by chest pain but rather by symptoms of acute left ventricular failure and chest tightness or by marked weakness or frank syncope. These symptoms may be accompanied by diaphoresis, nausea and vomiting (Muller *et al*, 1990). Both angina pectoris and the pain of AMI are thought to arise from nerve endings in ischemic or injured, but not necrotic, myocardium (Malliani and Lombardi, 1982). Thus in MI, stimulation of nerve fibers in an ischemic zone of myocardium surrounding the necrotic central area of infarction probably gives rise to the pain.

The pain of AMI may simulate the pain of acute pericarditis, which is usually associated with some pleuritic features, that is, it is aggravated by respiratory movements and coughing and often involves the shoulder, ridge of the trapezius and neck. An important feature that distinguishes pericardial pain from ischemic discomfort is that ischemic discomfort never radiates to the trapezius ridge, a characteristic site of radiation of pericardial pain (Spodick, 1995). Pleural pain is usually sharp, knife like, and aggravated in a cyclical fashion by each breath, which distinguishes it from the deep, dull, steady pain of AMI.

1.1.3.Silent MI and Atypical Presentation

Population studies suggest that between 20 and 60 percent of nonfatal MIs are unrecognized by the patient and are discovered only on subsequent routine electrocardiographic or postmortem examinations (Sigurdsson *et al*, 1995). Of these

unrecognized infarctions, approximately half are truly silent, with the patients unable to recall any symptoms whatsoever. Unrecognized or silent infarction occurs more commonly in patients without antecedent angina pectoris (Sigurdsson *et al*, 1995) and in patients with diabetes and hypertension. Silent MI is often followed by silent ischemia.

1.1.4.Histological changes in Myocardium during MI

In some of the infarcts a pattern of wavy myocardial fibers may be seen 1 to 3 hrs after onset, especially at the periphery of the infarct (Antman and Braunwald, 1997). After 8 hrs, oedema of the interstitium becomes evident, as do increased fatty deposits in the muscle fibers, along with infiltration of neutrophilic polymorphonuclear leukocytes and red blood cells. By 24 hrs there is clumping of the cytoplasm and loss of cross-striations, with appearance of irregular cross bands in the involved myocardial fibers. Gross alterations of the myocardium are difficult to identify until at least 6 to 12 hrs following the onset of necrosis (Antman and Braunwald, 1997).

1.1.5.Diagnosis of MI

The diagnosis of AMI, as formally established by the World Health Organisation, requires at least two of the following criteria: a history of chest pain, evolutionary changes on the ECG, and elevation of the serial cardiac enzymes (proteins). Often, the examinig physician is fairly certain after obtaining a patient's history and completing a physical examination and performing ECG that an MI has occurred. When the ECG fails to demonstrate an AMI, the cardiac markers must be used (Apple, 1989).

1.1.6.Risk factors for Acute Myocardial Infarction

A search for the origin, cause and subsequent prevention of AMI and CAD has proceeded long back. The term risk factors describe those characteristics found in healthy individuals, which are independently related to the subsequent occurrence of coronary artery diseases. It includes modifiable lifestyles and biochemical / physiological characteristics as well as non-modifiable personal characteristics, such as age, gender, family history of early-onset and coronary artery diseases (Wood *et al*, 1998). The role of various factors in AMI is explained below.

1.1.6.1.Age and Gender

Coronary artery diseases increase with age in both men and women. It is rare in the first two decades of life, becoming more prevalent after the age of 30 and much more marked

in males than in females below the age of 60 years. CAD in females increases at an accelerated rate and after the seventh decade the rate approaches that in males. It is unclear whether atherosclerosis is a result of the ageing process or the cumulative effect of the known risk factors exerting their effect over time (Grace, 1997).

1.1.6.2.Body mass Index (BMI) and waist-hip ratio (WHR)

Epidemiological studies have revealed a strong predictive relationship between the lipoprotein fractions and physical parameters like BMI and WHR ultimately leading to coronary artery diseases. In recently conducted studies (Rabkin *et al*, 1997 and Pachocka *et al*, 1997), it was observed that subjects with higher BMI had raised triglyceride and cholesterol levels with low HDL cholesterol values. Similarly BMI was also found to be positively associated with total cholesterol and HDL cholesterol levels in another group of study subjects (Shrikrushna *et al*, 1998). Abdominal adiposity judged by WHR was reported to be significantly associated with increased blood lipid profile (Rinc *et al*, 1996 and Lue *et al*, 1994), which in turn could lead to higher incidence of CVD risk. Further, in a study carried out on South Asians living in Britain, it was reported that increased WHR was an important coronary risk factor both in males and females (Keiue *et al*, 1991). Thus, persons having more than the desirable weight and abdominal obesity have high risk for CAD.

1.1.6.3.Obesity

Obesity has an adverse influence on a number of other vascular risk factors, including BP, lipids and glucose tolerance, which could partly explain effects on CVD risk. Furthermore, obesity was independently associated with left ventricular hypertrophy, while weight loss can reduce left ventricular mass. It should be mentioned that central obesity with an increased intraobesity fat mass is associated with a particularly adverse effect on these risk factors and is also linked to insulin resistance (Lasko, 1996). The thinnest people show some excess risk compared to those who are 'normal' or slightly over weight, but with increasing obesity all-cause mortality increases and this is largely due to an increase in cardiovascular mortality (Larson, 1992). The best estimate of obesity is BMI.

1.1.6.4.Lack of physical exercise

The evidence is increasing that lack of physical exercise is a contributory factor to coronary artery diseases. Studies on Harward graduates have shown that life long exercise protects against coronary events, independent of smoking, obesity, hypertension or parental

death from heart disease (Paffenberger *et al*, 1978). Although earlier studies had suggested that exercise had to be vigorous in order to be effective, more recent studies have suggested that only moderate exercise is needed to achieve a benefit. It has been found that undertaking at least 20-30 minutes of exercise sufficient to cause slight breathlessness three times a week is necessary for this purpose (Harris *et al*, 1989).

Can physical exercise trigger heart attack? Convincing evidence that it can trigger and this has been provided by two recent studies (Mittleman *et al*, 1993 and Willich *et al*, 1993), which showed that strenuous physical exercise at least doubled the risk of heart attack. This increased risk, however, was confined to those who did not exercise regularly. In those who did, strenuous exercise did not appear to precipitate heart attack.

1.1.6.5.Cigarette smoking

Smoking can significantly increase CAD mortality and morbidity; an adverse effect related to the amount of tobacco smoked daily and the duration of smoking (Manson *et al*, 1992 and Wilhelmsen, 1998). Passive smoking has also been found to increase CAD risk and the impact of smoking on CAD risk is modified by plasma lipid levels. (Yano *et al*, 1984). Nicotine stimulates release of adrenaline leading to increased serum concentrations of fatty acids (Shepherd *et al*, 1978). Free fatty acid is a stimulant of hepatic secretion of LDL and triglycerides (TG). The free fatty acid can also stimulate hepatic synthesis and release of cholesterol (Banonome *et al*, 1992). In addition to this cigarette smoking can alter coagulation system, produce various free radicals; all of which may contribute to atherosclerosis. The benefit of smoking cessation is seen regardless of how long and how much the person previously smoked (Kawachi, 1993).

1.1.6.6.Diabetes mellitus

The atheroma of diabetics is more extensive and diffuse compared to those of non-diabetics. Three quarters of diabetics die from large vessel (macrovascular disease) and a half from coronary artery disease (CAD), with significant contributions from cerebrovascular disease and peripheral vascular disease (Betteridge and Morell, 1998). Indiviuals with diabetes have an increased risk of coronary artery disease (CAD) morbidity and mortality (Stamler *et al*, 1993). People with type-2 diabetes experience higher rates of ischemia and death after a first myocardial infarction (Miettinen *et al*, 1998), and in the absence of established CAD, they experience the burden of CAD at a rate equal to that of the

nondiabetic individuals with established CAD (Malmberg *et al*, 2000). The clustering of other cardiac risk factors with diabetes suggests an increased prevalence of atherosclerosis (Gracy *et al*, 1998). The mechanisms for increased clinical CAD burden in diabetics are not fully defined. To this end, contrasting and speculative reports point toward more severe anatomic CAD or increased frequency of clinical events due to microvascular disease or coagulation disturbances (Burchfield *et al*, 1993).

1.1.6.7.Hypertension

Studies demonstrate that systemic hypertension is a significant risk factor for the development of heart failure (Levy *et al*, 1996). Part of the explanation for the association between heart failure and hypertension arises from the increased risk of coronary artery disease (CAD) and myocardial infarction among hypertensives. However, clinical observations continue to suggest that hypertension may be an apparent cause for heart failure. The importance of elevated blood pressure (BP) as a risk factor for CAD, heart failure, cerebrovascular disease and renal failure in both men and women has been classified in a large number of epidemiological studies (Kannel, 1996). Systolic BP, at least act as a powerful risk factor as the diastolic BP, and isolated systolic hypertension is also established as a major hazard for coronary artery disease and stroke (Wilkings *et al*, 1988). Clinical trials of BP lowering using different drugs have clearly shown that the risks associated with increased BP can be substantially reduced, especially for stroke, but also for CAD and heart failure: a goal BP of <140/90 mm Hg is appropriate for primary and secondary prevention (Hebert, 1993).

1.1.6.8.Alcohol consumption

There is now good evidence that moderate consumption of alcohol is associated with a lower risk of coronary artery disease than either teetotalism or heavy drinking (Steinberg, 1991). However, the evidence is not such as to encourage nondrinkers to take up drinking. Increased alcohol consumption is associated with an increased risk of cardiovascular diseases due to hypertension and haemorrhagic stroke or sudden arrhythmic death (Kauhnen *et al*, 1997).

1.1.6.9.Hypertriglyceridemia

Although the relation between plasma triglycerides and coronary artery disease (CAD) is not as well established as the relation between plasma cholesterol and CAD, epidemiological

evidence suggests that triglyceride plays an important role in determining CAD risk. In prospective studies, univariated analysis has established a direct association, often weaker in multivariate analysis and may disappear in analysis controlling for HDL-cholesterol (Austin, 1991). Primary cause of high triglycerides (TG) include familial hypercholestrolemia and secondary effects can arise due to carbohydrates, alcohol and diabetes induced high TG, obesity, chronic renal failure, nephrotic syndrome, excessive stress, etc (Mysore P Ramaprasad, 1995).

1.1.6.10.Stress

Some consider stress to be an important risk factor, none more so than the patient who has suffered a myocardial infarction. The difficulty of assessing stress lies in defining what is 'stress' for an activity that may be stressful for one person may be regarded as a positive challenge to another. The possible mechanisms by which stress exerts its negative effects on CHD risk have been cited as increases in blood pressure, heart rate, increased plasma cholesterol levels and adverse effects on coagulation and fibrinolysis (Johnston, 1993). In life style counseling, it is important to include an assessment of the level of stress perceived by patients. Individualized strategies to reduce stress can improve patient well being greatly.

1.1.6.11.Left Ventricular Hypertrophy (LVH)

LVH is defined as left ventricular mass exceeding 131 gm/m^2 of the body surface area in men and 100 gm/m^2 in women and is the response of the heart to chronic pressure or volume overload. Its incidence increases with age, blood pressure and obesity. LVH is independently associated with increased incidence of cardiovascular disease, and cause mortality and stroke. (Bikkina *et al*, 1994). Effective blood pressure control in hypertensive patients, along with non-pharmacological interventions such as weight reduction, sodium restriction and aerobic physical exercise can reduce left ventricular mass (Ghali *et al*, 1997).

1.1.6.12.Homocysteine

High plasma homocysteine concentrations were initially thought to be associated with advanced atherosclerosis on the basis of autopsy findings in patients with homozygous defects in enzyme necessary for homocysteine metabolism, such as cystathionine beta synthase or methylene tertahydrofolate reductase (Nehler *et al*, 1997 and Nygard *et al*, 1997). In patients with such defects, severe atherosclerosis develops in childhood, and may have their first MI by the age of 20 years (Nehler *et al*, 1997 and Nygard *et al*, 1997).

Homocysteine is toxic to endothelium (Harker *et al*, 1976) and is prothrombotic (Hajjar, 1993), and it increases collagen production (Majors *et al*, 1997) and decreases the availability of nitric oxide (Upchurch *et al*, 1997).

Plasma homocysteine concentrations are slightly elevated in many patients who have no enzymatic defects in homocysteine metabolism. These patients have an increased risk of symptomatic atherosclerosis of the coronary, peripheral and cerebral arteries (Verhoef and Stampfer, 1995).

1.1.6.13.Infection

Several reports have shown a correlation between the incidence of atherosclerosis and the presence of at least two types of infectious microorganisms, herpes virus and C.pneumoniae (Libby *et al*, 1997 and Hendrix *et al*, 1990). Both organisms have been identified in atheromatous lesions in coronary arteries and in other organs obtained at autopsy (Jackson *et al*, 1997). Increased titers of antibodies (Thom *et al*, 1991) to these organisms have been used as a predictor of further adverse events in patients who have had a myocardial infarction (Melnick *et al*, 1993). Nonetheless, there is no direct evidence that these organisms can cause the lesions of atherosclerosis (Nicholson and Hajjar, 1998). Although these organisms are ubiquitous in many tissues and organs, the fact that lesions cannot be induced experimentally in animals by injection of the organisms leaves their role as etiologic agents in question. It is nevertheless possible that infection, combined with other factors may be responsible for the genesis of the lesions of atherosclerosis in some patients (Danesh *et al*, 1997).

1.1.6.14.Lipoprotein (a) [Lp (a)]

There is always a risk association between increased Lp(a) level and acute myocardial infarction (Schafer *et al*, 1994). Many experimental trials have confirmed a positive correlation between Lp(a) and atherosclerosis. In hyperlipidaemic patients with increased Lp(a) levels, the decrease in LDL cholesterol levels is followed by neutralization of the atherogenic potential of Lp(a) (Maher *et al*, 1995). Lp(a) has got almost the similar structure as that of LDL. The Apo(a) that is present in Lp(a) is homologous to plasminogen. It is possible that Lp(a) might bind and prevent fibrinolysis either by blocking activation of plasminogen or by preventing initial access and binding of plasminogen. Lp(a) is now

recognized as the most powerful and most prevalent risk factor for cardiovascular diseases in the diverse population.

It has gradually become more evident that CHD mortality could not be explained solely on the basis of the effect of single risk factor. Analysis of existing data to examine the effects on CHD mortality when more than one risk factor is present revealed that the risk factors interacted synergistically, to increase the risk of CHD. Observational and intervention studies in populations were conducted to examine the effect of multiple risk factors on subsequent risk of CHD.

1.2.AMI and Atherosclerosis

One of the major causes of AMI is the coronary atherosclerosis. Atherosclerosis leading to coronary artery disease is complex in origin. Involved in the pathogenesis of atherosclerosis are hemodynamic, thrombotic, and carbohydrate – lipid metabolic variables, along with intrinsic characteristics of the arterial wall (Ross,1993). These physiologic and biochemical factors underlie the clinical events that may eventually occur. Environmental factors such as smoking or a sedentary life style also contribute to this process. The progression of atherosclerotic disease and the increasing severity of atherosclerosis relate not only to the presence and extent of cardiovascular risk factors but also to the persistence of risk factors over time (Stamler *et al*, 1993). Sudden death may occur in a young person with only a single lesion complicated by a coronary thrombus, without extensive vessel disease. Consequently, the extent of vascular lesions may not be directly related to the occurrence of clinical events, such as myocardial infarction. Morbidity due to coronary artery disease, however, is generally related to the extent of vascular lesions (Roberts, 1989). In this regard, clinical risk factors are considered to be useful in predicting the severity of atherosclerosis.

The lesions of atherosclerosis occur principally in large and medium-sized elastic and muscular arteries and can lead to ischemia of the heart, brain or extremities resulting in infarction (Ross, 1999). They may be present throughout a person's lifetime. In fact, the earliest type of lesion, the so-called fatty streak, which is common in infants and young children (Napoli *et al*, 1997), is a pure inflammatory lesion consisting only of monocyte-derived macrophages and T lymphocytes. In persons with hypercholesterolemia, the influx of these cells is preceded by the extracellular deposition of amorphous and membranous lipids (Napoli *et. al*, 1997).

1.3.AMI and Free radicals

Free radicals or reactive oxygen species (ROS), which are formed by various biochemical reactions, are thought to initiate atherosclerosis by damaging blood vessel walls. It was towards the end of the 18^{th} century that oxygen emerged as the paragon among the elements which sustained life, promoted physical health and stimulated mental vigor. But, too much of even the best is bad and it is now known that oxygen in high concentrations can damage the brain, lungs and other organs. The phenomenon of oxygen toxicity in the early days mainly referred to the toxic effects of oxygen at high pressure (as during diving, hyperbaric oxygen therapy, aerospace travel, etc). Free radicals are an unstable and extremely reactive chemical species, which have an unpaired electron in their structure. The most important free radicals are the by-products of energy generation and are formed during oxidation, such as that occurs in the electron transport chain.

The term reactive oxygen species (ROS) collectively describes free radicals such as superoxide radical ($O_2^{\cdot}$), hydroxyl radical ($OH^{\cdot}$), and hypochlorous acid (HOC1). These reactive oxygen intermediates may participate in reactions, which give rise to free radical species. Unstable free radical species attack cellular components, causing damage to lipids, proteins and DNA, which can initiate a chain of events which results in the onset of disease. Oxidative stress results when the balance between the reactive oxygen species overrides the antioxidant capability of the target cell. ROS may interact with and modify cellular protein, lipid and DNA, which results in altered, target cell function. Free radicals are extremely reactive. Their half-life is only a few milli-seconds. When a radical reacts with a new compound, more free radicals are generated. This chain reaction leads to thousands of events. Peroxidation of polyunsaturated fatty acids (PUFA) in plasma membranes results in the inhibition and loss of membrane functions such as absorption and secretion, inhibition of protein and enzyme synthesis and indirectly cause cell death.

Free radicals can originate in various ways.

i) Mostly by biochemical redox reactions involving oxygen, which occur as a part of normal metabolism. eg. $O_2^{\cdot}$, $NO^{\cdot}$, H_2O_2.

ii) By phagocytes as part of a controlled inflammatory reaction. eg. HOCI, $O_2^{\cdot}$.

iii) Occasionally in response to exposure to ionizing radiation, UV light,

environmental pollution, cigarette smoke, hyperoxia, excessive exercise and ischaemia eg. $O_2^{\cdot}$, $OH^{\cdot}$, $ROO^{\cdot}$.

Many free radicals and ROS have been implicated in disease development.

$OH^{\cdot}$ is a highly reactive radical, which can attack all biological molecules.

$O_2^{\cdot}$ is a less reactive radical, which can travel in the blood and attack a number of biological targets. $O_2^{\cdot}$ can also acts as a vasodilator and may have a role in intracellular signaling and growth regulation.

$NO^{\cdot}$ acts on smooth muscle cells in vessel walls causing relaxation.

H_2O_2 crosses cellular membranes easily and may cause alteration in the expression of virus genes in infected cells. eg. HIV. This ROS has only a few cellular targets but can result in the production of hydroxyl radicals.

Transition metals are thought to promote free radical reactions, including the Fenton reaction, which results in the formation of hydroxyl radicals.

$$Fe^{2+} + H_2O_2 \rightarrow OH^{\cdot} + OH^{\cdot} + Fe^{3+}$$

It is now widely believed that ROS play an important role in the development, and perhaps sometimes in the initiation of atherosclerosis which leads to AMI. Shear stress and turbulent blood flow can cause oxidative damage to vascular endothelial cells. They may respond by upregulating several enzymes, including superoxide dismutase (SOD) and nitric oxide synthase (NOS). The stress may lead to damage, or the enzyme upregulation may render the cells more resistant to subsequent insult (Topper *et al*, 1996). Perhaps this is one reason why regular exercise improves the functioning of the cardiovascular system. The reactive oxygen species (ROS) might activate the latent forms of matrix metalloproteinases released by macrophages. This may contribute to weakening of atherosclerotic plaques and cap rupture, often the precipitating event in myocardial infarction (Halpert, 1996).

Endothelial injury, a major event in CAD is followed by attachment of monocytes from the circulation, which enters the vessel wall and develop into macrophages. Activated monocytes and macrophages could injure neighbouring cells by secreting $O_2^{\cdot}$, H_2O_2, hydrolytic enzymes and possibly NO. Nitric Oxide is always produced by vascular endothelium and it is also possible that endothelium generates low levels of $O_2^{\cdot}$ (eg. by xanthine, NADH or NADPH oxidases). Indeed, Xanthine oxidase has been identified in

human atherosclerotic lesions (Swain and Gutteridge, 1995). Endothelial $O_2^{\cdot}$ production may be accelerated by injury, eg. in hypercholesterolaemic rabbits it has been reported that $O_2^{\cdot}$ formation, apparently involving xanthine oxidase, is elevated in endothelial cells. Whether $O_2^{\cdot}$ arises from endothelial cells and / or from monocytes and macrophages (Darley- Usmar and Halliwell, 1996), it might then react with NO to give peroxy nitrite (ONOO).

1.3.1.Biologically Relevant ROS

Superoxide radical

Hypochlorous acid

Hydrogen peroxide

Hydroxyl radical

Peroxyl radical

Nitric oxide

Peroxides

Peroxynitrite

Heme proteins

Singlet Oxygen.

Involvement of oxygen free radical in the pathophysiology of inflammation, ischaemia and in reperfusion damage in a number of organs and tissues were also reported (Parks *et al*, 1983). ROS generated at the time of re-flow may be responsible for the occurrence of specific myocardial damage other than that resulting from ischaemia period itself (Ambrosio and Chiariello, 1991). Studies have shown increased rate of neutrophil-derived free radicals in the genesis of reperfusion damage (Bell *et al*, 1990).

Though all classes of biomolecules may be attacked by free radicals, lipids are the most susceptible. The human cells are rich in polyunsaturated fatty acids (PUFA) and hence are readily attacked by oxidizing radicals by a process known as "lipid peroxidation", which is a highly damaging self-perpetuating chain reaction. This reaction leads to endothelial damage. AMI is one important clinical condition in which this free radical mediated endothelial damage is said to play a major role.

1.3.2.The antioxidant system

The body possesses a number of mechanisms both to control the production of ROS and to limit or repair the damage to tissues. The integrated antioxidant systems consists of i)

preventive antioxidants, which prevent the formation of new ROS [eg. ceruloplasmin (Cu), metallothionine (Cu), albumin (Cu), transferrin (Fe), ferritin (Fe) and myoglobin (Fe)], ii) scavenging antioxidants, which remove ROS once formed, thus prevent free radical chain reactions [eg. superoxide dismutase (SOD), catalase (CAT) glutathione peroxidase (GPx) and glutathione reductase (GR)] and iii) repair enzymes, which repair or remove ROS-damaged biomolecules (eg. DNA repair enzymes and methionine sulphoxide reductase). The first defense against ROS is mainly by the antioxidant enzymes. By the combined action of these enzymes, the free radicals are removed very effectively.

1.3.2.1.Antioxidant enzymes

The antioxidant enzymes are known to posses the free radical scavenging activity. The first line of defence against ROS is mainly done by the following enzymes.

1.3.2.2.Superoxide dismutase

Superoxide dismutase (SOD) is a metalloenzyme that convert superoxide radical to hydrogen peroxide

$$O^{\cdot}_2 + O^{\cdot}_2 + 2H^+ \rightarrow H_2O_2 + O_2$$

SOD is the most important enzyme because it is found virtually in all aerobic organisms. SOD is found in four different isoforms, copper dependent (Cu-SOD), copper-zinc dependent (Cu-Zn-SOD), manganese dependent (Mn-SOD) and iron dependent (Fe-SOD). Human SOD is the Cu-Zn-SOD. The transition metals of the enzyme react with oxygen radical by abstracting its electron (Oberley and Oberley, 1984). The only known substrate for SOD is the superoxide radical, which is converted to hydrogen peroxide by the action of the enzyme.

1.3.2.3.Catalase

Catalase also serves as a free radical scavenging enzyme. It is present in almost all the cells especially in erythrocytes. Catalase catalyses the decomposition of hydrogen peroxide to water and oxygen.

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Thus, the toxic hydrogen peroxide formed by the action of SOD on superoxide radical is catalyzed to water by catalase. Catalase is a haem containing protein and is found to act 10^4 times faster than peroxidases. It is localized mainly in mitochondria and in subcellular respiratory organelle (Pryor, 1986). Catalase is also present in peroxisomes and cytosol. It

has four protein subunits each containing a haem Fe (2^+)-protoporphyrin group bound to its active site.

1.3.2.4.Glutathione peroxidase

Glutathione peroxidase (GPx) is another well-known enzyme defense against oxidative stress, which in turn requires glutathione as a cofactor. Among the many functions of glutathione it is involved in the generation of nucleotide precursors of DNA via the reduction of ribonucleotides to deoxyribonucleotides (Meister, 1994). GPx catalyses the oxidation of GSH to GSSG at the expense of hydrogen peroxide.

$$ROOH + 2GSH \rightarrow ROH + H_2O + GSSG$$

The reverse reaction is catalyzed by glutathione reductase (GR) to retain the reduced glutathione

$$NADPH + H^+ + GSSG \rightarrow NADP^+ + 2GSH$$

By the combined action of these enzymes the oxidative stress induced by free radicals has been eliminated. Other antioxidant molecules such as glutathione, albumin, bilirubin and uric acid also found to defense against the oxidative stress induced by free radicals or ROS.

1.3.3.Antioxidant vitamins

The vitamins such as tocopherol (vitamin E), ascorbic acid (vitamin C) and beta carotene (vitamin A) are shown to possess the antioxidant properties and they are included to the second line of defence against free radicals. They are included under the category of micronutrients, which are essential for the body.

1.3.3.1.Vitamin E

This is a fat-soluble vitamin. Vitamin E occurs in plasma as a variety of tocopherols of which the alpha- and gamma- isomers are usually the predominant ones. All vitamin E in the human body is derived from the diet. Vitamin E is thought to be an important chain breaker and prevents lipid peroxidation via the formation of tocopherol radical. Vitamin E can directly act with a variety of ROS including the peroxy radical, hydroxyl radical, oxygen radical and singlet oxygen (Rimm *et al*, 1993).

1.3.3.2.Vitamin C

Vitamin C is an important water-soluble antioxidant in biological fluids and an essential micronutrient required for normal metabolic function of the body (Enstrom *et al*, 1992). It readily oxidizes to dehydroascorbic acid. This will interact with oxygen radical and

hydroxyl radical in plasma, thus preventing damage to red cell membrane. It probably assists alpha-tocopherol in inhibiting lipid peroxidation by recycling the tocopherol radical. Vitamin C has also been shown to regulate urate, glutathione and beta carotene *in vitro* from their respective one electron oxidation product. Further, vitamin C neutralizes ROS and reduces oxidative DNA damage and genetic mutation (Frei *et al*, 1989).

Vitamin A, a fat-soluble vitamin is also found to be an excellent scavenger of singlet oxygen, produced during photosensitivity (Bates, 1997). The ROS induced- oxidative damage is found to affect the proteins, lipids and nucleic acids. The antioxidant system tries to maintain the normal cellular functions by removing the ROS by various means. However, the free radical attack on lipid molecules will directly lead to the complications of AMI via atherogenesis by LDL oxidation.

The oxidative modification of LDL by free radicals appears to have an important role in foam-cell formation and atherogenesis, which is considered to be the basic cause of AMI. The link between the oxidation of LDL and atherogenesis provides a convenient and simple rationale for the cause and incidence of coronary artery disease. A number of clinical studies and studies in animals have explored this link between LDL oxidation and atherogenesis. This is well explained by the oxidative modification hypothesis.

1.3.4.Oxidative Modification Hypothesis

According to the oxidative modification hypothesis, LDL initially accumulates in the extracellular subendothelial space of arteries and through the action of resident vascular cells is mildly oxidized to a form known as minimally modified LDL (Navab *et al*, 1996) (Fig.1). This minimally modified LDL induces local vascular cells to produce monocyte chemotactic protein 1 and granulocyte and macrophage colony stimulating factors, which stimulate monocyte recruitment and differentiation to macrophages in arterial walls (Parhami *et al*, 1993). The accumulating monocytes and macrophages stimulate further peroxidation of LDL. The products of this reaction make the protein component of LDL (apolipoprotein B-100) more negatively charged. By virtue of its increased negative charge, this completely oxidized LDL is recognized by scavenger receptors on macrophages and internalized to form so-called foam cells (Henriksen *et al*, 1981). In contrast to the uptake of native (unoxidised) LDL by the LDL (apolipoproteins B and E) receptors on macrophages, the uptake of

oxidized LDL by the scavenger receptor pathway is not subject to negative-feed back regulation and thus results in massive uptake of cholesterol (from oxidized LDL) by the macrophages.

In addition to promoting the formation of foam cells, oxidized LDL has direct chemotactic activity for monocytes (Quinn *et al*, 1988) and stimulates the binding of monocytes to the endothelium (Frostegard *et al*, 1991). Once monocytes cross the endothelial layer, they become trapped in the subendothelial space, partly because oxidized LDL inhibits their egress from the arterial wall (Quinn *et al*, 1987). Oxidized LDL is also cytotoxic to vascular cells (Schwartz *et al*, 1991 and Cathcart *et al*, 1985), thus promoting the release of lipids and lysosomal enzymes into the intimal extracellular space and enhancing the progression of atherosclerotic lesions (Schwartz *et al*, 1991).

The oxidative-modification hypothesis is supported by evidence that LDL oxidation occurs *in vivo* and contributes to the clinical manifestations of atherosclerosis. Antibodies raised against oxidized LDL react with atherosclerotic lesions but not with normal arterial segments (Palinski *et al*, 1989). LDL extracted from the human atherosclerotic lesion, but not plasma derived LDL, resembles LDL that has been oxidatively modified *in vitro* (Yla–Herttuala *et al*, 1989). Patients with carotid atherosclerosis have higher levels of autoantibodies to oxidized LDL than do age-matched normal subjects (Salonen *et al*, 1992). Plasma concentrations of immunoreactive oxidized LDL are higher in patients with acute myocardial infarction than in normal subjects (Holvoet *et al*, 1995).Thus, oxidative modification of LDL appears to have an important role in foam-cell formation and atherogenesis. A number of clinical studies and studies in animals have explored this link between LDL oxidation and atherogenesis.

The release of copious foam cell lipids to the extracellular compartment induces a second cascade of inflammatory responses within the vascular intimal layer. In particular, granulomatous foci involving macrophages, lymphocytes, and multinucleate giant cells surround and invade the extracellular lipid. Besides the foam cell death, other mechanisms can also account for the formation of extracellular lipid deposits. New lines of evidence suggest that lipoproteins, particularly LDL, aggregate and then fuse with one another in the extracellular space to form microscopically evident lipid deposits (Tirzui *et al*, 1995). Structures resembling lipoprotein aggregates have been visualized in human atherosclerosis

by electron microscopy, and lipid aggregates containing apolipoprotein B (apo B) have also been isolated.

A number of proteins and peptides have been detected in relative abundance within or near the atherosclerotic core. Many of the proteins in this region are hydrophobic, including the apolipoproteins, C-reactive protein, and the 70- and 60-kDa heat shock proteins (HSPs). Cells that border and penetrate the atherosclerotic core not only participate in the deposition (or removal) of core lipids but can also be influenced by the accumulating lipids and proteins. Complement components have been found in relative abundance in the core, and both toxic and chemotactic responses may be generated via activation of complement. Antigenic markers of complement activation, including C3D and the terminal C5B-9 neoantigen, have been found in the atherosclerotic core, and terminal C5B-9 has been detected coincident with the cholesterol-rich vesicles in the sub-endothelium (Seifert *et al*, 1989).

1.4.Plaque Rupture

The clinical expression of atherosclerotic disease activity is determined by pathologic events leading to coronary thrombosis. In this regard, there are two key factors

(i) The propensity of plaques to rupture, and

(ii) The thrombogenicity of exposed plaque components.

The morphologic characteristics of plaques that determine their propensity to rupture have been determined from analysis of lesions exhibiting disruption. Observational studies conducted by pathologists using necropsy and atherectomy tissue samples have shown convincingly that plaques causing intraluminal thrombosis are rich in extracellular lipid and that the lipid core of these "vulnerable or rupture-prone" plaques occupies a large proportion of the overall plaque volume. The degree of cross-sectional stenosis involving the vessel lumen is typically < 50% (Little *et al*, 1988). In addition to the predominant lipid core, vulnerable plaques are characterized by a thin fibrous cap and high macrophage density (Davies and Thomas, 1985). Whereas most individuals with atherosclerotic coronary artery disease exhibit a diversity of plaque types, most have a preponderance of one specific type

Figure 2: Plaque formation and Plaque rupture.

1. Excess LDL particles accumulate in the artery wall and undergo chemical alterations. The modified LDLs then stimulate endothelial cells to display adhesion molecules, which latch onto monocytes and T cells in the blood. The endothelial cells also secrete "chemokines", which lure the snared cells into the intima.

2. In the intima, the monocytes mature into active macrophages. The macrophages and T cells produce many inflammatory mediators, including cytokines and factors that promote cell division. The macrophages also display so-called scavenger receptors, which help them ingest modified LDLs.

3. The macrophages feast on LDLs, becoming filled with fatty droplets. These frothy-looking, fat-laden macrophages (foam cells) and the T cells constitute the fatty streak, the earliest form of atherosclerotic plaque.

4. Inflammatory molecules can promote further growth of the plaque and formation of a fibrous cap over the lipid core. The cap develops when the molecules induce smooth muscle cells of the media to migrate to the top of the intima, multiply and produce a tough, fibrous matrix that glues the cells together. The cap adds to the size of the plaque but also walls it off safely from the blood.

5. Later, inflammatory substances secreted by foam cells can dangerously weaken the cap by digesting matrix molecules and damaging smooth muscle cells, which then fail to repair the cap. Meanwhile the foam cells may display tissue factor, a potent clot promoter. If the weakened plaque ruptures, tissue factor will interact with clot-promoting elements in the blood, causing a thrombus, or clot, to form. If the clot is big enough, it will halt the flow of blood to the heart, producing a heart attack - the death of cardiac tissue.

(vulnerable or nonvulnerable). The genetic and acquired determinants of plaque types are subjects of intense investigation.

The lipid core of an advanced atherosclerotic plaque is bounded in its luminal aspect by a fibrous cap, at its edges by the shoulder region, and on its abluminal side by the plaque base. Because the lipid core contains a substantial amount of prothrombotic substrate, the fibrous cap, separating the core from circulating blood components within the vessel's lumen, determines the overall stability of the plaque. In turn, the extra cellular matrix of the fibrous cap, consisting of several proteinacious macromolecules, including collagen (type I and III) and elastin secreted by transformed smooth muscle cells, determines its integrity. The core size and fibrous cap thickness are not related to absolute plaque size or to the degree of luminal stenosis. The determinants of core size have not been fully elucidated, although death of lipid- filling macrophages by apoptosis is a possibility. Fibrous cap thickness appears to be related to macrophage and smooth muscle cell activity, particularly their production of metalloproteinases that degrade connective tissue (Figure 2).

1.5.AMI and MMPs

Matrix metalloproteinases (MMPs), part of a super family of enzymes that include collagenases, gelatinases and elastases require activation from proenzyme precursors to attain enzymatic activity. Under normal circumstances, tissue inhibitors hold these enzymes in check; however, exposure of smooth muscle cells to the cytokines, IL-1 and tumor necrosis factor - α (TNF-α) causes induction of interstitial collagenases and stromelysin. Macrophages exposed to inflammatory cytokines also stimulate the production of matrix-degrading enzymes (Galis *et al*, 1995). Coronary atherectomy specimens from patients with acute coronary syndromes have been shown to contain a 92-kDa gelatinase that is produced predominantly by macrophages and smooth muscle cells (Brown *et al*, 1995). Within the atherosclerotic plaques, the highest stress regions have a two-fold greater matrix metalloproteinase (MMP-1) expression than the lowest stress regions. Over expression of MMP-1 in vulnerable plaques is associated with a substantial increase in circumferential stress. Degradation and weakening of the collagenous extracellular martrix at critical points of high shear stress may play an important role in the pathogenesis of plaque rupture.

Fibrous cap thickness can be maintained by smooth muscle cell mediated collagen synthesis (local repair); however, interferon - γ (IFN - γ) , an inflammatory cytokine found within atherosclerotic plaques, decreases the ability of smooth muscle cells to express the collagen gene . Because only T- lymphocytes can elaborate IFN - γ (Amento *et al*, 1991) , it has been suggested that chronic immune stimulation within atherosclerotic plaques leads to the production of IFN-γ from T- cells that subsequently inhibits collagen synthesis in vulnerable regions of the fibrous cap . IFN - γ can also contribute to apoptosis and, therefore, may be a key biochemical determinant of plaque vulnerability. The recent observation that mast cells may be involved with macrophage / foam cell development has raised questions concerning their potential involvement in plaque rupture. Human mast cells contain proteoglycans, proteolytic enzymes including chymase and tryptase. In normal coronary arteries, mast cells amount to 0.1 % of all nucleated cells; however, within the fibrous cap, lipid core, and shoulder regions of atheromatous lesions, there are 5 - 5 - and 10 fold-increased densities, respectively (Kaartinen *et al*, 1994). Electron and light microscopic studies of mast cells in the plaque shoulder region have revealed evidence of degradation, a sign of activation that may contribute to matrix degradation and plaque rupture in acute coronary syndromes
(Constantinides, 1995).

1.5.1.Matrix metalloproteinases (MMPs)

Extracellular proteinases are required for numerous developmental and disease related processes. The ability to degrade extracellular proteins is essential for any individual cell to interact properly with its immediate surroundings and for multicellular organisms to develop and function normally. This was obvious long before it was first shown that diffusible enzymes produced by fragments of involuting tadpole tail could degrade gels made of native fibrillar collagen (Gross and Lapiere, 1962). Since then, a family of related enzymes has been identified in species from hydra to humans and collectively called matrix metalloproteinases (MMPs) because of their dependence on metal ions for catalytic activity, their potent ability to degrade structural proteins of the extracellular matrix (ECM), and specific evolutionary sequence considerations that distinguish them from other closely related metalloproteinases (Stocker *et al*, 1995). In addition to their ECM substrates, MMPs also cleave cell surface molecules and other pericellular non-matrix proteins, thereby

regulating cell behaviors in several ways (Sternlicht *et al*, 2000). Thus like the many proteins they modify, the MMPs influence diverse physiologic and pathologic processes, including aspects of embryonic development, tissue morphogenesis, wound repair, and inflammatory diseases such as cancer and cardiovascular diseases (Nelson *et al*, 2000 and Sternlicht *et al*, 2001).

With the ability to alter cell fates and developmental outcomes comes the necessasity for higher levels of control. Nevertheless, it took nearly a decade from the time collagenolytic activities were first demonstrated to realize that MMPs are synthesized as inactive zymogens that require activation (Harper *et al*, 1971), and even longer to demonstrate the existence of the first of at least four endogenous metalloproteinase inhibitors, now called tissue inhibitors of metalloproteinases or TIMPs (Bauer *et al*, 1975). Since then, other levels of MMP regulation have been elucidated, although several layers of complexity are still left to unravel. In addition to being differentially regulated at the level of transcription, MMPs can be controlled at the protein level by their endogenous activators and inhibitors and by factors that influence their secretion, their cell surface localization, and their own degradation and clearance. Moreover, higher organisms express multiple MMPs, each with its own profile of expression, localization, activation, inhibition, and clearance, as well as its own, sometimes broad, range of preferred substrates. Thus multiple modifiers, each with its own regulatory inputs, control different MMP functions *in vivo*.

The multiplicity of MMPs with distinct but somewhat overlapping functions probably acts as a safeguard against any loss of regulator control. Although such redundant and compensatory mechanisms are advantage to the organism, they often confound efforts to fully understand how MMPs function *in vivo*. As the field has grown, the unforeseen complexity of MMPs has emerged, often revealing surprising mechanism of action. Frequently, the belief that an MMP is involved in a given biologic process and the existence of plausible mechanisms and then tested using various experimental approaches. For example, MMPs are invariably upregulated in rheumatoid arthritis and malignant disease, with more severe increases often indicating a worse prognosis. Moreover, a major hallmark of these diseases is the capacity of cells to cross tissue boundaries. Thus, ECM degrading enzymes must be present to break down the structural barriers to invasion. Indeed, extensive experimental evidence supports this straight forward and conceptually appealing supposition,

but the mechanisms may be more complex than originally thought. In arthritis, the loss of certain MMPs surprisingly exacerbates rather than alleviates the disease (Harper *et al*, 1971). Considerable evidence implicates MMPs as important players in several biologic processes, yet the actual mechanisms underlying their influence are mostly unsolved. It is hoped that understanding these processes will result in a more rational approach toward reducing or entirely alleviating the ill effects of MMPs in disease while maintaining their necessary and beneficial function

1.5.1.1.The Metzincin Super Family

Proteolytic enzymes are classified as either exopeptidases or endopeptidases based on whether they cleave terminal or internal peptide bonds, respectively. Most endopeptidases are classified as serine, cysteine, aspartic or metalloproteinases based on their catalytic mechanism and inhibitor sensitivities, and the metalloproteinases are further separated into five super families based on sequence considerations. Of these, the metzincin super family is distinguished by a highly conserved motif containing three histidines that bind zinc at the catalytic site and a conserved methionine turn that sits beneath the active site zinc (Stocker *et al*, 1995). Their signature zinc-binding motif reads HEBXHXBGBXHZ, where histidine (H), glutamic acid (E) and glycine (G) residues are invariant, B is a bulky hydrophobic residue, X is a variable residue, and Z is a family- specific amino acid. The metzincins are further subdivided into four multigene families, the serralysins, astacins, ADAMS / adamalysins, and MMPs, based primarily on the identity of the Z residue, which is serine in all but a few MMPs (Stocker *et al*, 1995).

The serralysins, which have a proline in the Z position, are large bacterial enzymes that often play an important role in bacterial virulence and pathogenicity (Sternlicht and Werb, 1999). Astacins, on the other hand, contain glutamic acid in the Z position. They include bone morphogenetic protein-1 (BMP-1), which is the procollagen C-proteinase that removes the C-terminal propeptides of fibrillar procollagens, Drosophila and mammalian tolloid and tolloid like proteins, which activate certain growth factors, and secreted and transmembrane meprins A and B that can process peptide hormones (Sternlicht and Werb, 1999).

1.5.1.2.Structure and function of MMP

At present 25 vertebrate MMPs and 22 human homologues have been identified (Nagase and Woessner, 1999, Sternlicht and Bergers, 2000, and Lohi *et al*, 2001). In addition, several nonvertebrate MMPs have been identified, incluing the embryonic sea urchin hatching enzyme envelysin (Lepage and Gache, 1990); Caenorhabditis elegans MMPs C31 , H 19 , and Y 19 (Wada *et al*, 1998) ; a Drosophila MMP ; an MMP in hydra that regulates cell differentiation and foot process development (Leontovich *et al*, 2000) ; soybean leaf metalloendopeptidase-1 (Mc Geehn *et al*, 1992) ; an MMP in the flowering mustard plant Arabidopsis thaliana (Maidment *et al*, 1999) ; and gamete lytic enzyme from green algae (Kinoshita *et al*, 1992). Each of the vertebrate MMPs has distinct but often overlapping substrate specificities, and together they can cleave numerous extracellular substrates, including virtually all ECM proteins (Sternlicht *et al*, 2001). In addition to their conserved zinc-binding motif and "Met turn", the MMP share added stretches of sequence homology, giving them a fairly conserved overall structure (Stocker *et al*, 1995).

Historically, the MMPs were divided into collagenases, gelatinases, stromelysins and matrilysins on the basis of their specificity for ECM components; and the common names of the MMPs reflect this classification (Table A). Individual MMPs are referred to by their common names according to a sequential numeric nomenclature reserved for the vertebrate MMPs as given in the table. In addition they are often grouped according to their modular domain structure (Figure 3). In this regard; all MMPs have an N-terminal signal sequence (or "Pre" domain) that is removed after it directs their synthesis to the endoplasmic reticulam. Thus, most MMPs are secreted; however, six display transmembrane domains and are expressed as all surface enzymes. The pre-domain is followed by a propeptide "Pro" domain that maintains enzyme latency until it is removed or disrupted, and a catalytic domain that contains the conserved zinc-binding region (Nagase and Woessner, 1999). The catalytic domain dictate cleavage site specificity through its active site cleft, through specificity sub-site pockets that bind amino acid residues immediately adjacent to the scissile peptide bond, and through secondary substrate-binding exosites located outside the active site itself (Overall, 2001).

With the exception of MMP7 (matrilysin), MMP26 (endometase/matrilysin-2), and MMP23, all MMPs have a hemopexin/ vitronectin-like domain that is connected to the

Table:A The vertebrate MMPs

MMP	Common name(s)	Domain organization
MMP1	Collagenase 1	B
MMP2	Gelatinase A	C
MMP3	Stromelysin 1	B
MMP7	Matrilysin	A
MMP8	Collagenase 2	B
MMP9	Gelatinase B	C
MMP10	Stromelysin 2	B
MMP11	Stromelysin 3	D
MMP12	Macrophage metalloelastase	B
MMP13	Collagenase 3	B
MMP14	MT1-MMP	E
MMP15	MT2-MMP	E
MMP16	MT3-MMP	E
MMP17	MT4-MMP	F
MMP18	Collagense 4 (Xenopus)	B
MMP19	RASI 1	B
MMP20	Enamelysin	B
MMP21	XMMP (Xenopus)	G
MMP22	CMMP (Chiken)	B
MMP23		H
MMP24	MT5-MMP	E
MMP25	MT6-MMP	F
MMP26	Endometase, Matrilysin 2	A
MMP27		B
MMP28	Epilysin	D

catalytic domain by a hinge or linker region (Figure 3). MMP7 and MMP26 merely lack these extra domains, where as MMP23 has unique cysteine-rich, proline rich, and IL-1 type II receptor-like domains instead of a hemopexin domain (Gururajan *et al*, 1998 and Park *et al*, 2000). When present, the hemopexin domain influences TIMP binding, the binding of certain substrates, membrane activation, and some proteolytic activities. For example, chimeric enzyme studies indicate that both ends of MMP1 (collagenase-1) are required for it to cleave native fibrillar collagens (Sanchez-Lopez *et al*, 1993). This collagenolytic activity requires the initial binding and orientation of the collagen fibril, local unwinding of its triple-helical structure, and sequential cleavage of each α-chain individually because the catalytic cleft is too narrow to accommodate the entire triple helix (Overall, 2001). Apparently, the hemopexin domain participates in all but the last of these steps. The hinge region, in turn, varies in length and composition among the various MMPs and also influences substrate specificity (Knauper *et al*, 1997). Gelatinase A and B (MMP2 and MMP9, respectively) are further distinguished by the insertion of three head-to-tail cysteine-rich repeats within their catalytic domain. These inserts resemble the collagen-binding type II repeats of fibronectin and are required to bind and cleave collagen and elastin (Shipley *et al*, 1996). In addition, MMP9 has a unique type V collagen-like insert of unknown importance at the end of its hinge region. Finally, the membrane type (MT) MMPs have a single-pass transmembrane domain and a short cytoplasmic C-terminal tail (MMPs 14,15,16, and 24) or a C-terminal hydrophobic region that acts as a glycophosphatidyl inositol (GPI) membrane-anchoring signal (MMP17 and MMP25) (Itoh *et al*, 1999 and Kojima *et al*, 2000). These domains play an essential role in the localization of several important proteolytic events to specific regions of the cell surface.

1.5.1.3.Regulation of MMP activity

The MMPs are synthesized as inactive zymogens (pro-MMPs). They are kept inactive by an interaction between a cysteine-sulphhydril group in the propeptide domain and the zinc ion bound to the catalytic domain: activation requires proteolytic removal of the propeptide domain (Sternlicht and Werb, 2001). Most of the MMPs are activated outside the cell by other activated MMPs or serine proteinases. However, MMP11, MMP28 and the MT-MMPs

can also be activated by intracellular furin-like serine proteinases before they reach the cell surface (Sternlicht and Werb, 2001).

MMP2 is activated at the cell surface through a unique multistep pathway that involves MMP14 (MT1-MMP) and the tissue inhibitor of melalloproteinases2 (TIMP-2) (Strongin *et al*, 1995): TIMP-2 binds MMP14 at its amino terminus and pro-MMP2 at its carboxyl terminus, which allows an adjacent, noninhibited MMP14 to cleave the bound proMMP2. MMP14 does not fully activate MMP2 and another, already activated; MMP2 is required to remove a residual portion of the MMP2 propetide (Deryugina *et al*, 2001). Pro MMP2 might also be activated by MMP15 by means of a mechanism that does not require TIMP2 (Morrison, 2001).

MMP activity is tightly controlled by endogenous inhibitors. The main inhibitor of MMPs in tissue fluid is α_2- macroglobulin, an abundant plasma protein (Sottrup-Jensen and Birkedal-Hansen, 1989). α_2- Macroglobulin binds to MMPs and the α_2- macroglobulin-MMP complex then binds to a scavenger receptor and is irreversibly cleared by endocytosis. In a similar way to α_2- macroglobulin, thrombospondin-2 forms a complex with MMP-2 and facilitates scavenger-receptor- mediated endocytosis and clearance (Yang *et al*, 2001). By contrast, thrombospondin-1 binds to Pro-MMP2 and -9 and directly inhibits their activation (Bein and Simons, 2000, and Rodriguez-Manzaneque, 2001). Curiously, thrombospondin-1 has also been reported to increase MMP2 and-9 activation (Taraboletti, 2000). The best-studied endogenous MMP inhibitors are TIMPs-1,2,3 and 4, which reversibly inhibit MMPs in a 1:1 stoichiometric fashion (Edwards, 2001). They differ in tissue-specific expression and ability to inhibit various MMPs (Edwards, 2001). Studies with TIMP2- deficient mice indicate that the dominant physiological function of TIMP2 is activation of MMP2 (Wang *et al*, 2000). MMP inhibitors that contain sub domains with structural similarity to the TIMPs also exist, and these include the carboxy-terminal fragment of the procollagen C-terminal proteinase enhancer protein (Mott *et al*, 2000) and the NC1 domain of collagen type IV (Netzer *et al*, 1998). Finally, RECK (reversion-inducing cysteine rich protein with Kazal motifs) is the only known membrane-bound MMP inhibitor (Oh *et al*, 2001).

To accomplish their normal (or pathologic) functions, MMPs must be present in the right cell type and pericellular location at the right time, and in the right amount, and they must be activated or inhibited appropriately. Thus MMP3 are tightly regulated at the transcriptional and post-transcriptional levels and are also controlled at the protein level via their activators, their inhibitors, and their cell surface localization.

1.5.1.4.Transcriptional Regulation

The biologic function of individual MMPs is largely dictated by their differential patterns of expression. Indeed differences in the temporal, spatial, and inducible expression of MMPs are often indicative of their unique roles. Accordingly, most MMPs are closely regulated at the level of transcription, with the notable exception of MMP2 as described earlier, which is often constitutively expressed and controlled through a unique mechanism of enzyme activation (Strongin *et al*, 1995) and some degree of post-transcriptional mRNA stabilization (Overall *et al*, 1991). Nevertheless, data indicate that the basal expression of MMP2, MMP14 (MT-MMP), and TIMP2 is coregulated, which is consistent with their cooperation during MMP2 activation and with specific similarities in their gene promoters (Lohi *et al*, 2000). Otherwise, MMP gene expression is regulated by numerous stimulatory and suppressive factors that influence multiple signaling pathways. Type I collagen acts as a ligand for discoid in domain containing receptor-like tyrosine kinases that induce MMP1 expression when they are activated by intact collagen and become inactive when they bind MMP1-cleaved collagen (Vogel *et al*, 1997 and Shrivastava *et al*, 1997). Thus MMP1 expression can be induced by its own substrate and specifically repressed once it cleaves that substrate and is no longer needed. In addition MMP expression is regulated by several cytokines and growth factors, including interleukins, interferons, EGF, KGF, NGF, basic FGF, VEGF, PDGF, TNF-α TGF-β and the extracellular matrix metalloproteinase inducer EMMPRIN (Lohi et al, 2000). Many of these stimuli induce the expression and or activation of c-fos and c-jun proto-oncogene products, which heterodimerize and bind activator protein (AP-1) sites within several MMP gene promoters.

Although AP-1 complexes play a critical role in the regulation of several MMP genes, other factors are also involved. In some cases, one signal may co-ordinately regulate some MMP genes and differentially regulate others. For example, TGF-β suppresses the

transcription of the MMP1 and MMP3 (stromelysin-1) genes but induces the expression of MMP13 (collagenase-3) (Uria *et al*, 1998). In addition, some MMPs are expressed in only a small repertoire of all types, eg. MMP20 expression appears to be confined to the enamel organ of developing teeth (Sternlicht *et al*, 2000), and normal MMP9 expression is largely limited to osteoclasts, macrophages, trophoblast cells, hippocampal neurons, and migrating keratinocytes at the margins of healing wounds (Mohan *et al*, 1998 and Munaut *et al*, 1999). Basal and inducible levels of MMP gene expression can also be influenced by genetic variations that may, in turn, influence the development or progression of several diseases. Common bi-allelic single-nucleotide polymorphisms (SNPs) that influence the rate of transcription have been identified in several MMP gene promoters (Ye, 2000).

1.5.1.5.Post Transcriptional Regulation

Post-transcriptional mechanisms can also influence MMP expression. For example, mRNA transcripts that encode MMP1 and MMP3 are stabilized by phorbol esters and EGF, where as MMP13 transcripts are stabilized by PDGF and glucocorticoids and destabilized by TGF-β (Delany *et al*, 1995 and Vincenti, 2001). The turnover of MMP1 mRNA is apparently regulated by AU-rich sequences in the 3, untranslated region, and similar sequences may also regulate the stability of other MMP transcripts (Vincenti, 2001). In addition, a soluble and proteolytically active form of MT3-MMP is generated by alternative mRNA splicing rather than membrane shedding, where as the multiple transcripts of MMP13, MMP17, and MMP20 probably result from alternative polyadenylation (Sternlicht and Werb, 1999).

1.5.1.6.Regulation of MMP Secretion

Most MMPs are constitutively secreted once they become translated. Conspicuous instances of secretory control do exist, MMP8 (collagenase-2, neutrophil collagenase) and MMP9 are synthesized by differentiating granulocytes in the bone marrow, stored in the specific and gelatinase (teriary) granules of circulating neutrophils, respectively and released following neutrophil activation by inflammatory mediators (Hasty *et al*, 1990). In macrophages, plasmin and thrombin induce the secretion of MMP12, but do not alter its rate of transcription (Raza *et al*, 2000). Instead, post-translational release of pre-formed MMP12

from macrophages occurs in response to protein kinase C activation down stream of the G-protein-coupled thrombin receptor PAR-1 (proteinase-activated receptor-1), which becomes activated after binding a ligand that is generated from its own N-terminal end by thrombin-dependent cleavage.

1.5.1.7.Activation of Latent Metalloproteinases

MMPs are first synthesized as inactive proenzymes or zymogens. Their latency is maintained by an unpaired cysteine sulfhydryl group near the C- terminal end of the propeptide domain. This sulfhydryl acts as a fourth ligand for the active zinc ion, and activation requires that this cysteine-to-zinc switch be opened by normal proteolytic removal of the propeptide domain or by ectopic perturbation of the cysteine-zinc interaction (Van wart and Birkedal-Hanses, 1990). Once displaced, the thiol group is replaced by a water molecule that can then attack the peptide bonds of MMP targets.

Although most MMPs are secreted as latent zymogens , MMP11 (stromelysin 3) , MMP27 (epilysin), and the MT-MMPs contain an RXK / RR furin-like enzyme recognition motif between their propeptide and catalytic domains. This allows them to be activated by intracellular subtilisin-type serine proteinases before they reach the cell surface or are secreted (Pei and Weiss, 1995). MMP23 also has a furin-susceptible cleavage site and is a likely target of intracellular proprotein convertases , but unlike all other MMPs , it lacks the conserved cysteine that is required for enzyme latency in the first place (Gururajan *et al*, 1998). All other MMPs lack a furin- susceptible insert and are thus activated outside the cell following their secretion.

The extra cellular activation of most MMPs can be initiated by other already activated MMPs or by several serine proteinases that can cleave peptide bond within MMP prodomains (Woessner and Nagase, 2000). However, MMP2 is refractory to activation by serine proteinases and is instead activated at the cell surface through a unique multi step pathway involving MT-MMPs and TIMP2 (Strongin et al, 1995). Indeed, MT1-MMP is a particularly efficient MMP2 activator, where as MT-MMPs that are unable to activate MMP2 (Miyamori *et al*, 2000, and English *et al*, 2000). First, a cell surface MT-MMP binds and is inhibited by the N-terminal domain of TIMP2, and the C-terminal domain of the bound

TIMP2 acts as a receptor for the hemopexin domain of pro MMP2. Then, an adjacent, uninhibited MT-MMP cleaves and activated the tethered proMMP2. Following the initial clearage of proMMP2 by MT1-MMP, a residual portion of the MMP2 propeptide is removed by another MMP2 molecule to yield a fully active, mature form of MMP2 (Deryugina *et al*, 2001)

The role of TIMP2 in MMP2 activation is its dominant in vivo function, as shown by targeted mutagenesis in mice (Wang *et al*, 2000). Nevertheless, while the C-terminal domain of TIMP2 participates in the cell surface docking and activation of MMP2, its N-terminal domain is an MMP inhibitor, Not surprisingly, low-to-moderate levels of TIMP2 promote the activation of MMP2, where as higher levels inhibit its activation by saturating free MT-MMPs that are needed to remove the MMP2 prodomain (Strongin *et al*, 1995). TIMP2 protein levels are reduced and MMP2 activation is enhanced in the presence of the MMP2 substrate, type IV collagen (Maquoi *et al*, 2000). Further more, the ability of collagen to induce MMP2 activation on demand probably results from TIMP2 degradation because there are no accompanying changes in MMP2, MT1-MMP,or TIMP2 mRNA expression or in the synthesis or activation of MT1-MMP. Therefore, local accumulation of type IV collagen may trigger its own degradation by some how lowering local TIMP2 concentrations to levels that favor MMP2 activation.

1.5.1.8.MMP Substrates

Historically, MMPs were thought to predominantly degrade structural components of the ECM, there by facilitating cell migration. But because cells have receptors for structural ECM components (for example, integrins), cleavage of ECM proteins by MMPs also affects cellular signaling and functions (Streuli, 1999). Cleavage of ECM components by MMPs can also generate fragments with new functions: cleavage of laminin-5 and collagen type IV results in exposure of cryptic sites that promote migration (Xu-J, 2001). Moreover, cleavage of insulin-like growth-factor-binding protein (IGF-BP) and prelecan releases IGFs and fibroblast growth factors (FGFs), respectively (Manes *et al*, 1997).

In addition to cleaving structural ECM components, MMPs and the related proteinases, the ADAMS (a disintegrin and metalloproteinases), participate in the release of

cell-membrane-bound precursor forms of many growth factors, including transforming growth factor- α (TGF- α) (Peschon, 1998). Bioavailability of TGF-β is regulated differently: it is released by MMP-2 and MMP-9 from an inactive extracellular complex (Yu, 2000).

Growth factor receptors are also MMP substrates. The FGF receptor 1 is cleaved by MMP2 (Levi, 1996), whereas two members of the epidermal-growth-factor receptor EGFR) family-HER2/neu (also known as ERBB2) and HER4 (also known as ERBB4)- and the hepatocyte-growth-factor receptor C-MET are substrates for unidentified MMPs or ADAMs (Nath *et al*, 2001). In all cases, extracellular domains of the receptors are released, and these might function as decoy receptors for the respective ligands. Cell adhesion molecules are also MMP substrates. Cleavage of E-cadherin and CD44 results in the release of fragments of the extracellular domains and in increased invasive behaviour (Kajita, 2001) and cleavage of the α V integrin subunit precursor by MMP14 enhances cancer cell migration (Deryugina *et al*, 2001). Finally, the MMPs cleave and activate their own zymogen forms and, in addition, cleave other MMPs and proteinase inhibitors such as serpins (Sternlicht and Werb, 2001).

1.5.1.9.MMP catabolism and Clearance

An obvious means of regulating MMPs is via their own proteolytic inactivation and physical clearance. Although considerable progress has been made in understanding the progressive proteolytic processing of MMP propeptides, relatively little is known about the further auto proteolysis of active MMPs. Nevertheless, it is clear that some cleavages inactivate MMPs, Whereas others, such as those that specifically remove the hemopexin domain, can generate truncated enzymes that lose their ability to cleave some substrates but retain their ability to cleave others (Woessner and Nagase, 2000). Such processing can also diminish their affinity for and ability to be inhibited by TIMPs, as occurs with C-terminally truncated MMP2. Removal of the hemopexin-like domain also cancels the ability of certain MMPs to localize to the cell surface. In addition, MT-MMPs can be secreted if they are cleaved at a juxtamembrane site before or after they reach the cell surface (Imai *et al*, 1996). Thus factors that influence MMP degradation can alter the steady-state concentrations of

MMPs, their substrate speificities, their localization, and their ability to be activated or inhibited.

Another means of regulating extracellular MMP levels is by the direct clearance of intact enzymes. Most MMPs cleave the bait region of α 2- macroglobulin, thereby initiating a conformational change in the large tetrameric macroglobulin that irreversibly traps the enzymes (Sottrup-Jenson and Birkedal-Hanson, 1989). Although the catalytic activity of the MMP is not inhibited per se, its physical entrapment keeps the enzyme from interacting with natural substrates, and the α2-macroglobulin/MMP complex is eventually endocytosed and permanently cleared.

Thrombospondin2 (TS2) has also been implicated in the clearance of MMPs. Interestingly, TS2-deficient mice exhibit a number of connective tissue abnormalities, and their fibroblasts have an adhesion defect that is the result of increased MMP2 levels (Yang *et al*, 2000). The increased MMP2 levels occur because TS2 normally binds both latent and active MMP2 and because TS2 is normally endocytosed by the low-density lipoprotein receptor-related protein (LRP) and probably carries any bound MMP2 with it (Yang *et al*, 2001). The cellular internalization of TS2/MMP2 complexes by the LRP scavenger receptor may therefore play an important role in regulating MMP2 levels outside fibroblasts and other cells. Evidence also indicates that MMP13 is rapidly cleaved after it binds to an MMP13-specific 170-KDa high-affinity receptor present on various cell types (Barmina *et al*, 1999). The binding requires calcium, and the subsequent internalization and degradation of MMP13 requires LRP because LRP-null cells bind MMP13 but fail to internalize it. More over, the internalization of both MMP13 and TS2/MMP2 complexes is inhibited by the 39-KDa receptor-associated protein RAP, which binds and inhibits LRP. Thus MMPs are tightly regulated by several variously characterized mechanisms during virtually every aspect of their life span, from their induction to their ultimate destruction.

1.5.2.MMP and AMI

It is evident hat MMPs are involved in the process of plaque repture in atherosclerosis, resulting the further complications of the disease. Mainly MMP2 (72 KDa) and MMP9 (92 KDa) are involved in this process. A recent study has shown that the

polymorphisms in the promoter regions of MMP2, MMP3, MMP9 and MMP12 genes also determines the severity of coronary artery disease (Nicolas Lamblin *et al*, 2002). Once the plaque has ruptured, blood clots tend to form over ruptured plaques and can then occlude arteries, leading to further atherosclerotic complications as acute myocardial infarction and stroke.

1.6.Aim of the Present Study

From the above review it is evident that oxidative stress, antioxidant scavengers and metalloproteinases have a major role to play in the pathogenesis of atherosclerosis and its sequele acute myocardial infarction. Our present study is aimed to study the oxidative stress, the antioxidant scavengers and metalloproteinases in patients with acute myocardial infarction.

1.7.Relevance of the Study

The incidence of coronary artery disease (CAD) is very high in India. Studies conducted at Thiruvananthapuram in urban and rural setting also pointed out high prevalence of this disease in Kerala. Acute Myocardial Infarction, an important presentation of CAD causes significant mortality and morbidity. It affects people at their prime and often takes away the breadwinner thereby shattering the economy of many families. Even after recovery, most people cannot return to their job, thereby enhancing the common hardships. All these cause a great burden on the state's economy too. A study which can throw light on the causative factors of myocardial infarction will definitely help in managing those patients better. An increasing trend has recently been observed on the incidence of acute coronary syndromes in Indian population. This shows that acute myocardial infarction is fast emerging as a major cause of death in developing countries, especially in India. Mechanisms involved in the formation and rupture of atheromatous plaque is not fully established. It is considered important to study the role of inflammation and oxidative stress on the incidence of myocardial infarction. The activities of enzymes metalloproteinase 2 and metalloproteinase 9, which are considered to be important factors leading to plaque rupture are to be assayed in serum of patients admitted with myocardial infarction. The assays of scavenger enzymes of reactive oxygen species- superoxide dismutase (SOD), catalase

(CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) are also important to study the role of oxidative stress in the incidence of acute myocardial infarction. It is thought that elevated levels of matrix metalloproteinases (MMP2 and MMP9) and an increased oxidative stress may lead to plaque rupture, which would lead to acute myocardial infarction, and this study may give us an opportunity to test this hypothesis.

Hence, the objectives of the present study are to find out

1) Whether there is increased oxidative stress demonstrated by altered levels of scavenger enzymes of reactive oxygen species,

2) Whether there is elevated matrix metalloproteinases in acute myocardial infarction patients in general or a subgroup of patients in particular,

3) Whether this information will be useful in identifying a high-risk subset of patients after acute myocardial infarction.

EVALUATION OF THE ROLE OF MATRIX METALLOPROTEINASES AND OXIDATIVE STRESS IN THE PATHOGENESIS AND COURSE OF ACUTE MYOCARDIAL INFARCTION

THESIS SUBMITTED TO THE UNIVERSITY OF CALICUT FOR THE DEGREE OF

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(FACULTY OF MEDICINE)

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CHAPTER 2

MATERIALS AND METHODS

2.0.Chemicals

Nicotinamide adenine dinucleotide phosphate-reduced (NADPH), nitroblue tetrazolium (NBT), 1-chloro 2,4-dinitrobenzene (CDNB), 5 5'-dithio-bis 2 nitrobenzoic acid (DTNB) and reduced-glutathione (GSH) were purchased from Sisco Research Laboratories, Mumbai. Chloroform, thiobarbituric acid (TBA) and thiourea were purchased from E-Merck (India), Mumbai, India. p-phenylene diamine was obtained from Sigma -Aldrich, Bangalore. Drabkin's reagent was purchased from AGAPPE Diagnostics, Mumbai, α,α'-dipyridyl from Loba Chemie, Mumbai and heparin from Gland Pharma Ltd. Hyderabad. All other chemicals and reagents used for this study were of analytical reagent grade.

2.1. Diagnostic kits

Plasma glucose	SPAN Diagnostics Ltd, Surat.
Urea	Ecoline, E-Merck Ltd, Mumbai.
Creatinine	AUTOPAK, Bayer Diagnostics India Ltd, Baroda.
Uric acid	Ecoline, E-Merck Ltd, Mumbai.
Bilirubin	SPAN Diagnostics Ltd, Surat.
Albumin	Ecoline, E-Merck Ltd, Mumbai.
Cholesterol	Ecoline, E-Merck Ltd, Mumbai.
Triglycerides	Ecoline, E-Merck Ltd, Mumbai.
Cholesterol HDL	Ecoline, E-Merck Ltd, Mumbai.
Zinc	Chema Diagnostica, Argentina.
Magnesium	Raichem, San Diego, California.
Ceruloplasmin	SPINREACT, Santa Coloma, Spain.
Ferritin	General Biological Corp, Taiwan.

Glutathione peroxidase (GPx)	RANDOX Laboratories Ltd, United kingdom.
Glutathione Reductase (GR)	RANDOX Laboratories Ltd,United Kingdom.
MMP-2	CHEMICON International, Temecula, California.
MMP-9	CHEMICON International, Temecula, California.

2.2.Instruments

Spectrophotometer	ELICO (SL159 and SL177)
Cooling centrifuge	Remi
Deep freezer (-70°C)	Remi
Cell-Dyne 1700	ABOTT Diagnostics
Automatic analyzer	Hitachi
ECS 2000	SM Diagnostics
Easy Lyte Plus	Medica EasyLyte
Microlab 200	Merck
pH meter	ELICO
ELISA reader	Organon Teknika
ECG Machine	BPL

2.3.Patients

Patients admitted to the Intensive Coronary Care Unit (ICCU) of Amala Cancer Hospital with a diagnosis of acute myocardial infarction presenting within 24 hours of onset of chest pain were included in the study. Myocardial infarction was diagnosed by at least 0.1-mv ST segment elevation in two or more contiguous limb leads or 0.2-mv ST segment elevation in two or more chest leads associated with typical chest pain. Patients with cardiogenic shock, cerebrovascular

accident and significant hepatic or renal disease were excluded. Patients with clear evidence of infection anywhere in the body were also excluded.

In patients included in the study, detailed history was taken and complete physical examination was carried out by the cardiologist. A 12 lead ECG with V_3R and V_4R was recorded immediately on admission and repeated after 2hrs, 6hrs, 12hrs, 24hrs, 48hrs and pre-discharge. 10 ml blood was withdrawn for laboratory analysis. Serial creatine kinase assays were also done to confirm the myocardial infarction and it was done at 2hrs, 6hrs, 12hrs, 24hrs, and 48hrs after admission. Chest X- ray was done at the time of discharge from the ICCU. An echocardiographic examination was performed at the time of discharge or at the end of the first week or early second week after admission. All patients were seen in the cardiology out-patient department 4 to 6 weeks after discharge, when a detailed history was taken and complete physical examination was again carried out. A symptom limited treadmill test was done as per the Bruce protocol and maximum heart rate (HR), blood pressure (BP), double product, time to 1 mm ST depression, metabolic equivalences (METs) achieved, duration of exercise, angina, dyspnoea and arrhythmias were recorded along with ST segment changes.

The normal volunteers had no past history or evidence of cardiovascular disease, hypertension or diabetes mellitus. The present study does not include control subjects with a history of neoplastic, hepatic, infectious or autoimmune disease or any surgical procedure in the preceeding 6 months.

The patients were allowed to relax and on the second day they were subjected to an oral questionnaire as described in our Proforma, in order to collect the history of these patients. They were asked about the type of chest pain, the time of onset of the pain, radiation to other parts of the body and any previous history of chest pain. They were also asked about the symptoms

associated with the chest pain, history of diabetes, history of hypertension, habits of smoking or alcohol or pan chewing, food habits (vegetarians or non vegetarians) and any positive family history of AMI. Then these patients were categorized according to the following risk factors and based on these risk factors the study has been designed.

1) Age and sex
2) Time of onset of chest pain
3) History of diabetes and hypertension
4) A cholesterol value of <200 mg/dl and >200 mg/dl
5) Habits of smoking and alcohol intake
6) Food habits and a positive and negative family history of AMI

2.4. Laboratory investigations

The following laboratory investigations were carried out.

2.4.1. Hemoglobin

The hemoglobin (oxyhemoglobin, methemoglobin, carboxyhemoglobin) is converted to cyanmethemoglobin by reaction with potassium ferricyanide and potassium cyanide. The absorbance of cyanmethemoglobin formed is proportional to the hemoglobin concentration (Drabkin and Austin, 1932)

The reaction mixture contained 10 µl of whole blood or standard (60mg/dl) and 2.5 ml of reagent, which contained potassium ferricyanide, potassium cyanide and monopotassium phosphate. Mixed well and kept at room temperature for five minutes and the absorbance against the reagent blank was taken at 546nm.

2.4.2. WBC

WBC was counted using a Von Behrens WBC transducer. A whole blood sample was aspirated from the sample collection tube and sent to the sample rotor valve. Then 6µl sample (measured in the sample rotor valve) was diluted to 1:250 with 0.994 ml of diluent and 0.5ml of WBC lyse reagent. The diluted sample was then sent to the WBC detector. After approximately 13 sec in this condition, the red blood cells were demolished and platelets contracted. 400µl of the diluted sample was aspirated through the aperture (total volume was controlled by the WBC manometer). The WBC detector counts and totals WBC using the DC detection method (Robert.H.Carman, 1993).

2.4.3. ESR

ESR was determined by Westergren's method (Robert.H.Carman, 1993). The blood and anticoagulant were mixed by rotating the bottle on the surface of a table. Sucked the blood into the Westergrens tube and allowed it to empty into the bottle twice, finally sucking the blood to the 'O' mark. Keeping the finger over the open end, transfered the tube in the Westergren stand. Placed the lower end of the tube firmly over the rubber cork, and fixed its upper end on a spring fitted to the stand that was holding and keeping the tube in a vertical position. Without leakage of blood from lower end allowed the tube to remain in this position for one hour at the end of which, took the readings as mm of clear plasma column above the red cells.

2.4.4. Sodium

Sodium in the serum was detected by using flame photometry (Velapoldi *et al*, 1978), in which the sample is diluted with a known concentration of a reference ion (usually lithium or cesium), is aerosolized and passed through a flame which excites the cations. They re-emit the

energy as light of different frequencies; and the amplitude of this emission is proportional to the ion concentration in the sample.

The sodium was also measured by EasyLyte, using ion selective electrode technology. The flow through sodium electrode contains glass tubing, specially formulated to be sensitive to sodium ions. The potential of the electrode is measured relative to a fixed, stable voltage established by the silver/silver chloride reference electrode. An ion selective electrode develops a voltage that varies with the concentration of ion to which it responds.

A comparative method of measurement was utilized. First, the analyzer measured the potentials developed when the sample was pumped through the electrodes. Next, standard solution was pumped through the electrodes. The difference in the two potentials is related logarithmically to the concentration of sodium ion in the sample divided by it's respective concentration in the standard solution. Since the difference in potentials and the concentration of sodium ion in the standard solution were known, the computer could calculate the concentration of the ions in the sample solution in accordance with the Nernst equation.

2.4.5. Potassium

Potassium in the serum was detected by using flame photometry (Velapoldi *et al,* 1978), in which the sample is diluted with a known concentration of a reference ion (usually lithium or cesium), is aerosolized and passed through a flame which excites the cations. They re-emit the energy as light of different frequencies; and the amplitude of this emission is proportional to the ion concentration in the sample.

The potassium was also measured by EasyLyte, using ion selective electrode technology. The flow through potassium electrode employs a plastic tube, incorporating valinomycin as the selective element. The potential of the electrode is measured relative to a

fixed, stable voltage established by the silver/silver chloride reference electrode. An ion selective electrode develops a voltage that varies with the concentration of ion to which it responds.

A comparative method of measurement was utilized. First, the analyzer measures the potentials developed when the sample was pumped through the electrodes. Next, standard solution was pumped through the electrodes. The difference in the two potentials is related logarithmically to the concentration of potassium ion in the sample divided by it's respective concentration in the standard solution. Since the difference in potentials and the concentration of potassium ion in the standard solution were known, the computer could calculate the concentration of the ions in the sample solution in accordance with the Nernst equation.

2.4.6. Urea

Urea was detected by the Urease method (Lespinas *et al*, 1989). The reaction mixture contained 10µl of sample or standard (50mg/dl) and 1ml of reaction solution which contained Tris, pH 7.8 (120mmol/l), 2-oxoglutarate (7mmol/l), ADP (0.6mmol/l), Urease (>6 KU/l), GLDH (>1KU/l) and NADH (0.25mmol/l). Mixed well and incubated for 60 seconds at 30°C. Then the absorbance was taken at 340nm.

2.4.7. Creatinine

Creatinine was done by Picrate method (Jaffe's reaction) (Cook, 1975), in which 50 µl of the sample was mixed with 1ml of the working solution which contained Picric acid (49mmol/l) and sodium hydroxide (28mmol/l). The concentration of the standard was 2.0mg/dl. The readings were taken at 510nm immediately after the addition of the reagent.

2.4.8. Random blood glucose

Random glucose was detected by GOD-PAP method (Trinder, 1969), in which

10 µl of the sample was mixed with 1ml of working reagent, which contained phosphate buffer pH 7.4 (100mmol/l), phenol (10mmol/l) glucose oxidase (≥10000U/l), peroxidase (≥600U/l) and 4-aminoantipyrine (270Umol/l). The absorbance was taken at 532nm after an incubation of 20 min at 37°C. The readings were taken against the blank. The standard concentration was 100mg/dl.

2.4.9. Creatine kinase (CK)

Creatine kinase (CK) was determined by the optimized standard method (Gruber, 1978). The reaction mixture contained 100µl of the sample, 2.5ml of the reagent solution, which contained imidazole buffer (pH 6.7, 0.1 mol/l), glucose (20mmol/l), Mg ++ (10mmol/l), EDTA (2mmol/l), hexokinase (2.5U/ml), G6PDH (1.5U/ml), ADP (2mmol/l), AMP (5mmol/l), diadenosine pentaphosphate (10µmol/l), NADP (2mmol/l), creatine phosphate (30mmol/l) and N-acetylcysteine (20mol/l). Mixed well and allowed to stand for 3 min at 25^{0}C. The readings were taken repeatedly after1, 2 and 3 min at 340nm.

2.4.10. Creatine kinase isoenzyme (CK-MB)

CK-MB was assayed by kinetic immunoinhibition method (Apple, 1992). The procedure involves measurement CK activity in the presence of an antibody to CK-M monomer. This antibody completely inhibits the activity of CK-MM and half of the activity of CK-MB while not affecting the B subunit activity of CK-MB and CK-BB. Then the CK method is used to quantitatively determine the CK-B activity. The CK-MB activity is obtained by multiplying the CK-B activity by two.

40µl of the serum sample was mixed with 1.0 ml of the reconstituted reagent, which contained imidazole (pH 6.7, 100 mmol/l), magnesium acetate (10mmol/l), glucose (20mmol/l), EDTA (2mmol/l), ab.anti-CK-M (2000U/l), ADP (2mmol/l), AMP (5mmol/l),

NADP (2mmol/l), diadenosine-5-P (10mmol/l), hexokinase (2500U/l), G-6-PDH (1500U/l), creatine phosphate (30mmol/l) and N-acetylcysteine (20mmol/l). Mixed well and the readings were taken at 340 nm immediately.

2.4.11.Cholesterol

Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolyses the esters. In the subsequent enzymatic oxidation by cholesterol oxidase, H_2O_2 is formed. This is converted into a colored quinonimine in a reaction with 4-amino antipyrine and phenol catalysed by peroxidase (Duncan *et al*, 1982).

The reaction mixture contained 10µl of serum or plasma or standard (200mg/dl), 1ml reaction solution which contained, PIPES buffer pH: 7.5 (99mmol/l), salicylic alcohol (3.96mmol/l), 4-Aminoantipyrine (0.5mmol/l), peroxidase (≥100U/l), cholesterol oxidase (≥100U/l) cholesterol esterase (≥100U/l). Mixed well and incubated for 5min at 37°C.Then the absorbance was measured at 546nm.

2.4.12.HDL-Cholesterol

HDL-Cholesterol was estimated from the supernatant after precipitation by phosphotungstic acid and magnesium chloride (1.4mmol/l phosphotungstic acid and 8.6mmol/l magnesium chloride) (Harris *et al* 1996). 0.1ml of the supernatant was mixed with 1ml of reaction solution (same as that for cholesterol). Mixed well and incubated for 5min at 37°C. The absorbance was measured against the reagent blank at 546nm.

2.4.13.Triglycerides

Triglycerides in presence of lipase are converted to glycerol and fatty acid. This glycerol by the action of glycerokinase is converted to glycerol-3-phosphate. Glycerol-3-phosphate will react with glycerol-3-phosphate oxidase (GPO) to form dihydroxy acetone phosphate and H_2O_2.

H_2O_2 generated in this step will react with aminoantipyrine and 4-chlorphenol in presence of peroxidase (POP) to form chinonimine, which is read at 546nm (National Cholesterol Education Program 1995).

10µl of serum or plasma was mixed with 1ml of reaction solution which contained Good's buffer pH 7.2 (50mmol/l), 4-chlorphenol (4mmol/l), ATP (2mmol/l), Mg2+ (15mmol/l), glycerokinase (≥0.4KU/l), peroxidase (≥2KU/l), lipoprotein lipase (≥2KU/l), 4-aminoantipyrine (0.5mmol/l), glycerol-3-phosphate oxidase (≥1.5KU/l). 10µl of triglycerides (200mg/dl) was used as the standard. Mixed and incubated at 37°C for 10min. The absorbance was taken at 546nm.

2.4.14. LDL-Cholesterol

LDL-Cholesterol was calculated by using Friedewald's formula (Friedewald *et al* 1972) ie., LDL-Cholesterol (mg/dl) = Total cholesterol - (HDL-Cholesterol + TG/5)

2.4.15. Albumin

Albumin forms blue-green complex with bromocresol green at slightly acidic pH, which is measured photometrically at 600nm (Doumas and Peters, 1997).

The reaction mixture contained 10µl of serum and 1ml of reaction solution (citrate buffer pH 4.2 (30mmol/l) and bromocresol green (0.26mmol/l)). Mixed, incubated for 10 min at 37°C. The absorbance was red at 600nm. 10µl of albumin (5g/dl) was used as the standard.

2.4.16. Uric acid

Uric acid by the action of the enzyme uricase is converted to allantoin and hydrogen peroxide. Hydrogen peroxide can react with 2,4,6-tribromo-3-hydroxy benzoic acid and 4-aminoantipyrine to give chinonimine which is measured at 546nm (Whelton *et al*, 1993).

20µl of serum was mixed with 1ml of reagent 1 (phosphate buffer pH:7(100mmol/l), 2,4,6-tribromo-3-hydroxy benzoic acid(1mmol/l)) and incubated at 37°C for 5min. 250µl of reagent 2 (phosphate buffer pH:7 (100mmol/l), 4-aminoantipyrine 0.3mmol/l, K_4[Fe(CN)6] (10µmol/l), peroxidase (>2KU/l) and uricase (>30U/l)). Incubated at 37°C for 10min and the absorbance was read at 546nm within 30min. 20µl of uric acid solution (6mg/dl) was used as standard.

2.4.17. Bilirubin

Direct bilirubin couples with diazotized sulfanilic acid, forming azobilirubin, a red purple colored product in acidic medium. The intensity of the color is measurd spectrophotometrically and it is proprtional to the concentration of the bilirubin (Doumas *et al*, 1973).

The reaction mixture contained 100µl of serum / plasma, 0.9ml of distilled water, 0.25ml Diazo reagent and 1.25ml methanol. Mixed well and kept the tubes in dark at room temperature for 30 minutes and then the absorbence was taken at 540nm.

2.4.18.Ceruloplasmin

The determination of human ceruloplasmin is based on the reaction between ceruloplasmin as antigen and the specific antiserum as antibody. This reaction forms an insoluble complex producing a turbidity, which is measured spectrophotometrically at 340nm (Milne, 1994).

The reaction mixture contained 50µl of diluted sample (1:21 with 0.9% NaCl) and 1ml of the working reagent, which contained TRIS/PEG buffer (pH 7.5), and antiserum anti-

ceruloplasmin. Mixed well and the absorbance was taken against the blank after 10 minutes at 340nm.

2.4.19. Superoxide Dismutase (SOD)

SOD is measured by the degree of inhibition of formazan dye, which is formed by the reduction of nitroblue tetrazolium (NBT) in presence of riboflavin (Mc Cord and Fridovich, 1969).

Whole blood was obtained by venipuncture prevented from coagulation by heparin. 0.1ml of blood was hemolysed by 0.9ml of cold (4°C) water. Hemoglobin was removed by 0.25ml of chloroform and 0.5ml of ethanol with rigorous mixing (Tsuchihashi's method). The mixture was centrifuged at 18,000 X g for 60 min. The clear supernatant was used for SOD assay. Different microliters of supernatant was pipetted into test tubes. The volume was made up to 2.65ml with phosphate buffer (pH 7.8). 2.65ml of phosphate buffer was taken as blank. Into these tubes 0.2ml of EDTA / NaCN followed by 0.1ml of NBT were added. Just before taking the reading 0.05ml of riboflavin was added into each tube and the absorbance was noted at 560 nm. After taking the initial readings the tubes were kept for illumination for at least 15 min. Then the tubes were removed and the final readings were again taken at 560 nm. The difference between initial and final readings were used for the determination of SOD values.

2.4.20. Catalase

In the UV range H_2O_2 shows a continued increase in absorption with decreasing wave length. The decomposition of H_2O_2 can be followed directly by the decrease in extinction at 240nm (E_{240}). The difference in extinction (ΔE_{240}) per unit time is a measure of the catalase activity (Aebi, 1983).

The assay system consisted of phosphate buffer (50mM, pH:7) 1ml, hemolysate 2ml in control and 2ml hemolysate and 1ml H_2O_2 solution (30mM) in test. The reaction occurred by the addition of H_2O_2. The decrease in extinction was recorded at 240 nm at 15 sec intervals for 3 minutes.

2.4.21.Reduced-Glutathione (GSH)

The glutathione was estimated by the reaction of GSH with DTNB to give a yellow colored complex with absorption maximum at 412 nm (Moron *et al*, 1979).

The reaction mixture contained 0.5ml of hemolysate and 125µl of 25% trichloroacetic acid (TCA) to precipitate the proteins. The tubes were cooled on ice for 5min and the mixture was further diluted with 0.6ml of 5% TCA. Centrifuged for 10min and 0.3ml of the resulting supernatant was taken for the GSH estimation. The volume of the aliquot was made up to 1ml with 0.2M phosphate buffer (pH 8) and 2ml of freshly prepared DTNB (0.6mM) was added to the tubes and the intensity of yellow color formed was read at 412nm.

2.4.22.Glutathione Reductase (GR)

Glutathione reductase activity is determined by the amount of NADPH consumed in the conversion of oxidised glutathione (GSSG) to reduced glutathione (GSH). The reaction is catalysed by glutathione reductase (Racker *et al*, 1955).

The reaction mixture contained 40 µl of sample (serum, plasma or blood) and 1000 µl of GSSG (2.2mmol/l). The reaction commenced with the addition of 200 µl of NADPH and the decrease in absorbance/min was noted and followed at every 1min interval for 5min at 340nm.

2.4.23. Glutathione peroxidase (GPX)

Glutathione peroxidase was estimated by the method of Paglia and Valentine (Paglia and Valentine, 1967).

The reaction mixture contained 50 µl of blood, 50 µl of distilled water, 2.5ml of reagent (GSH 4mmol/l, GR≥0.5U/l and NADPH 0.34mmol/l) and 10 µl of cumene hydroperoxide (4.3mmol/l). The absorbance was taken at 1min interval for 3min at 340nm at 37°C.

2.4.24.Glutathione-S -transferase (GST)

Glutathione-S-transferase was estimated by the method of Habig et al (Habig *et al*, 1974). The reaction mixture contained 2.79ml phosphate buffer (0.1M, pH 6.5), 100 µl of GSH (30mM), 10 µl of hemolysate and 100 µl of CDNB (30mM). The absorbance was noted for 3min at 1min interval at 340nm at 37^0C.

2.4.25. Lipid peroxidation

Lipid peroxidation is a chain reaction initiated by the attack on the membrane lipids by free radicals that has sufficient reactivity to abstract a hydrogen atom from the methylene group. This leaves behind an unpaired electron on the carbon atom. The carbon radical is stabilised by molecular rearrangement to produce conjugated diene, which then reacts with an oxygen molecule to form a peroxy radical. Peroxy radical can form cyclic peroxide and cyclic endoperoxide. Fragmentation of these peroxides leads to the formation of malondialdehyde (MDA) (TBARS). This reacts with thiobarbituric acid to form pink colored complex, which is measured at 532 nm (Yoshioka *et al*, 1979).

0.2ml of the serum was mixed with 1ml of 20% trichloroacetic acid (TCA). To the mixture 0.4ml of 0.67% thiobarbituric acid (TBA) was added, shaken and kept for 30 minutes in

a boiling water bath. After cooling to room temperature, 1.6ml of butanol was added and the mixture was shaken. The organic mixture was separated by centrifugation and its absorbance was measured at 532nm. The break down product of 1,1,3,3-tetramethoxy propane was used as standard.

2.4.26. Vitamin E

Plasma tocopherol is assayed using the Emmeric Engel reaction, which is based on the reduction by tocopherols of ferric to ferrous ions which then form a red complex with α,α'-dipyridyl. Tocopherols and carotenes are first extracted into xylene and the extinction read at 460nm to measure the carotenes. A correction was made for these after adding ferric chloride and readings were taken at 520nm (Baker and Frank, 1968).

Into 3 stoppered centrifuge tubes measured 0.3ml serum, 0.3ml standard and 0.3ml water (blank) respectively. To test and blank added 0.3ml ethanol and to the standard 0.3 ml water. Then added 0.3ml of xylene to all the tubes. Stoppered, mixed well and centrifuged. Transferred 0.2ml of the xylene layers into other stoppered tubes taking care not to include any ethanol or protein. Added 0.2ml α,α'-dipyridyl reagent to each tube, stoppered and mixed and read the extinction of test and standard against the blank at 460nm . Then, beginning with the blank 0.07ml ferric chloride solution was added, mixed and after exactly 1.5min test and standard were read against the blank at 520nm

2.4.27. Vitamin C

Ascorbic acid in plasma is oxidised by Cu^{2+} to form dehydroascorbic acid, which reacts with acidic 2,4-dinitrophenyl hydrazine to form a red bis-hydrazone, which is measured at 520nm. The heparinised blood is collected and the plasma is used immediately for analysis (Donald *et al*, 1999).

Into 0.1ml of heparinised plasma 0.4ml of freshly prepared metaphosphoric acid was added, centrifuged and 0.24ml of the clear supernatent was used for the analysis. 0.24ml of metaphosphoric acid was taken as the blank. Into both tubes 0.08ml of DTCS (dinitrophenyl hydrazine, thiourea and copper sulphate) reagent was added and the tubes were incubated in a water bath at 37° C for 3hrs. Then tubes were removed and chilled for 10min. Into the tubes then added 0.4ml of cold sulphuric acid (12mol/l), capped and mixed in a vortex mixer.The absorbance was noted at 520nm. A standard graph was plotted by taking different concentrations of ascorbic acid.

2.4.28.Zinc

Zinc was detected by the Nitro-PAPS method (Akita and Smiko, 1989) in which Nitro-PAPS reacts with zinc in alkaline solution to form a purple colored complex. The absorbence was taken at 575nm. Interference from copper and iron are virtually eliminated by pH and chelating additives.

The reaction mixture contained 100µl standard (zinc nitrate 200µg/dl) or sample and 2ml of the reagent which contained borate buffer (370mM, pH 8.2), salicylaldoxime (12.5mM), dimethylglyoxime (1.25mM), surfactants, preservatives and Nitro-PAPS buffer (0.4mM). Mixed well and incubated at 30°C for 5minutes and the absorbance was taken at 575nm.

2.4.29.Magnesium

The sodium salt of Xylidyl blue I forms a red complex in alkaline solution with magnesium. The absorbence at 520nm of the red Xylidyl blue I: magnesium complex is proportional to the concentration of magnesium in the sample (Yong, 1990).

20 µl of sample or standard (2.mg/dl) was mixed with 3ml of reagent (Xylidyl Blue I 0.14mmol/l), mixed gently by inversion and incubated for 5minutes at 30°C and the absorbance was taken at 520nm.

2.4.30.Iron

Ferrous iron gives a pink color with dipyridyl. A solution of dipyridyl in acetic acid is added to serum followed by a reducing agent. Proteins are removed by heating in boiling water and then centrifuging (Varley, 1988).

The reaction mixture contained equal volumes of serum, 0.1M sodium sulphite and dipyridyl reagent and were mixed in a glass stoppered tube and centrifuged, heated in boiling water bath for five minutes, cooled and 1ml of chloroform was added, stoppered and shaken vigorously and again centrifuged and the supernatent was taken for the iron assay. The absorbance was taken at 520nm.

2.4.31.Total Iron Binding Capacity (TIBC)

For TIBC estimation 100µl of serum was mixed with 200µl of ferric chloride. After standing for five minutes 200µg magnesium carbonate was added, shaken frequently for thirty minutes. The supernatent was used for the TIBC assay by using the same procedure as for iron. Solution containing 100µg of iron per ml was used as the standard (Varley, 1988).

2.4.32. %Transferrin saturation

Percentage saturation of transferrin was derived from the formula given by Gupta *et al* (Gupta *et al*, 2000) and is given as

$$\%\text{Transferrin saturation} = \frac{\text{(Serum iron)}}{\text{TIBC}} \times 100$$

2.4.33.Ferritin

The ferritin quantitative test is based on a solid phase enzyme linked immunosorbent assay (ELISA) (Virgil *et al*, 1999). The assay system utilized one rabbit anti-ferritin antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-ferritin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample was allowed to react simultaneously with the antibodies, resulting in the ferritin molecules being sandwiched between the solid phase and the enzyme linked antibodies. After a 45 minute incubation at room temperature, the wells were washed with water to remove unbound-labeled antibodies. A solution of TMB was added and incubated at room temperature for 20 minutes, resulting in the development of a blue color. The reaction was stopped with the addition of stop solution, and the color changed to yellow and was measured spectrophotometrically at 450nm. The concentration of ferritin is directly proportional to the color intensity of the test sample.

2.4.34. Matrix Metalloproteinase-2 (MMP 2)

The assay of MMP2 is based on the principle that the antibodies immobilized on a bead matrix, in combination with enzyme-labeled antibodies, directed against different antigenic sites on the same MMP2 molecule (Fujimoto *et al* 1993). Upon addition of an MMP2 containing specimen, the result is an MMP2 molecule being sandwiched between the solid phase and enzyme labeled antibodies. After removing unbound enzyme-labeled antibody, the bead containing the sandwich is incubated with enzyme substrate and O-phenylenediamine, resulting in the development of color. The activity of the peroxidase enzyme is proportional to the amount of antigen, MMP2, so that MMP2 concentration in specimens can be determined from a standard curve.

The assay mixture contained 50µl of standard or specimen with 300µl enzyme labeled antibody solution and one anti-MMP2 coated bead. The mixture was incubated at 17-27^{0}C for 1 hour. The reaction was then stopped by the addition of 3ml of washing solution and it was aspirated and this was repeated at least 3 times. Each washed bead was then transferred in to a clean fresh tube and 300µl of coloring solution was added and incubated at 17-27^{0}C for 1 hour. The reaction was then stopped by the addition of 1.5ml of stop solution. Using deionized water as blank, the absorbance for the standard curve solutions and specimens were taken at 492 nm (A_{492}).

2.4.35. Matrix Metalloproteinase-9 (MMP 9)

The assay of MMP9 is based on the principle that the antibodies immobilized on a bead matrix, in combination with enzyme-labeled antibodies, directed against different antigenic sites on the same MMP9 molecule (Fujimoto *et al* 1994). Upon addition of an MMP9 containing specimen, the MMP9 molecule is sandwiched between the antibody coated on the bead and enzyme-labeled antibodies. After removing unbound enzyme-labeled antibody, the bead containing the MMP9 complex is incubated with enzyme substrate and o-phenylenediamine, resulting in the development of a colour. The resultant colour of the enzyme reaction is proportional to the amount of MMP9 present in the specimen. The concentration of MMP9 in specimens can be determined from a standard curve.

The assay mixture contained 50µl of standard or specimen with 300µl enzyme labeled antibody solution and one anti-MMP9 coated bead. The mixture was incubated at 17-27^{0}C for 1 hour. The reaction was then stopped by the addition of 3ml of washing solution and it was aspirated and again 3ml of washing solution was dispensed and aspirated, and this was repeated at least 3 times. Each washed bead was then transferred in to a clean fresh tube

and 300µl of coloring solution was added and incubated at 17-27^{0}C for 1 hour. The reaction was then stopped by the addition of 1.5ml of stop solution. Using deionized water as blank, the absorbance for the standard curve solutions and specimens were taken at 492 nm (A_{492}).

2.5.Statistical Analysis

The statistical analysis of the values obtained was performed by using the 'z- test'and by the analysis of variance (ANOVA) (Puri, 1984). The 'z' value was determined by using the formula,

$Z = X_1^* - X_2^* / \sqrt{(S_1^2/n_1) + (S_2^2/n_2)}$: Provided $n_1 + n_2$ is more than 30. Here X_1^* is the mean of the values in control group, X_2^* is mean of the values in affected group, S_1^2 is the sum of variances in the first group (control), and S_2^2 is the sum of the variances in the second group (affected group). And these are given by

$S_1^2 = 1/n_1\text{-}1\ (X_1 - X_1^*)^2$ and $S_2^2 = 1/n_2\text{-}1\ (X_2 - X_2^*)^2$

Where X_1 and X_2 are the individual values of control and affected groups respectively. The results obtained by 'z- test' were expressed in terms of probability (p). 'z' value $\geq$ 2.88 ($p<0.01$) and $\geq$1.96 ($p<0.05$) were considered to be statistically significant.

The inter group comparison have been done by using the one-way analysis of variance (ANOVA). The results of ANOVA were expressed in terms of alphabets. The same alphabets denote the homogeneity between the values and different alphabets denote the heterogeneity between the values. Always the highly significant value is expressed by the alphabet 'a' followed by b, c and d. And 'd' denotes the least significant value among the four.

PROFORMA FOR ACUTE MYOCARDIAL INFARCTION

Name : Age : Sex :

Address : Date of Admission :

Occupation :

Socio-economic status :

Financial Status : Educational Status :

Religion :

PRESENTING SYMPTOMS

1. CHEST PAIN

I) Site: a) Restrosternal b) Left sided c) Right sided
d) Any other site:

II) Duration : a) < 1 hr. b) 1-2 hr c) 2-6 hr
d) 6-12 hr e) 12-24 hrs f) > 24 hrs.

III) Time of onset of present pain a) 12 Mid night - 6 am b) 6 am-12 noon c) 12 noon - 6 pm
d) 6 pm - 12 mid night
Time of Max. intensity a) 12 Mid night - 6 am b) 6 am - 12 noon c) 12 noon - 6 pm
d) 6 pm - 12 mid night

IV) Radiation : a) Throat b) Lt arm c) Rt arm
d) both arms e) Abd. f) Other sites

V) Autonomic symptoms a) Sweating b) Vomiting
c) Bladder/Bowel Symptoms

VI) a) Similar Pain/Angina a) Within 24 hrs b) Within 48 hrs
c) 2 days - 1 week d) 1 - 4 weeks
b) ACP a) Within 24 hrs b) Within 48 hrs
c) 2 days - 1 week d) 1 - 4 weeks

VII) Precipitating factor :

a) Emotional Upsets (anger, upsetting life events)	<1 hr	1-2 hrs	2-6 hrs	Within 24 hrs	Within 48 hrs
b) Unaccustomed Exercise	<1 hr	1-2 hrs	2-6 hrs	Within 24 hrs	Within 48 hrs

VIII) Relation to heavy in take of food/alcohol/smoking: a) < 1 hr. b) 1-2 hrs c)1-6 hrs

IX) Relaton to weather Hot/Humid cold Rainy

X) Others

2. OTHER CARDIAC SYMPTOMS

I.	Breathlessness	II.	PND	III.	Syncope	IV.	Palpitation
V.	CNS Symptoms	VI.	Dyspeptic Symptoms	VII.	Fatigue		
VIII.	Excessive sweating	IX.	Nausea & Vomiting	X.	Silent.		

PAST HISTORY

1. ANGINA

I). Stable H/o TMT +ve/-ve

II) Unstable

Duration: a) < 1month b) 1-6 months c) 6-1 year d) 1-3 years e) > 3 years

Treatment : Regular/Irregular

2. PAST HISTORY OF MI QMI/NON Q SITE

Duration: a). <1 month b) 1-6 months c) 6-1 year d) 1-3 years e) > 3 years

Treatment : Regular/Irregular.

3. DIABETES MELLITUS

I) Duration : Control: Good/Poor On OHA

II) Duration : Control: Good/Poor On Insulin

4. HYPERTENSION

i) Duration: Controlled/Uncontrolled

ii) Primary/Secondary

5. HYPERLIPIDEMIA Recent Reading (3 months) < 1 Year 1 - 5 years

I) S. Cholesterol

II) HDL

III) LDL

IV) TG

V) S. Chol./HDL

VI) LP (a)

6. ASTHMA/CHRONIC BRONCHITIS

7. OTHER VASCULAR PROBLEMS

I) CVA II) TIA III) PVD

8. RENAL DISEASE

9. LIVER DISEASE

10. HEAMATOLOGICAL PROBLEMS

11. RECENT INFECTIONS: Throat Dental Respiratory

12. PAST HISTORY OF SURGICAL PROCEDURE

CARDIAC a) PTCA + Stenting Vessel Duration:

b) CABG LIMA+SVG Duration:

NON-CARDIAC: Blood Loss: Yes/No

PERSONAL HISTORY

1. SMOKING EX.SMOKER

I) Duration: When:

II) Number:

III) Cigaratte/Beedi:

2. ALCOHOL INTAKE

3. PAN CHEWING

4. MENSTURAL HISTORY

I) Premenopausal II) Post-menopausal On Hormones/ Off Hormones III) Hysterectomy/ Oopherectomy

5. PHYSICAL ACTIVITY

I) Sedentary

II) Active Level of Exercise:

6. PERSONALITY Tense Calm

II) RHYTHM

a) Sinus Tachycardia b) Sinus Bradycardia c) CHB d) Atrial fibrillation
e) SV ectopics f) Ventricular ectopics g) VT h) VF

III) OLD MI

IV) CONDUCTION DEFECT LBBB RBBB
1° AV Block LAHB LPHB

COURSE IN HOSPITAL

Fully evolved MI Time for ST to become isoelectric
Fresh arrhythmia/Heart block Reinfarction

CHEST X-RAY

I) Cardia: Normal sized / Cardiamegally
II) Lung
III) Aortic Knuckle Calcification

BIOCHEMICAL REPORT

Hb	RBS (F) & (PP)	Blood Urea	CRP	
Tc	S.Chol	S. Creatinine	CPK-MB	Trop T
Dc	HDL	Blood Group	SGOT	
ESR	TG-(F)&(R)	S. Uric acid	SGPT	
	LDL			
Repeat:	S.Chol	RBS	After 6 weeks	
	Lipoprotein			
	HIV	HBS Ag.	S.Electrolytes	

ECHOCARDIOGRAM

RWMA: Yes/No LVH: Yes/No
LVEF: On day 1: day 7: 42 days
LV apical clot: Yes/No

PRE EFFUSION

MR	1	2	3	4
TR	1	2	3	4
VSD				

OTHER CO-EXISTING DISEASE

CART REPORT

LMCA:				
LAD:	Type	1	2	3
DI				
LCX:	Dominant/Non dominant/Co-dominant			
OM_1				
OM_2				
RCA:				
PDA:				
PLV:				
RWMA:				
LV Angio:	EF			

FOLLOW UP:

I) ECG:
11) Echo: at 6 weeks
RWMA
EF
III) S. Cholesterol HDL RBS FTG

TREAD MILL REPORT: On drugs/Off drugs

Bruce/modified bruce
Ex. Duration:
Cause for termination
HR achieved
-ve/+ve
+vity (1mm ST depression at HR /mt): St elevation
Recovery changes: Yes/No Duration: min.
Arrhythmia
BP response

TREATMENT DETAILS

THROMBOLYTIC THERAPY: Yes/No
If No, Contradiction
Window

Thrombolytic therapy		Agent used		SK/UK	IF UK - dose:			
Arrhythmias observed	1 hr:	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	12 hrs	24hrs
BP behaviour								
Chest pain relief								
ST changes								
Allergy								
Bleeding tendencies								
LVF								

ADJUVANT THERAPY S/C/ IV (Infusion / intermittent) Oral

I. HEPARIN
II. LMWH
III. NITRATES
IV. BETA BLOCKERS
V. ASPIRIN
VI. ACE INHIBITORS
VII. CA+ANTAGONIST
VIII. DIURETICS
IX. DIGOXIN
X. ANTIARRHYTHMIC AGENTS

COURSE IN HOSPITAL

Discharged On day

Complication:
Arrhythmia
Reinfarction
Angina
LV dysfunction
Cardiogenic shock
VSD
MR
Pericardial effusion
CVA

Died On day

Cause of death

EVALUATION OF THE ROLE OF MATRIX METALLOPROTEINASES AND OXIDATIVE STRESS IN THE PATHOGENESIS AND COURSE OF ACUTE MYOCARDIAL INFARCTION

THESIS SUBMITTED TO THE UNIVERSITY OF CALICUT FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY IN BIOCHEMISTRY
(FACULTY OF MEDICINE)

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CHAPTER 3

ROUTINE HAEMATOLOGICAL AND BIOCHEMICAL INVESTIGATIONS IN ACUTE MYOCARDIAL INFARCTION

3.0.Introduction

The inflammatory response is fundamental to the ability of the higher organisms to protect themselves against exposure to infective agents and to respond to injury. Despite the salutary and essential role of inflammation in host defense, it is a double-edged sword because of its potential to cause tissue damage. Inflammation occurs in vascularized tissue and involves a complex set of interactions among blood cells, plasma mediator systems, and the microvasculature (Robbins *et al*, 1984). The lesions of atherosclerosis occur principally in large and medium-sized elastic and muscular arteries and can lead to ischemia of the heart, brain or extremities, resulting in infarction. They may be present throughout a person's lifetime. In fact, the lesions of atherosclerosis represent a series of highly specific cellular and molecular responses that can best be described, in aggregate, as an inflammatory disease (Ross, 1986).

Hemodynamic changes and increase in vascular permeability are hallmarks of the acute inflammatory response and largely account for the classic local clinical signs (swelling, heat, redness and pain) of acute inflammation. Inflammation, as evident by a high white blood cell count, a high platelet count and a high erythrocyte sedimentation rate (ESR), has been thought to reflect the body's response to tissue injury in patients with acute myocardial infarction. In the early 1980s Kotis et al (Kotis *et al*, 1984) and Lowe et al (Lowe *et al*, 1985) suggested that total leucocytes count during acute myocardial infarction independently predicts the frequency of early ventricular fibrillation. Central to the pathogenesis of atherosclerosis or AMI is the deposition of cholesterol in the arterial walls. Nearly all lipoproteins are involved in this process.

Epidemiological observations have shown that there is a strong positive relationship between the concentration of circulating cholesterol, especially LDL cholesterol fraction and the risk of AMI. The relationship is non-linear and depends strongly on the presence of other risk factors including sex, arterial hypertension, cigarette smoking, diabetes mellitus and positive familial or personal history of ischaemic heart disease, electrocardiographic and echocardiographic observations. Lipids, in particular oxidized low-density lipoprotein (LDL), interfere with most of the normal endothelial functions in a dose-dependent and potentially reversible way. Studies in both animals and humans have shown that oxidized LDL results in a reduction of effective quantities of endothelium-derived nitric oxide, owing

both to impaired production and to an increase in its degradation (Creager *et al*, 1990). Atherogenic lipids such as oxidized LDL effect a wide range of adverse effects on endothelial function as well as proatherogenic effects on other cells such as monocytes and platelets (Dinerman and Mehta, 1990). Oxidized LDL has important effects on cell division and growth within the vessel wall. There is evidence that oxidized LDL triggers apoptosis of endothelial cells via reactive oxygen species (Dimmeler *et al*, 1997). Oxidized LDL disrupts normal regulatory functions of the endothelium that are important in both stimulating and inhibiting vascular growth, via the secretion of the NO and a variety of cytokines (Ross, 1993). This can lead to intimal thickening via the up-regulation of growth promoting genes, which involves the proliferation of smooth muscle cells and monocytes (Simari *et al*, 1996).

Reduced endothelium-dependent dilatation has been described in a number of conditions associated with hypertriglyceridemia, such as the obesity/insulin resistance syndrome (Steinberg, 1996), and in non-insulin-dependent diabetes mellitus (Clarkson *et al*, 1996). This reduction may be related to the abnormal and atherogenic lipids that frequently coexist. Raised triglycerides levels are frequently accompanied by small, dense LDL particles that are more oxidized, and by lower HDL. For example, Chowienczyk et al examined peripheral artery small vessel function in subjects with isolated hypertriglyceridemia associated with lipoprotein lipase dysfunction, and they found no relationship between triglyceride levels and the degree of endothelial dysfunction (Chowienczyk *et al*, 1997). Recent evidence, however, suggests that triglycerides rich particles may directly damage the endothelium by stimulating the expression of cell adhesion molecules and plasminogen activator inhibitor. Reduced endothelium-dependent dilatation has also been demonstrated in human subjects with postprandial hypertriglyceridemia (Sattar *et al*, 1998).

In addition to the beneficial effects that HDL cholesterol has on reverse cholesterol transport and thrombosis, increased HDL levels may attenuate the negative effects of hypercholesterolemia on endothelium-dependent dilatation. *In vitro* studies of rabbit aorta preparations have yielded inconclusive results; HDL has not consistently helped to retain endothelium-dependent response to acetyl choline (Takahashi *et al*, 1990). HDL can down-regulate endothelial expression of a variety of cell adhesion molecules such as ICAM-1, VCAM-1 and E-selectin (Cockerill *et al*, 1995), as well as reversing some of the changes

induced by LDL on platelets (Cockerill *et al*, 1995). Studies of patients have shown that the plasma level of HDL ameliorates the adverse effects of plasma LDL on endothelial function (Zeiher, 1996).

In the present chapter the hematological parameters such as hemoglobin, total WBC count and erythrocyte sedimentation rate (ESR) in the blood of both normal as well as acute myocardial infarction patients were studied. Routine biochemical investigations such as serum urea, creatinine, blood glucose, electrolytes (sodium and potassium), creatine kinase and creatine kinase isoenzyme (CKMB) were also done in these patients as well as in the controls. The lipid profile - cholesterol, triglycerides, HDL cholesterol and LDL cholesterol were also estimated in the blood of these patients.

3.1.Materials and Methods

The hemoglobin was estimated in the blood by using cyanmethemoglobin method. The total WBC count was detected by using a Von Behrens WBC transducer in an automated haematology analyzer (Hitachi), ESR was done by using the Westergren's method and Na+ and K+ were done by flame photometry. The urea was done by the urease method, creatinine was done by using the Jaffe's reaction method and random blood glucose was done by the GOD-PAP method. Creatine kinase was done by the optimized standard method as described by Gruber and CKMB was estimated by using the kinetic immunoinhibition method. Cholesterol was done by the CHOD-PAP method; HDL-cholesterol was done by the precipitation using phosphotungstic acid and magnesium chloride. Triglycerides were done by the GPO-PAP method and LDL-cholesterol was estimated by using the Friedewald's formula. The detailed procedures adopted for the analysis are given in Chapter 2.

3.2.Results

Table 1 represents the routine haematological and biochemical parameters in normal and in AMI patients. The values of total WBC count, ESR, urea, creatinine, random blood glucose, CK and CK-MB were found to be significantly increased ($p<0.01$) in all the AMI patients when compared to that of the normal controls. The value of sodium was found to be slightly increasedd in AMI patients ($p<0.05$). However, values of hemoglobin and potassium were not statistically significant in AMI patients when compared to that of the normal controls.

Table 1(a) represents the values of routine haematological and biochemical parameters in normal and in AMI patients according to the age and sex. The AMI patients with age groups ≤40 years showed elevated values of total WBC count, ESR, potassium, random blood glucose, CK and CK-MB which were found to be statistically significant ($p<0.01$). The value of creatinine was also found to be slightly elevated in this group ($p<0.05$). However, the values of hemoglobin, sodium and urea were not statistically differing in this age group of AMI patients when compared to their normal counterparts. A comparison of the routine biochemical parameters in AMI patients, age group 40-60 years with their normal controls showed statistically significant elevated ($p<0.01$) levels of total WBC count, ESR, urea, creatinine, random blood glucose CK and CK-MB. The values of hemoglobin, sodium and potassium were found to be not statistically significant in this age group of AMI patients when compared to their normal controls. Significantly increased values ($p<0.01$) of total WBC count, ESR, CK and CK-MB were also observed in AMI patients with age ≥60 years when compared to their normal controls. The value of creatinine was also found to be slightly elevated ($p<0.05$) in this group. But, the values of hemoglobin, sodium, potassium, urea and blood sugar were found to be not statistically significant in this age group of AMI patients when compared to those of their normal controls.

A comparison of the routine haematological and biochemical parameters among these three age groups by ANOVA has shown a significant total WBC count in ≥60 years age group of AMI patients when compared to the other two age groups. However, the values of ESR, potassium and creatinine were found to be significantly increased in ≤40 years age group of patients, when compared to the other two groups. A significantly increased value of the random blood glucose was observed in 40-60 years age group of AMI patients when compared to the other two groups. However, all the other parameters did not show any significant difference among these three groups.

The values of total WBC count, ESR, urea, creatinine, random blood glucose, CK and CK-MB were found to be significantly elevated ($p<0.01$) in all the AMI males when compared to the normal males. The value of sodium was also found to be slightly elevated ($p<0.05$) in the AMI group. However, the values of hemoglobin and potassium did not show any statistical significance. In the case of females with AMI the values of hemoglobin, total WBC count, ESR, CK and CK-MB were found to be significantly increased ($p<0.01$) when

compared to those of the normal females. The value of potassium was found to be significantly decreased ($p<0.05$) in AMI females when compared to those of the normal females. The values of sodium, urea, creatinine and random blood glucose did not show any statistical significance in this group. The comparisons of AMI males with AMI females have shown the significantly increased values of hemoglobin, potassium, random blood glucose and CK-MB in AMI males when compared to AMI females. However, the other parameters did not show any statistically significant difference.

Table 1 (b) represents the values of routine haematological and biochemical parameters in normal and in AMI patients according to the time of onset of chest pain. The values of total WBC count, ESR, creatinine, random blood glucose, CK and CK-MB were found to be significantly elevated ($p<0.01$) in AMI patients when the onset of chest pain is from 12 midnight to 6 am as compared to that of the normal controls. The value of potassium was also found to be significantly elevated ($p<0.05$) in this group of patients, whereas the values of hemoglobin, sodium and urea were not found to be statistically significant in this group when compared to that of the normal controls. The AMI patients with the onset of chest pain from 6 am to 12 noon showed statistically significant elevation ($p<0.01$) in the values of total WBC count, ESR, urea, creatinine, random blood glucose, CK and CK-MB. The value of sodium was also found to be significantly elevated ($p<0.05$) in this group when compared to the normal controls. However, the value of hemoglobin was found to be significantly decreased ($p<0.01$) in this group of AMI patients when compared to that of the normal controls. But, the value of potassium did not show any significant change in this group of patients. The values of total WBC count, ESR, creatinine, random blood glucose, CK and CK-MB were found to be significantly elevated ($p<0.01$) in AMI patients with onset of chest pain from 12noon to 6pm when compared to that of the normal controls. The value of urea was also found to be elevated ($p<0.05$) in this group. However, the values of hemoglobin, sodium and potassium did not show any statistical significance in this group. The AMI patients with onset of chest pain from 6pm to 12midnight showed significantly elevated values of total WBC count, ESR, urea, random blood glucose, CK and CK-MB. The values of hemoglobin and sodium were also found to be significantly elevated ($p<0.01$) in this group of AMI patients when compared to that of the normal controls. The values of potassium and creatinine did not show any statistically significant change in this group of

AMI patients. A comparison between the routine biochemical investigations in AMI patients with different time of onset of chest pain showed significantly increased values of hemoglobin, creatinine, random blood glucose and CK-MB in patients with onset of chest pain from 12 mid night to 6 am when compared to the other three groups. A significantly elevated value of potassium has been observed in AMI patients with onset of chest pain from 6 am to 12 noon when compared to the other three groups. However, other parameters did not show significant change.

Table 1 (c) represents the values of routine biochemical parameters in normal and in AMI patients with the history of diabetes and hypertension. Here the values of total WBC count, ESR, urea, creatinine, random blood glucose, CK and CK-MB were found to be significantly elevated ($p<0.01$) in AMI patients both with and without the history of diabetes when compared to that of the normal controls. The value of sodium was found to be significantly increased ($p<0.05$) in AMI patients without the history of diabetes but it did not show any statistical significance in AMI patients with the history of diabetes. However, the values of both hemoglobin and potassium did not show any statistically significant difference in AMI patients both with and without the history of diabetes. A comparison between the diabetic and non-diabetic AMI patients have shown significantly increased values of creatinine and random blood glucose in AMI patients with diabetes than in AMI patients without diabetes. The values of total WBC count, ESR, creatinine, random blood glucose, CK and CK-MB were found to be significantly increased ($p<0.01$) in AMI patients both with and without the history of hypertension when compared to that of the normal controls. The value of potassium was found to significantly elevated ($p<0.05$) in AMI patients with the history of hypertension when compared to that of the normal controls. The values of hemoglobin and sodium did not show any statistically significant difference in this group of AMI patients. The value of hemoglobin was found to be significantly decreased ($p<0.05$) and the value of sodium was significantly increased ($p<0.05$) in AMI patients without the history of hypertension when compared to that of the normal controls. However, the value of potassium did not show any statistical significance in this group. A comparison of these parameters between AMI patients both with and without the history of hypertension by ANOVA has shown significantly increased values of creatinine and random blood glucose in

AMI patients with the history of hypertension than in patients without the history of hypertension.

Table 1 (d) represents the values of routine biochemical parameters in normal and in AMI patients according to the values of cholesterol. The values of total WBC count, ESR, creatinine, random blood glucose, CK and CK-MB were found to be significantly elevated ($p<0.01$) in AMI patients with the value of cholesterol both in >200 mg/dl group and <200 mg/dl group when compared to those of the normal controls. The value of potassium did not show any statistical significance in both these groups when compared to that of the normal. The values of urea ($p<0.01$) and sodium ($p<0.05$) were found to be significantly increased in AMI patients with the cholesterol value <200 mg/dl when compared to the normal. The hemoglobin showed a significantly decreased ($p<0.01$) value in AMI patients with cholesterol value <200 mg/dl when compared to that of the normal controls. A comparison of biochemical parameters in the AMI patients with cholesterol value <200 mg/dl and >200 mg/dl showed significantly increased values of creatinine, random blood glucose and CK-MB in AMI patients with cholesterol value >200 mg/dl than in patients with cholesterol value <200 mg/dl.

Table 1 (e) represents the values of routine biochemical parameters in normal and in AMI patients with the habit of smoking and alcohol intake. The total WBC count, ESR, CK and CK-MB were found to be significantly elevated ($p<0.01$) in AMI patients both with and without the habit of smoking. The values of urea, creatinine, and random blood glucose were found to be significantly increased ($p<0.01$) in AMI patients with the habit of smoking when compared to that of the normal controls. The value of sodium was also found to be significantly elevated ($p<0.05$) in this group. However, the values of hemoglobin and potassium did not show any statistical significance in this group when compared to those of the normal healthy individuals. AMI patients without the habit of smoking showed slightly increased values of urea and random blood glucose ($p<0.01$). The values of hemoglobin, sodium, potassium and creatinine did not show statistically significant values in this group when compared to that of the normal controls. A comparison between the AMI patients both with and without the habit of smoking have showed significantly increased values of creatinine, random blood glucose and CK-MB in AMI patients with the habit of smoking than without the habit of smoking. The values of total WBC count, ESR, random blood

glucose, CK and CK-MB were found to be significantly elevated ($p<0.01$) in AMI patients both with and without the habit of alcohol intake. The values of sodium and creatinine were found to be significantly elevated ($p<0.05$) in AMI patients with the habit of alcohol intake when compared to that of the normal controls. But the values of hemoglobin, potassium and urea did not show any statistical significance in this group of patients when compared to those of the normal controls. The values of urea and creatinine were found to be significantly increased ($p<0.01$) in AMI patients without the habit of alcohol intake when compared to those of the normal controls. However, the value of hemoglobin showed a statistically significant reduction ($p<0.05$) in this group when compared to that of the normal controls. The values of sodium and potassium did not show any statistical significance in this group of patients. A comparison between AMI patients both with and without the habit of alcohol intake have shown significantly increased values of creatinine and random blood glucose in AMI patients with the habit of alcohol intake than in the patients without the habit of alcohol intake.

Table 1 (f) shows the values of routine biochemical parameters in normal and in AMI patients according to the food habits and family history of AMI. The values of total WBC count, ESR, CK and CK-MB were found to be significantly elevated ($p<0.01$) in AMI patients: both vegetarians and nonvegetarians. The values of hemoglobin, sodium, potassium, urea, creatinine and random blood glucose did not show statistically significant variation in vegetarian AMI patients when compared to that of the normal controls. The values of urea, creatinine and random blood glucose were found to be significantly elevated ($p<0.01$) in nonvegetarian AMI patients when compared to that of the normal controls. The value of sodium showed significant increase ($p<0.05$) and hemoglobin showed a slight decrease in this group of patients when compared to that of the normal controls. However, the value of potassium did not show any significant change in this group. The values of total WBC count, random blood glucose and CK-MB were found to be significantly increased in non-vegetarian AMI patients than vegetarian patients. The AMI patients both with and without the family history of AMI showed significantly elevated ($p<0.01$) values of total WBC count, ESR, creatinine, random blood glucose, CK and CK-MB when compared to that of the normal controls. However, the value of hemoglobin is found to be significantly reduced ($p<0.01$) in both these groups when compared to that of the normal. The value of urea has

shown a slight increase ($p<0.05$) in AMI patients with the family history of AMI when compared to that of the normal. The value of urea ($p<0.01$) and sodium ($p<0.05$) were found to be significantly increased in AMI patients without the family history of AMI when compared to that of the normal controls. However, the value of potassium did not show any statistical significance in both these groups when compared to that of the normal controls. A comparison between these two groups have shown significantly increased values of creatinine, random blood glucose and CK-MB in AMI patients with the family history of AMI than in patients without the family history of AMI.

Table 2 shows the values of lipid profile parameters in normal and in AMI patients. The values of cholesterol, triglycerides, and LDL were found to be significantly increased in AMI patients when compared to that of the normal controls. The value of HDL was found to be significantly decreased ($p<0.01$) in AMI patients when compared to that of the healthy individuals.

Table 2 (a) represents the values of lipid profiles in normal and in AMI patients according to the age and sex. The value of cholesterol was found to be significantly increased in all the three age groups when compared to that of the normal controls. The value of triglycerides was significantly increased in 40-60 age ($p<0.01$) groups and in ≤ 40 years age groups ($p<0.05$) when compared to their respective normal controls. And it did not show any significant change in ≥ 60 years age group of patients. The value HDL was found to be significantly decreased in ≤ 40 years ($p<0.05$) and 40-60 years ($p<0.01$) age groups of patients when compared to that of the normal controls. The values of LDL was found to be significantly elevated in 40-60 years ($p<0.01$), ≤ 40 years ($p<0.05$) and ≥ 60 years ($p<0.05$) age groups of patients when compared to that of the normal controls. A comparison of the lipid profiles among these three age groups have shown significantly increased value of cholesterol in ≤ 40 years age group of patients when compared to that of the other two groups. The values of cholesterol was found to be significantly elevated ($p<0.01$) in both males and in females with AMI when compared to their respective controls. And the value was found to be more significant in males than in females. The other parameters such as triglycerides and LDL were also found to be significantly elevated ($p<0.01$) in males with AMI when compared to that of the normal controls. The HDL showed a significantly decreased value

($p<0.01$) in AMI males when compared to that of the normal. In females with AMI these parameters did not show any significant change.

Table 2 (b) represents the values of lipid profile parameters in normal and in AMI patients according to the time of onset of chest pain. The values of cholesterol, triglycerides and LDL were found to be significantly increased in all the four groups when compared to that of the normal controls. However, HDL showed a significant reduction ($p<0.01$) in all the four groups. The comparison of the lipid profiles among the four groups have shown the significantly increased value of cholesterol in patients with time of onset of chest pain from 12 midnight to 6am when compared to that of the other three groups.

Table 2 (c) represents the values of lipid profiles in normal and in AMI patients according to the history of diabetes and hypertension. The values of cholesterol, triglycerides and LDL were found to be significantly increased ($p<0.01$) in AMI patients with and without the history of diabetes when compared to that of the normal controls. The value of HDL was found to be significantly decreased in both diabetic and non-diabetic AMI patients when compared to that of the normal controls. A comparison between the diabetic and non-diabetic AMI patients have shown significantly increased value of cholesterol in AMI patients with diabetes than in patients without diabetes. The AMI patients with and without hypertension have shown elevated values of cholesterol, triglycerides and LDL when compared to that of the normal controls. The value of HDL was found to be significantly decreased ($p<0.01$) in both these groups. A comparison between the AMI patients with and without the history of hypertension has shown significantly increased value of cholesterol in AMI patients with the history of hypertension than in patients without hypertension.

Table 2 (d) represents the values of lipid profiles in normal and in AMI patients according to the values of cholesterol. The values of triglycerides and LDL were found to be significantly increased ($p<0.01$) in AMI patients with cholesterol value of both >200 mg/dl and <200 mg/dl. The value of HDL was found to be significantly decreased ($p<0.01$) in AMI patients with cholesterol value of both >200 mg/dl and <200 mg/dl groups when compared to that of the normal controls.

Table 2 (e) shows the values of lipid profiles in normal and in AMI patients according to the habits of smoking and alcohol intake. The values of cholesterol, triglycerides and LDL were found to be significantly increased in AMI patients with and without the habit of

smoking when compared to that of their normal controls. The value of HDL was found to be significantly decreased ($p<0.01$) in both the groups when compared to that of the normal controls. A comparison between AMI patients with and without the habit of smoking has shown significantly increased value of cholesterol in AMI patients with the habit of smoking than in patients without the habit of smoking. The AMI patients both with and without the habit of alcohol intake have shown significantly elevated ($p<0.01$) values of cholesterol and triglycerides. However, the value of HDL was found to be significantly decreased in both the groups when compared to that of the normal. The value of LDL was significantly elevated ($p<0.01$) in AMI patients without the habit of alcohol intake when compared to that of the normal. A comparison between AMI patients with and without the habit of alcohol intake have shown a significantly increased value of cholesterol in AMI patients with the habit of alcohol intake than in patients without the habit of alcohol intake.

Table 2 (f) represents the values of lipid profiles in normal and in AMI patients according to the food habits and family history of AMI. The values of cholesterol, triglycerides and LDL were found to be significantly elevated in AMI patients with the habit of both vegetarian and non-vegetarian food intake. The value of HDL was found to be significantly decreased ($p<0.01$) in AMI patients with non-vegetarian food intake. A comparison between the values of lipid profiles between the AMI patients with vegetarian and non-vegetarian food intake have shown significantly increased value of cholesterol in AMI patients with non-vegetarian food intake than in patients with vegetarian food intake. The values of cholesterol, triglycerides and LDL were found to be significantly elevated ($p<0.01$) in AMI patients with and without the family history of AMI. The value of HDL was found to be significantly decreased ($p<0.01$) in both the groups when compared to that of the normal controls. A comparison between AMI patients with and without the family history of AMI have shown significantly increased value of cholesterol in AMI patients without the family history of AMI than in patients with the family history of AMI.

Table 3 represents the values of hemoglobin, total WBC count, ESR, sodium, potassium, urea, creatinine, random blood glucose, CK and CK-MB in normal, in patients with AMI and in patients belong to different risk groups with AMI. The values of hemoglobin and potassium did not show any statistical significance between the different risk groups studied. However, the value of total WBC count and CK-MB were found to be

significantly increased in AMI patients with a positive family history of AMI. The values of ESR and creatinine were found to be significantly increased in AMI patients with age ≤40 years and the value of sodium was found to be significantly increased in AMI patients with time of onset of chest pain from 6 am to 12 noon. The values of urea and CK were found to be significantly increased in AMI patients with the time of onset of chest pain from 6 pm to 12 midnight. The value of random blood glucose was found to be significantly increased in AMI patients with time of onset of chest pain from 12 midnight to 6am.

Table 4 represents the values of lipid profiles in normal, in AMI patients and in patients belong to different risk groups with AMI. The value of cholesterol was found to be significantly increased in AMI patients with cholesterol value > 200 mg/dl and the value of triglycerides were found to be significantly increased in AMI patients with the habit of vegetarian food intake. The values of HDL was found to be significantly decreased in AMI patients with time of onset of chest pain from 6 pm to 12 midnight and the value of LDL was found to be significantly increased in AMI patients with age ≤40 years.

3.3.Discussion

Eventhough elevation of certain lipid parameters can be considered as a risk factor of coronary artery disease, plaque rupture and thrombosis are notable complications of advanced lesions that lead to unstable coronary syndromes or myocardial infarction (Ross, 1993). Total white blood cell count is performed in a variety of inflammatory conditions. Elevation of the leukocyte count can be a marker for the prediction of CAD (Lowe *et al*, 1985). Formed partially in the bone marrow and partially in the lymph glands, their site of action is the area of infection and inflammation. Hence, they can be considered as an important inflammatory marker. Leukocytes are of different types, each having a discrete function. The ubiquitous monocytes, the precursor of macrophages in all tissues, are present in every phase of atherogenesis. Monocytes derived macrophages are scavenging and antigen presenting cells, and they secrete cytokines, chemokines, growth regulating molecules and other hydrolytic enzymes. The ability of macrophages to produce cytokines, proteolytic enzymes and growth factors may be critical in the role of these cells in the damage and repair that ensue as the lesion progresses. Monocytes and granulocytes protect the body against invading organisms by phagocytosis. Lymphocytes and plasma cells neutralize the antigens by producing antibodies.

ESR is increased in any condition associated with an increase in plasma fibrinogen and globulins, which include chronic infection like tuberculosis and kala azar. Inflammatory disease of the tissues like heart attack, where there has been tissue break down, the elevation of ESR may be a simple way of judjing the disease. Other biochemical parameters such as urea, creatinine and random blood glucose, were also important in assessing the disease state. The deposition of cholesterol in the arterial intima is considered to be the primary event in the process of atherogenesis. Oxidized LDL act as the mediator for the formation of foam cells. Deposition of cholesterol in the arterial wall is enhanced by the chemokines and macrophages as part of the inflammatory reaction. The LDL particles composed of fatty molecules (lipids) and proteins, transport cholesterol from their source in the liver and intestines to other organs. The excessive amounts of LDL and cholesterol promote CAD. Oxidized LDL can attack the endothelial cells of the major arteries and can initiate the formation of foam cells, which ultimately lead to the blockage of the blood vessels (Peter Libby, 2002).

The results of the present study have shown elevated values of total WBC count, ESR, Na^+, urea, creatinine, random blood glucose, creatine kinase (CK) and creatine kinase isoenzyme (CKMB) in the serum of patients with AMI when compared to that of the normal controls. The value of potassium did not change significantly in AMI patients when compared to that of the controls. The total WBC count, ESR, creatinine, CK and CKMB were found to be elevated in AMI patients with different age groups as compared to that of the normal controls. Among the different age groups the value of total WBC count was found to be more in AMI patients with age $\geq$60 years when compared to the other two age groups. The values of ESR, K^+ and creatinine, were found to be more in AMI patients with age $\leq$40 when compared to that of the other two age groups. The value of random blood glucose was found to be more in AMI patients with age ranges from 40-60 years. Blood hemoglobin was found to be decreased in both AMI males and females when compared to their normal controls and the value was found to be less in AMI females when compared to AMI males. The values of K^+, random blood glucosse, and CKMB were found to be high in AMI males when compared to that of the AMI females. Based on the time of onset of chest pain the values of hemoglobin, creatinine, random blood glucose, and CKMB were found to be more in AMI patients with time of onset of chest pain from 12midnight to 6am, when compared to

that of the other groups. The value of K^+ was found to be more in AMI patients with time of onset of chest pain from 6 am to 12 noon, when compared to that of the other groups. The values of creatinine and random blood glucose were found to be more in AMI patients with the history of diabetes and hypertension when compared to that of the AMI patients without the history of diabetes and hypertension. The values of creatinine, random blood glucose and CKMB were found to be more in AMI patients with the cholesterol value >200 mg/dl when compared to that of the AMI patients with cholesterol value <200 mg/dl. The values of creatinine and random blood glucose were found to be more in AMI patients with the habits of smoking and alcohol intake when compared to that of the AMI patients without the habits of smoking and alcohol intake. The value of CKMB was found to be more in AMI patients with the habit of smoking when compared to that of the AMI patients without the habit of smoking. The value of hemoglobin was found to be more in AMI patients with the habit of vegetarian food intake when compared to that of the AMI patients of nonvegetarian food intake. The value of total WBC count, random blood glucose and CKMB were found to be more in nonvegetarian AMI patients when compared to that of the vegetarian AMI patients. The values of creatinine, random blood glucose and CKMB were found to be more in AMI patients with the family history of AMI when compared to that of the AMI patients without the family history of AMI.

The values of the lipid profiles such as cholesterol, triglycerides (TG) and LDL were found to be significantly increased and the value of HDL was found to be significantly decreased in AMI patients when compared to that of the normal healthy individuals irrespective of the risk factors taken into account. The value of cholesterol was found to be more in AMI patients with age ≤40 years when compared to that of other two groups. The cholesterol value was found to be more in AMI males when compared to that of the AMI females. Further, the cholesterol value was found to be more in AMI patients with time of onset of chest pain from 12 midnight to 6 am when compared to other groups, and in patients with the history of diabetes and hypertension when compared to patients without the history of diabetes and hypertension. The value of cholesterol was found to be more in AMI patients with the habits of smoking, alcohol intake and nonvegetarian food intake when compared to that of the AMI patients without the habits of smoking, alcohol intake and with the habit of vegetarian food intake. Further the value of cholesterol was found to be more in AMI patients

without the family history of AMI when compared to that of the AMI patients with the family history of AMI. However, other parameters did not show significant alteration among different groups.

Of the various haematological and biochemical parameters studied in AMI patients, the value of total WBC count and CK-MB were found to be significantly elevated in patients with a positive family history of AMI. Similarly, the values of ESR and creatinine were found to be increased in AMI patients with age $\leq$40 years. Further, the present study indicate the increased value of urea and CK in AMI patients with time of onset of chest pain from 6 pm to 12 midnight and the value of sodium was significantly increased in AMI patients with time of onset of chest pain from 6 am to 12 noon. The value of random blood glucose was significantly increased in patients with time of onset of chest pain from 12 midnight to 6 am. However, the values of hemoglobin and potassium did not show any statistical significance.

The present study clearly indicates elevated values of total WBC count, ESR, creatinine, random blood glucose, creatine kinase and its isoenzyme in AMI patients when compared to that of the normal controls. The lipid profile parameters such as cholesterol, TG and LDL-chol were found to be significantly elevated in AMI patients and HDL-chol was found to be significantly decreased when compared to that of the normal controls. The cholesterol value was found to be significantly elevated in different risk groups studied. The incidence of AMI was found to be more in nonvegetarians. Other important findingsof the present study are the significantly elevated values of ESR in patients with age $\leq$ 40 years, CK in AMI patients with the time of onset of chest pain from 6pm to 12 midnight and total WBC count and CKMB in patients with a positive family history of AMI.

Table : 1 (a)

Values of routine haematological and biochemical parameters in normal and in AMI patients according to the age and sex.

Parameters	Age (years)						Sex			
	≤ 40		40 - 60		≥ 60		Male		Female	
	Normal (n=22)	AMI (n=27)	Normal (n=54)	AMI (n=160)	Normal (n=24)	AMI (n=113)	Normal (n=85)	AMI (n=261)	Normal (n=15)	AMI (n=39)
Hb (gm/dl)	11.80±1.73	11.47±2.67	11.94±1.78	11.46±2.72	12.34±1.55	11.86±3.01	12.13±1.74	11.91±2.84^{a}	11.28±1.46	9.66±1.82^{b**}
TC(Count/cmm)	6638.32 ± 1485.54	10538.52 ± 2610.37^{c**}	7192.35 ± 1325.36	11591.25 ± 1943.12^{ab**}	7540±775.44	11681.95 ± 1919.21^{a**}	7116.59 ± 1350.40	11588.70 ± 1965.64**	7365.33 ± 877.48	11142.31 ± 2368.99**
ESR (mm/Hr)	16.00±3.85	67.11±24.17^{a**}	16.70±5.25	59.60 ± 19.47^{ab**}	17.08±5.58	56.43 ± 18.73^{bc**}	16.84±5.06	59.30±20.13**	15.53±5.01	57.67±18.09**
Na+(mEq/l)	133.82±9.11	136.41±4.08	135.76±8.04	137.87±7.78	135.92±8.51	137.10±7.38	134.89±8.60	137.47±7.55*	138.07±6.89	137.31±6.22
K+(mEq/l)	3.55±0.59	4.37±0.57^{a**}	4.26±0.87	4.19±2.16^{ab}	4.08±0.68	4.06±0.74^{bc}	4.02±0.84	4.20±1.75^{a}	4.28±0.67	3.88±0.69^{b*}
Urea(mg/dl)	30.59±11.73	33.77±17.78	30.70±9.31	35.93±13.73**	33.71±8.65	37.67±13.28	30.93±9.80	36.28±14.10**	34.07±9.58	37.54±13.43
Creatinine (mg/dl)	1.13±0.40	1.50±0.84^{a*}	0.96±0.32	1.22±0.43^{ab**}	0.98±0.29	1.15±0.43^{bc*}	1.01±0.34	1.23±0.54**	0.94±0.33	1.09±0.43
Blood glucose(mg/dl)	99.36±24.75	143.41±16.22^{b**}	102.02±24.20	151.67 ± 63.79^{a**}	120.50±21.00	129.73±31.66^{c}	103.95±25.24	145.79 ± 53.91^{a**}	116.73±20.53	121.74±25.48^{b}
CK(IU/L)	135.57±14.52	1778.37 ± 379.77**	136.00±18.38	1959.31 ± 292.69**	122.58 ± 24.73	2018.29 ± 156.61**	135.20±18.73	1962.46 ± 282.58**	118.93±22.58	1983.85 ± 141.57**
CKMB(IU/L)	30.45±7.98	134.15 ± 36.22**	35.37±9.63	129.98 ± 30.99**	30.96±8.69	126.25 ± 20.99**	33.38±9.40	130.19 ± 29.25^{a**}	32.40±9.05	120.64 ± 19.05^{b**}

Values are mean±SD

**p<0.01, *p<0.05

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values

Table: 1 (b)

Values of routine haematological and biochemical parameters in normal and in AMI patients according to the time of onset of chest pain.

Parameters	Time of Onset of chest pain				
	Normal (n=100)	12midnight to 6am (n=50)	6am to 12 noon (n=94)	12noon to 6pm (n=90)	6pm to 12 midnight (n=66)
Hb (gm/dl)	12.00 ± 1.73	12.37 ±2.63[a]	11.04±2.67[d]**	11.48±2.96[acd]	12.01±2.87[abc]*
TC (Count / cmm)	7153.90 ± 1293.61	11887.40±1817.65**	11543.72±1928.35**	11349.67±2263.94**	11488.64±1940.79**
ESR (mm/Hr)	16.64 ± 5.07	63.98±22.64**	58.16±19.27**	57.03±20.23**	59.49±17.27**
Na+ (mEq/l)	135.37 ± 8.44	136.58±6.05	138.09±7.97**	136.88±7.60	137.97±7.02*
K+ (mEq/l)	4.06 ±0.82	4.31±0.63[b]*	4.31±2.76[ab]	4.01±0.65[ad]	4.02±0.78[acd]
Urea (mg/dl)	31.40 ± 9.83	34.78±17.00	36.28±13.22**	36.30±13.63*	38.15±12.93**
Creatinine (mg/dl)	0.99 ± 0.34	1.46±0.87[a]**	1.22±0.42[ab]**	1.16±0.39[bc]**	1.11±0.41[cbd]
Blood glucose (mg/dl)	105.87 ± 25.01	184.76±85.35[a]**	138.35±45.60[bc]**	138.53±32.20[b]**	122.53±21.94[d]**
CK (IU/L)	132.76 ± 20.21	1860.78±396.68**	1979.30±289.79**	1959.53±190.87**	2032.15±190.87**
CKMB (IU/L)	33.23 ± 9.36	136.24±32.07[a]**	132.21±32.12[ab]**	126..44±25.40[abc]**	122.18±20.16[cd]**

Values are mean ± SD

**p<0.01, *p<0.05

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table:1 (c)

Values of routine haematological and biochemical parameters in normal and in AMI patients with the history of diabetes and hypertension.

Parameters	Normal (n=100)	Diabetes		Hypertension	
		AMI		AMI	
		Diabetic (n=101)	Non diabetic (n=199)	Hypertensive (n=62)	Non hypertensive (n=238)
Hb (gm/dl)	12.00 ± 1.73	11.49 ± 2.91	11.67±2.80	11.99±2.93	11.51±2.81*
TC (Count / cmm)	7153.90 ± 1293.61	11309.90±2142.63**	11642.71±1958.07**	11501.13±2000.95**	11538.36±2035.16**
ESR (mm/Hr)	16.64 ± 5.07	58.94±20.64**	59.16±19.49**	60.42±17.29**	58.74±20.50**
Na+ (mEq/l)	135.37 ± 8.44	137.39±6.58	137.48±7.77*	136.31±6.36	137.74±7.61*
K+ (mEq/l)	4.06 ±0.82	4.17±0.71	4.15±1.97	4.33±0.63*	4.06±0.82
Urea (mg/dl)	31.40 ± 9.83	37.50 ±13.84**	35.92±14.08**	34.03±16.60	37.08±13.19**
Creatinine (mg/dl)	0.99 ± 0.34	1.35 ± 0.69^{a}**	1.15±0.40^{b}**	1.47±0.81^{a}**	1.15±0.40^{b}**
Blood glucose (mg/dl)	105.87 ± 25.01	171.50±68.71^{a}**	126.85±30.19^{b}**	181.71±81.90^{a}**	132.49±33.58^{b}**
CK (IU/L)	132.76 ± 20.21	1964.61±290.46**	1965.56±256.74**	1880.47±377.56**	1987.33±226.77**
CKMB (IU/L)	33.23 ± 9.36	131.24±31.91**	127.78±26.22**	127.19±27.79**	129.40±28.39**

Values are mean ± SD

** p<0.01, *p<0.05

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table :1 (d)

Values of routine haematological and biochemical parameters in normal and in AMI patients according to the values of cholesterol.

Parameters	Cholesterol (mg/dl)		
	Normal (n=100)	AMI	
		> 200 (n=105)	< 200 (n=195)
Hb (gm/dl)	12.00 ± 1.73	11.79 ± 2.68	11.51±2.92**
TC (Count / cmm)	7153.90 ± 1293.61	11698.67±1792.96**	11440.21±2138.73**
ESR (mm/Hr)	16.64 ± 5.07	60.17±21.56**	58.50±18.90**
Na+ (mEq/l)	135.37 ± 8.44	136.91±7.35	137.74±7.39*
K+ (mEq/l)	4.06 ±0.82	4.05±0.73	4.21±1.98
Urea (mg/dl)	31.40 ± 9.83	34.77±15.12	37.35±13.30**
Creatinine (mg/dl)	0.99 ± 0.34	$1.35\pm0.68^{a**}$	$1.15\pm0.40^{b**}$
Blood glucose (mg/dl)	105.87 ± 25.01	$161.56\pm71.19^{a**}$	$132.65\pm33.10^{b**}$
CK (IU/L)	132.76 ± 20.21	1913.91±362.70**	1992.88±194.81**
CKMB (IU/L)	33.23 ± 9.36	$135.44\pm32.79^{a**}$	$125.45\pm24.89^{b**}$

Values are mean ± SD

** p<0.01, *p<0.05

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table :1 (e)

Values of routine haematological and biochemical parameters in normal and in AMI patients with the habits of smoking and alcohol intake.

Parameters	Smoking				Alcohol intake			
	Normal (n=62)	AMI (n=222)	Normal (n=38)	AMI (n=78)	Normal (n=20)	AMI (n=160)	Normal (n=80)	AMI (n=140)
	Yes		No		Yes		No	
Hb (gm/dl)	11.98±1.72	11.62±2.75	12.04±1.74	11.59±3.08	12.16±1.72	11.84±2.83	11.97±1.73	11.34±2.83*
TC (Cout/cmm)	6864.65±1474.46	11612.70 ± 1938.25**	7625.84±705.27	11297.18 ± 2248.39**	6692.70±1512.87	11712.13 ± 1845.45**	7269.20±1205.45	11323.29 ± 2200.43**
ESR (mm/Hr)	16.24±4.82	59.70±20.57**	17.29±5.40	57.32±17.70**	15.60±3.69	60.40±20.70**	16.90±5.33	57.58±18.81**
Na+ (mEq/l)	134.97±8.32	137.60±7.31*	136.03±8.61	137.01±7.58	133.15±9.21	137.56±7.49*	135.93±9.21	137.31±7.49
K+ (mEq/l)	3.96±0.84	4.21±1.87	4.24±0.75	4.01±0.75	4.41±0.87	4.16±2.16	3.98±0.78	4.15±0.72
Urea (mg/dl)	30.45±10.38	36.04±14.36**	32.95±8.65	37.60±12.92*	33.20±9.80	36.82±13.32	30.95±9.77	36.02±14.77**
Creatinine (mg/dl)	0.99±0.37	1.25±0.56^{a}**	1.00±0.29	1.12±0.41^{b}	1.08±0.38	1.29±0.60^{a}*	0.98±0.33	1.14±0.40^{b}**
Blood gluco-se(mg/dl)	101.58±23.19	149.51±57.29^{a}**	112.87±26.26	123.17±21.11^{b}*	96.80±24.47	151.67±63.79^{a}**	108.14±24.62	132.36±29.82^{b}**
CK (IU/L)	137.89±17.23	1947.59±296.10**	124.39±21.84	2015.47±156.42**	135.35±15.10	1929.64±328.83**	132.11±21.42	2005.93±166.99**
CKMB (IU/L)	34.19±9.26	130.85±30.65^{a}**	31.66±9.30	123.53±19.25^{b}**	30.20±8.02	130.06±31.01**	33.99±9.51	127.67±24.82**

Values are mean ± SD

**p<0.01, *p<0.05

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table : 1 (f)

Values of routine haematological and biochemical parameters in normal and in AMI patients according to the food habits and family history of AMI

Parameters	Food habits				Family history		
	Vegetarians		Non Vegetarians		Normal (n=100)	AMI	
	Normal (n=16)	AMI (n=27)	Normal (n=84)	AMI (n=273)		+ ve (n = 82)	- ve (n = 218)
Hb (gm/dl)	12.11±1.58	12.93±2.93[a]	11.99±1.76	11.47±2.80[b]*	12.00±1.73	11.89±2.75**	11.51±2.87**
TC (Cout/cmm)	6562.06±1552.99	10538.58 ± 2610.37[b]**	7266.63±1205.55	11628.79 ± 1933.78[a]**	7153.90±1293.61	11859.15±1817.11**	71407.15±2088.78**
ESR (mm/Hr)	15.25±4.97	58.19±20.28**	16.91±5.05	59.17±19.85**	16.64±5.07	56.83±17.72**	59.93±20.58**
Na+ (mEq/l)	138.19±6.69	137.67±5.74	134.83±8.63	137.31±7.49*	135.37±8.44	137.65±7.96	137.37±7.16*
K+ (mEq/l)	4.21±0.70	4.07±0.71	47.03±0.84	4.17±1.72	4.06±0.82	4.04±0.75	4.20±1.88
Urea (mg/dl)	33.31±9.73	36.70±14.83	31.04±9.80	36.42±13.94**	31.40±9.83	36.24±12.90*	36.52±14.42**
Creatinine (mg/dl)	0.99±0.39	1.08±0.42	1.00±0.33	1.23±0.53**	0.99±0.34	1.38±0.74[a]**	1.16±0.41**[b]
Blood glucose (mg/dl)	116.25±19.97	126.56±26.50[b]	103.89±25.38	144.25±53.34[a]**	105.87±25.01	171.10±75.99[a]**	131.96±33.09**[b]
CK (IU/L)	117.38±22.68	2032.11±215.17**	135.69±18.29	1958.63±272.39**	132.76±20.21	1963.78±217.35**	1965.79±285.46**
CKMB (IU/L)	31.75±9.12	119.37±17.47[b]**	33.51±9.38	129.89±28.99[a]**	33.23±9.36	136.42±31.20[a]**	126.14±26.61**[b]

Values are mean ± SD

** p< 0.01, *p<0.05

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table:2

Values of lipid parameters in normal and in AMI patients.

Parameters	Groups	
	Normal (n = 100)	AMI (n = 300)
Cholesterol (mg/dl)	137.20 ± 16.02	185.50 ± 37.85 **
TG (mg/dl)	111.64 ± 30.08	135.39 ± 56.47 **
HDL-chol (mg/dl)	51.82 ± 14.33	45.06 ± 10.32 **
LDL-chol (mg/dl)	107.80 ± 26.89	122.03 ± 30.52 **

Values are mean ± SD.
**p<0.01

Table:2 (a)

The values of lipid parameters in normal and in AMI patients according to the age and sex.

Parameters	Age (years)						Sex			
	≤ 40		40 - 60		≥ 60		Male		Female	
	Normal (n=22)	AMI (n=27)	Normal (n=54)	AMI (n=160)	Normal (n=24)	AMI (n=113)	Normal (n=85)	AMI (n=261)	Normal (n=15)	AMI (n=39)
Cholesterol (mg/dl)	135.91±16.99	225.22 ± 17.08^{a**}	135.67±15.69	194.88 ± 40.93^{b**}	141.83±14.50	162.73 ± 18.52^{c**}	136.05±15.90	189.04 ± 38.84a**	143.73±14.45	162.53±18.26b**
TG (mg/dl)	93.00±29.19	128.11±65.97*	112.06±30.90	135.98 ± 54.79**	127.79±16.28	136.30±56.25	108.57±30.87	135.48 ± 57.54**	129.07±16.82	134.79±48.77
HDL-chol (mg/dl)	53.41±13.82	45.59±9.70*	51.96±15.30	44.81±10.29**	50.04±12.12	45.28±10.44	51.94±14.83	45.11±10.19**	51.13±10.91	44.72±10.82
LDL-chol (mg/dl)	109.59±26.10	129.41±32.51*	107.24±27.23	120.09 ± 29.75**	107.42±26.78	123.02±30.76*	107.47±26.87	121.59±30.52**	109.67±26.93	124.97±30.33

Values are mean ± SD

**p<0.01, *p<0.05

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table:2 (b)

Values of lipid parameters in normal and in AMI patients according to the time of onset of chest pain.

Parameters	Time of Onset of chest pain				
	Normal (n=100)	AMI			
		12mid night to 6am (n=50)	6am to 12 noon (n=94)	12noon to 6pm (n=90)	6pm to 12 mid night (n=66)
Cholesterol (mg/dl)	137.20 ± 15.93	229.42±21.32^{a**}	200.35±40.77^{b**}	163.39±18.23^{c**}	161.23±17.81^{cd**}
TG(mg/dl)	111.64±30.09	135.90±64.83*	133.92±57.53**	137.57±57.08**	134.14±46.31**
HDL-chol (mg/dl)	51.82±14.33	45.60±10.13**	44.78±10.19**	45.34±10.40**	44.65±10.52**
LDL-chol (mg/dl)	107.80±26.89	119.82±33.49*	122.87±30.39**	123.54±31.02**	120.44±27.28**

Values are mean ± SD

** p<0.01, *p<0.05

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table:2 (c)

Values of lipid parameters in normal and in AMI patients according to the history of diabetes and hypertension.

Parameters	Normal (n=100)	AMI			
		Diabetes		Hypertension	
		Diabetics (n=101)	Non diabetics (n=199)	Hypertensive (n=62)	Non hypertensive (n=238)
Cholesterol (mg/dl)	137.20 ± 15.93	227.38 ±25.20[a]**	164.25±22.27[b]**	228.16±22.80[a]**	174.39±32.76[b]**
TG (mg/dl)	111.64±30.09	135.34±56.36**	135.42±56.53**	134.02±60.59*	135.75±55.34**
HDL-chol (mg/dl)	51.82±14.33	44.96±10.02**	45.11±10.47**	44.86±10.30**	45.11±10.33**
LDL-chol (mg/dl)	107.80±26.89	122.25±30.95**	121.92±30.29**	120.00±32.22*	122.56±30.03**

Values are mean ± SD

** $p < 0.01$, *$p<0.05$

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table :2 (d)

Values of lipid parameters in normal and in AMI patients according to the values of cholesterol.

Parameters	Normal (n=100)	AMI	
		Cholesterol (mg/dl)	
		> 200 (n=105)	< 200 (n=195)
Cholesterol (mg/dl)	137.2 ± 15.93	229.65 ± 21.10[a]**	161.73 ± 18.71[b]**
TG (mg/dl)	111.64 ± 30.09	135.29 ± 56.60**	135.45 ± 56.40**
HDL-chol (mg/dl)	51.82 ± 14.33	45.15 ± 10.03**	45.01 ± 10.48**
LDL-chol (mg/dl)	107.80 ±26.89	122.07 ± 30.71**	122.01 ± 30.40**

Values are mean ± SD

** p<0.01, *p<0.05

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table : 2 (e)

Values of lipid parameters in normal and in AMI patients according to the habits of smoking and alcohol intake.

Parameters	Smoking				Alcohol intake			
	Yes		No		Yes		No	
	Normal (n=62)	AMI (n=222)	Normal (n=38)	AMI (n=78)	Normal (n=20)	AMI (n=160)	Normal (n=80)	AMI (n=140)
Cholesterol (mg/dl)	136.45±13.31	193.88±39.43[a]**	138.42±15.17	161.65±17.78[b]**	135.80±17.54	205.78±39.06[a]**	137.55±15.48	162.33±17.86[b]**
TG (mg/dl)	106.66±29.09	133.72±55.94**	119.76±29.93	140.15±57.71*	88.95±27.37	135.67±58.30**	117.31±28.00	135.07±54.31**
HDL-chol (mg/dl)	52.55±14.83	44.90±10.11**	50.63±13.4	45.51±10.91*	53.70±14.29	44.86±10.06*	51.35±14.31	45.29±10.61**
LDL-chol (mg/dl)	108.98±26.94	123.36±30.84**	105.87±26.68	118.24±29.24*	112.60±24.06	121.34±30.98	106.60±27.42	122.81±29.93**

Values are mean ± SD

**p<0.01, *p<0.05

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table : 2 (f)

Values of lipid parameters in normal and in AMI patients according to food habits and family history of AMI.

Parameters	Food habits				Family history		
	Vegetarians		Non Vegetarians		Normal (n=100)	AMI	
	Normal (n=16)	AMI (n=27)	Normal (n=84)	AMI (n=273)		+ ve (n = 82)	- ve (n = 218)
Cholesterol (mg/dl)	143.63±13.96	156.70±16.63^{b*}	135.98±15.98	188.35±38.17^{a**}	137.20±15.93	162.71±17.70^{b**}	194.07±39.81^{a**}
TG (mg/dl)	106.19±23.62	141.19±60.73*	112.68±31.06	134.82±56.00**	111.64±30.09	136.73±59.85**	134.89±55.14**
HDL-chol (mg/dl)	50.56±10.81	45.85±10.70	52.06±14.90	44.98±10.28**	51.82±14.33	45.09±10.66**	45.05±10.19**
LDL-chol (mg/dl)	108.94±26.21	127.56±30.59*	107.58±27.02	121.48±30.45**	107.80±26.89	121.66±31.35**	122.17±30.18**

Values are mean ± SD

** p<0.01, *p<0.05

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table : 3

A comparison of routine biochemical parameters in AMI patients in different risk groups.

	Groups	Parameters									
		Hb (gm%)	Tc(Count/cmm)	ESR (mm/Hr)	Na+ (mEq/l)	K+ (mEq/l)	Urea (mg/dl)	Creatinine(mg/dl)	Blood glucose (mg%)	CK (IU/L)	CKMB (IU/L)
1	Normal (n=100)	12.00±1.73	7153.90 ± 1293.61	16.64 ± 5.07	135.37 ± 8.44	4.06 ± 0.82	31.40 ± 9.83	0.99 ± 0.34	105.87 ± 25.00	132.76 ± 20.21	33.23 ± 9.36
2	AMI (n=300)	11.61±2.83	11530.67 ± 2028.19**	59.08 ± 19.89**	137.45 ± 7.39*	4.16 ± 1.66	36.45 ± 14.02**	1.22 ± 0.53**	142.66 ± 51.75**	1965.24 ± 268.57**	128.95 ± 28.33**
3	Age ≤40 years (n=22)	11.4 ±2.67	10538.52 ± 2610.37	67.11 ± 24.17*	136.41 ± 4.08	4.37 ± 0.57	33.77 ± 17.78	1.50 ± 0.84*	143.41 ± 16.22	1778.37 ± 379.77	134.15 ± 36.22
4	Age 40-60 years (n=160)	11.46±2.72	11591.25 ± 1943.12	59.60 ± 19.47	137.87 ± 7.78	4.19 ± 2.16	35.93 ± 13.73	1.22 ± 0.43	151.67 ± 63.79	1959.31 ± 292.69	129.98 ± 30.99
5	Age ≥ 60 years (n=113)	11.86±3.01	11681.95 ± 1919.21	56.43 ± 18.73	137.10 ± 7.38	4.06 ± 0.74	37.67 ± 13.28	1.15 ± 0.43	129.73 ± 31.66	2018.29 ± 156.61	126.25 ± 20.99
6	Male (n=261)	11.91±2.84	11588.70 ± 1965.64	59.30 ± 20.13	137.47 ± 7.55	4.20 ± 1.75	36.28 ± 14.10	1.23 ± 0.54	145.79 ± 53.91	1962.46 ± 282.58	130.19 ± 29.25
7	Female (n=39)	9.66 ± 1.82	11142.31 ± 2368.99	57.67 ± 18.09	137.31 ± 6.22	3.88 ± 0.69	37.54 ± 13.43	1.09 ± 0.43	121.74 ± 25.48	1983.85 ± 141.57	120.64 ± 19.05
8	Time of onset of chest pain from 12 midnight to 6 am (n=50)	12.37±2.63	11887.40 ± 1817.65	63.98 ± 22.64	136.58 ± 6.05	4.31 ± 0.63	34.78 ± 17.00	1.46 ± 0.87	184.76 ± 85.35*	1860.78 ± 396.68	136.24 ± 32.07
9	Time of onset of chest pain from 6 am to 12noon (n=94)	11.04±2.67	11543.72 ± 1928.35	58.16 ± 19.27	138.09 ± 7.97*	4.31 ± 2.76	36.28 ± 13.22	1.22 ± 0.42	138.35 ± 45.60	1979.30 ± 289.79	132.21 ± 32.12
10	Time of onset of chest pain from 12 noon to 6pm (n=90)	11.48±2.96	11349.67 ± 2263.94	57.03 ± 20.23	136.88 ± 7.60	4.01 ± 0.65	36.30 ± 13.63	1.16 ± 0.39	138.53 ± 32.20	1959.53 ± 190.87	126.44 ± 25.40
11	Time of onset of chest pain from 6pm to 12 midnight (n=66)	12.01±2.87	11488.64 ± 1940.79	59.49 ± 17.27	137.97 ± 7.02	4.02 ± 0.78	38.15 ± 12.93*	1.11 ± 0.41	122.53 ± 21.94	2032.15 ± 190.87*	122.18 ± 20.16
12	AMI with diabetes (n=101)	11.49±2.91	11309.90 ± 2142.63	58.94 ± 20.64	137.39 ± 6.58	4.17 ± 0.71	37.50 ± 13.84	1.35 ± 0.69	171.50 ± 68.71	1964.61 ± 290.46	131.24 ± 31.91
13	AMI without diabetes (n=199)	11.67±2.80	11642.71 ± 1958.07	59.16 ± 19.49	137.48 ± 7.77	4.15 ± 1.97	35.92 ± 14.08	1.15 ± 0.40	126.85 ± 30.19	1965.56 ± 256.74	127.78 ± 26.22

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14	AMI with hypertension (n=62)	11.99±2.93	11501.13 ± 2000.95	60.42 ± 17.29	136.31 ± 6.36	4.33 ± 0.63	34.03 ± 16.60	1.47 ± 0.81	181.71 ± 81.90	1880.47 ± 377.56	127.19 ± 27.79
15	AMI without hypertension (n=238)	11.51±2.81	11538.36 ± 2035.16	58.74 ± 20.50	137.74 ± 7.61	4.06 ± 0.82	37.08 ± 13.19	1.15 ± 0.40	132.49 ± 33.58	1987.33 ± 226.77	129.40 ± 28.39
16	Cholesterol >200mg/dl (n=105)	11.79±2.68	11698.67 ± 1792.96	60.17 ± 21.56	136.91 ± 7.35	4.05 ± 0.73	34.77 ± 15.12	1.35 ± 0.68	161.56 ± 71.19	1913.91 ± 362.70	135.44 ± 32.79
17	Cholesterol<200 mg /dl (n=195)	11.51±2.92	11440.21 ± 2138.73	58.50 ± 18.90	137.74 ± 7.39	4.21 ± 1.98	37.35 ± 13.30	1.15 ± 0.40	132.65 ± 33.10	1992.88 ± 194.81	125.45 ± 24.89
18	AMI with smoking (n=222)	11.62±2.75	11612.70 ± 1938.25	59.70 ± 20.57	137.60 ± 7.31	4.21 ± 1.87	36.04 ± 14.36	1.25 ± 0.56	149.51 ± 57.29	1947.59 ± 296.10	130.85 ± 30.65
19	AMI without smoking (n=78)	11.59±3.08	11297.18 ± 2248.39	57.32 ± 17.70	137.01 ± 7.58	4.01 ± 0.75	36.60 ± 12.92	1.12 ± 0.41	123.17 ± 21.11	2015.47 ± 156.42	123.53 ± 19.25
20	AMI with alcohol intake (n=160)	11.84±2.83	11712.13 ± 1845.45	60.40 ± 20.70	137.56 ± 7.49	4.16 ± 2.16	36.82 ± 13.32	1.29 ± 0.60	151.67 ± 63.79	1929.64 ± 328.83	130.06 ± 31.01
21	AMI without alcohol intake (n=140)	11.34±2.83	11323.29 ± 2200.43	57.58 ± 18.81	137.31 ± 7.49	4.15 ± 0.72	36.02 ± 14.77	1.14 ± 0.40	132.36 ± 29.82	2005.93 ± 166.99	127.67 ± 24.82
22	AMI with vegetarian food intake (n=27)	12.93±2.93	10538.58 ± 2610.37	58.19 ± 20.28	137.67 ± 5.74	4.07 ± 0.71	36.70 ± 14.83	1.08 ± 0.42	126.56 ± 26.50	2032.11 ± 215.17	119.37 ± 17.47
23	AMI with nonvegetarian food intake (n=273)	11.47±2.80	11628.79 ± 1933.78	59.17 ± 19.85	137.31 ± 7.49	4.17 ± 1.72	36.42 ± 13.94	1.23 ± 0.53	144.25 ± 53.34	1958.63 ± 272.39	129.89 ± 28.99
24	AMI with +ve family history (n=82)	11.89±2.75	11859.15 ± 1817.11*	56.83 ± 17.72	137.65 ± 7.96	4.04 ± 0.75	36.24 ± 12.90	1.38 ± 0.74	171.10 ± 75.99	1963.78 ± 217.35	136.42 ± 31.20*
25	AMI with -ve family history (n=218)	11.51±2.87	7147.15 ± 2088.78	59.93 ± 20.58	137.37 ± 7.16	4.20 ± 1.88	36.52 ± 14.42	1.16 ± 0.41	131.96 ± 33.09	1965.99 ± 285.46	126.14 ± 26.61

Group 2 is compared with group 1

Groups 3,4,5.....are compared with group 2.

**p < 0.01, *p < 0.05

Table: 4

Comparison of lipid parameters in AMI patients in different risk groups.

Groups		Parameters			
		Cholesterol(mg%)	TG(mg%)	HDL-chol (mg%)	LDL-chol (mg%)
1	Normal (n=100)	137.20 ± 16.02	111.64 ± 30.08	51.82 ± 14.33	107.80 ± 26.89
2	AMI (n=300)	185.50 ± 37.85**	135.39 ± 56.47**	45.06 ± 10.32**	122.03 ± 30.52**
3	Age ≤40 years (n=22)	225.22 ± 17.08	128.11 ± 65.97	45.59 ± 9.70	129.41 ± 32.51*
4	Age 40-60 years (n=160)	194.88 ± 40.93	135.98 ± 54.79	44.81 ± 10.29	120.09 ± 29.75
5	Age ≥ 60 years (n=113)	162.73 ± 18.52	136.30 ± 56.25	45.28 ± 10.44	123.02 ± 30.76
6	Male (n=261)	189.04 ± 38.84	135.48 ± 57.54	45.11 ± 10.19	121.59 ± 30.52
7	Female (n=39)	162.53 ± 18.26	134.79 ± 48.77	44.72 ± 10.82	124.97 ± 30.33
8	Time of onset of chest pain from 12 midnight to 6 am (n=50)	229.42 ± 21.32	135.90 ± 64.83	45.60 ± 10.13	119.82 ± 33.49
9	Time of onset of chest pain from 6 am to 12noon (n=94)	200.35 ± 40.77	133.92 ± 57.53	44.78 ± 10.19	122.87 ± 30.39
10	Time of onset of chest pain from 12 noon to 6pm (n=90)	163.39 ± 18.23	137.57 ± 57.08	45.34 ± 10.40	123.54 ± 31.02
11	Time of onset of chest pain from 6pm to 12 midnight (n=66)	161.23 ± 17.81	134.14 ± 46.31	44.65 ± 10.52*	120.44 ± 27.28
12	AMI with diabetes (n=101)	227.38 ± 25.20	135.34 ± 56.36	44.96 ± 10.32	122.25 ± 30.95
13	AMI without diabetes (n=199)	164.25 ± 22.27	135.42 ± 56.53	45.11 ± 10.47	121.92 ± 30.29

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14	AMI with hypertension (n=62)	228.16 ± 22.80	134.02 ± 60.59	44.86 ± 10.30	120.00 ± 32.22
15	AMI without hypertension (n=238)	174.39 ± 32.76	135.75 ± 55.34	45.11 ± 10.33	122.56 ± 30.03
16	Cholesterol >200mg/dl (n=105)	229.65 ± 21.10**	135.29 ± 56.60	45.15 ± 10.03	122.07 ± 30.71
17	Cholesterol<200 mg /dl (n=195)	161.73 ± 18.71	135.45 ± 56.40	45.01 ± 10.48	122.01 ± 30.40
18	AMI with smoking (n=222)	193.88 ± 39.43	133.72 ± 55.94	44.90 ± 10.11	123.36 ± 30.84
19	AMI without smoking (n=78)	161.65 ± 17.78	140.15 ± 57.71	45.51 ± 10.91	118.24 ± 29.24
20	AMI with alcohol intake (n=160)	205.78 ± 39.06	135.67 ± 58.30	44.86 ± 10.06	121.34 ± 30.98
21	AMI without alcohol intake (140)	162.33 ± 17.86	135.07 ± 54.31	45.29 ± 10.61	122.81 ± 29.93
22	AMI with vegetarian food intake (n=27)	156.70 ± 16.63	141.19 ± 60.73*	45.85 ± 10.70	127.56 ± 30.59
23	AMI with nonvegetarian food intake (n=273)	188.35 ± 38.17	134.82 ± 56.00	44.96 ± 10.28	121.48 ± 30.45
24	AMI with +ve family history (n=82)	162.71 ± 17.70	136.73 ± 59.85	45.09 ± 10.66	121.66 ± 31.35
25	AMI with -ve family history (n=218)	194.07 ± 39.81	134.89 ± 55.14	45.05 ± 10.19	122.17 ± 30.18

Group 2 is compared with group 1
Groups 3,4,5.....are compared with group 2.
**p < 0.01, *p < 0.05

EVALUATION OF THE ROLE OF MATRIX METALLOPROTEINASES AND OXIDATIVE STRESS IN THE PATHOGENESIS AND COURSE OF ACUTE MYOCARDIAL INFARCTION

THESIS SUBMITTED TO THE UNIVERSITY OF CALICUT FOR THE DEGREE OF

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CHAPTER 4

OXIDATIVE STRESS IN THE PATHOGENESIS AND COURSE OF ACUTE MYOCARDIAL INFARCTION

4.0.Introduction

The involvement of reactive oxygen species (ROS) in the pathogenesis of various diseases including CAD has been under extensive investigation during the past decade. ROS are continuously generated in cells exposed to an aerobic environment during the course of normal metabolism. ROS damage proteins, DNA and other biomolecules (Davies, 1993 and Hussain *et al*, 1994). It has been postulated that age-dependent diseases such as atherosclerosis, arthritis, neurodegenerative disorders and cancer involve ROS at least at some stage of their development.

The term ROS refers to forms of oxygen exhibiting high reactivity and having at least one unpaired electron. However, other reactive forms of oxygen are also known which are non-free radicals. Both of these forms are collectively referred to as reactive oxygen species (ROS) and include singlet oxygen, superoxide anion, hydrogen peroxide, hydroxyl radical etc. ROS are short-lived species that are generated *in situ* in normal cells under pathological conditions. In addition, the metabolism of xenobiotics and / or exposure to ionizing radiation also generates these species. An important feature of free radical reactions with non-radical species is the formation of new radical species. Free radical driven reactions are usually chain reactions (Halliwell and Gutteridge, 1984). Electron acceptors such as molecular oxygen react easly with free radicals become radical themselves, the ROS. This explains why, in aerobic life, where molecular oxygen is ubiquitous, ROS become the primary mediators of cellular free radical injury.

Excess free radicals, which enhance the oxidative stress, are thought to initiate atherosclerosis by damaging blood vessel walls. LDL-cholesterol has long been implicated in the development of heart disease and many clinicians report that lowering blood LDL-cholesterol is the most effective means of combating heart disease. However, LDL only possess a threat after oxidation by free radicals, as it is reported to migrate across endothelial membranes into the arterial wall. These oxidized components attract macrophages, which absorb and deposit cholesterol within the cell to form what has been referred to as 'foam cells'. These foam cells may initiate the formation of an atherosclerotic lesion, which can result in blockage of the vessel. Interruption of the blood supply causes severe pain known as angina pectoris, and may eventually cause death of the cardiac tissue. Hence, an increased oxidative stress can definitely lead to CAD including AMI.

The first line of cellular defense against oxidative stress by free radicals consists of enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidases and reductases. These enzymes react directly with the oxidizing radicals to yield non-radical products. Selenium dependent glutathione peroxidase (GPx) removes both H_2O_2 and lipid peroxides by catalyzing the conversion of lipid hydroperoxides to hydroxy acids in the presence of reduced-glutathione (GSH). SOD is a copper-containing enzyme, occurring widely in cells and tissues such as erythrocytes, liver and brain. It is a free radical metabolizing enzyme, catalyzing dismutation of superoxide anion to hydrogen peroxide. This protects the cell membrane from damage by this highly reactive species.

Catalase catalyses the dismutation of hydrogen peroxide. The hydrogen peroxide formed by the dismutation of superoxide anion by SOD is decomposed to water by catalase, thus neutralizing the deleterious effects of H_2O_2 in the body. Glutathione plays a major role in cellular protection against oxidative damage (Mallika *et al*, 2000). Conditions that perturb intracellular levels of glutathione have been shown to result in significant alteration in the cellular metabolism.

Other clinical analytes such as albumin, uric acid, bilirubin and ceruloplasmin were also considered to posses the free radical scavenging properties. Raised levels of these antioxidant molecules can indicate the persistent injuries to arteries and hence the progression of atherosclerotic heart diseases (Kuller *et al*, 1971).

The present chapter mainly deals with the role of these antioxidant enzymes and other antioxidant molecules along with lipid peroxidation as markers of oxidative stress induced by free radicals in the pathogenesis of AMI. The levels of SOD, CAT, GPx, GR, GST, lipid peroxidation products, albumin, uric acid, bilirubin and ceruloplsmin were done as indicators of oxidative damage as these parameters may change in altered stress conditions.

4.1.Materials and Methods

Superoxide dismutase activity was determined by the method of Mc Cord and Fridovich, catalase by the method of Aebi, reduced-glutathione by the method of Moron et al, glutathione reductase by the method of Racker, glutathione peroxidase by the method of Paglia and Valentine, glutathione-S-transferase by the method of Habig et al and lipid peroxidation was done by the method of Yoshioka et al. Albumin in the serum was estimated by the method of Doumas and Peters, uric acid by the uricase method, bilirubin by the

method described by Varley and ceruloplasmin was done by the immunoturbidimetric method. The detailed procedures used for the estimations are given in chapter 2.

4.2.Results

Table 5 represents the values of antioxidant enzymes and lipid peroxidation in normal and in AMI patients. The values of SOD, CAT, GSH, GPX, GR and GST were found to be significantly decreased ($p<0.01$) in all the AMI patients when compared to that of the normal healthy individuals. The value of lipid peroxidation was found to be significantly elevated ($p<0.0l$) in all the AMI patients when compared to that of the normal controls.

Table 5 (a) represents the values of antioxidant enzymes and lipid peroxidation products in normal and in AMI patients according to the age and sex. Irrespective of the age and sex the values of all the antioxidant enzymes were found to be significantly decreased ($p<0.01$) in AMI patients when compared to that of the normal controls whereas the values of lipid peroxidation products were found to be significantly increased ($p<0.01$) in AMI patients when compared to that of the normal controls. A comparison of the values of these antioxidant enzymes between different age groups of AMI patients have shown significantly decreased values of SOD and CAT in AMI patients with age ranges from 40-60 years when compared to that of the other two age groups. However, the values of GPx and GR were found to be significantly decreased in AMI patients with age $\leq$40 years when compared to that of the other two age groups of AMI patients. The values of GSH, GST and lipid peroxidation did not show any alteration between the different age groups of AMI patients. Comparisons between the values of antioxidant enzymes and lipid peroxidation between AMI males and females have shown significantly decreased values of SOD, GPx and GR in AMI males when compared to that of the AMI females. However, the values of CAT, GSH, GST and lipid peroxidation were did not show any significant variation between the groups.

Table 5 (b) shows the values of antioxidant enzymes and lipid peroxidation in normal and in AMI patients according to the time of onset of chest pain. Here the values of all the antioxidant enzymes were found to be significantly decreased ($p<0.01$) in all the AMI patients with different times of onset of chest pain when compared to that of the normal controls. The value of lipid peroxidation was found to be significantly increased ($p<0.01$) in all the AMI patients with different time of onset of chest pain when compared to that of the normal controls. A comparison of the values of antioxidant enzymes and lipid peroxidation

between the AMI patients with different time of onset of chest pain have shown significantly reduced value of SOD in AMI patients with time of onset of chest pain from 6am to 12noon when compared to that of the other times of onset of chest pain. The values of GPx and GR were found to be significantly decreased in AMI patients with time of onset of chest pain from 12 mid night to 6 am when compared to that of the other groups. However, the values of CAT, GSH, GST and lipid peroxidation products did not show significant difference between the groups.

Table 5 (c) represents the values of antioxidant enzymes and lipid peroxidation in normal and in AMI patients according to the history of diabetes and hypertension. The values of antioxidant enzymes were found to be significantly reduced ($p<0.01$) in AMI patients both with and without the history of diabetes and hypertension when compared to that of the normal healthy individuals. The value of lipid peroxidation was significantly increased ($p<0.01$) in AMI patients both with and without the history of diabetes and hypertension when compared to that of the normal controls. Activities of SOD and GR were found to be decreased in AMI patients with the history of diabetes when compared to that of the AMI patients without the history of diabetes. The values of CAT, GSH, GPx, GST and lipid peroxidation products did not show any significant alteration between these groups. A significantly decreased value of SOD was observed in AMI patients without the history of hypertension when compared to that of the AMI patients with the history of hypertension. The value of GR was found to be significantly decreased in AMI patients with the history of hypertension when compared to that of the AMI patients without the history of hypertension. The values of CAT, GSH, GPx, GST and lipid peroxidation did not show significant variation between the groups.

Table 5 (d) represents the values of antioxidant enzymes and lipid peroxidation in normal and in AMI patients according to the values of cholesterol. The values of all the antioxidant enzymes were found to be significantly decreased ($p<0.01$) in AMI patients with cholesterol value >200 mg/dl and <200 mg/dl when compared to that of the normal controls and the value of lipid peroxidation was found to be significantly increased ($p<0.01$) in AMI patients with cholesterol value >200 mg/dl and <200 mg/dl when compared to that of the normal healthy individuals. A comparison between the values of antioxidant enzymes and lipid peroxidation in AMI patients with cholesterol value >200 mg/dl and <200 mg/dl have

shown the significantly decreased value of SOD in AMI patients with cholesterol value <200 mg/dl when compared to that of the AMI patients with cholesterol value >200 mg/dl. The value of GR was found to be significantly decreased in AMI patients with cholesterol value >200 mg/dl when compared to that of the AMI patients with cholesterol value <200 mg/dl. The values of catalase, GSH, GPx, GST and lipid peroxidation did not show any significant difference between the groups.

Table 5 (e) represents the values of antioxidant enzymes and lipid peroxidation in normal and in AMI patients according to the habits of smoking and alcohol intake. The values of all the antioxidant enzymes were found to be significantly decreased ($p<0.01$) in AMI patients with and without the history of smoking and alcohol intake and the value of lipid peroxidation was found to be significantly increased ($p<0.01$) in all the AMI patients with and without the habits of smoking and alcohol intake when compared to that of their respective controls. A comparison between the values of antioxidant enzymes and lipid peroxidation in AMI patients with and without the habits of smoking have shown significantly decreased values of SOD, GPx and GR in AMI patients with the habit of smoking when compared to that of the AMI patients without the habit of smoking. However, the values of catalase, GSH, GST and lipid peroxidation did not show significant alteration between the groups. A comparison between the values of antioxidant enzymes and lipid peroxidation in AMI patients with and without the habit of alcohol intake have shown significantly decreased values of SOD, GPx and GR in AMI patients with the habit of alcohol intake when compared to that of the AMI patients without the habit of alcohol intake. Here also the values of catalase, GSH, GST and lipid peroxidation did not show any significant alteration between the groups.

Table 5 (f) represents the values of antioxidant enzymes and lipid peroxidation in normal and in AMI patients according to the food habits and a family history of AMI. The values of antioxidant enzymes were found to be significantly decreased ($p<0.01$) and the value of lipid peroxidation was found to be significantly increased ($p<0.01$) in all the AMI patients both with the habits of vegetarian and nonvegetarian food intake and in AMI patients with and without the family history of AMI when compared to that of their normal controls. A comparison between the values of antioxidant enzymes and lipid peroxidation in vegetarian and nonvegetarian have shown significantly decreased values of SOD, GPx and

GR in nonvegetarian AMI patients when compared to that of the vegetarian AMI patients. The values of CAT, GSH, GST and lipid peroxidation did not show any significant alteration between the groups. A comparison between the values of antioxidant enzymes and lipid peroxidation products in AMI patients both with and without the family history of AMI have shown significantly decreased values of SOD in AMI patients with a family history of AMI when compared to that of the AMI patients without the family history of AMI. The values of GSH and GR were found to be significantly decreased in AMI patients without the family history of AMI when compared to that of the AMI patients with the family history of AMI. The values of catalase, GPx, GST and lipid peroxidation did not show any significant variation between the groups.

Table 6 represents the values of albumin, uric acid, bilirubin and ceruloplasmin in normal and in AMI patients. Here the values of albumin and ceruloplasmin were found to be significantly decreased ($p<0.01$) in AMI patients when compared to that of the normal controls. The value of uric acid was significantly increased ($p<0.01$) in AMI patients when compared to that of the normal controls. However, the value of bilirubin remained unchanged in AMI patients when compared to that of the normal controls.

Table 6 (a) represents the values of albumin, uric acid, bilirubin and ceruloplsmin in normal and in AMI patients according to the age and sex. The values of albumin and ceruloplasmin were found to be significantly decreased ($p<0.01$) and the value of uric acid was found to be significantly increased ($p<0.01$) in all the AMI patients irrespective of the age and sex when compared to that of the normal controls. However, the value of bilirubin did not show any change in AMI patients according to the age and sex when compared to that of the normal controls. A comparison between the values of albumin, uric acid, bilirubin and ceruloplasmin in AMI patients with different age groups have shown significantly decreased values of albumin and ceruloplasmin in AMI patients with age ≤ 40 years when compared to that of the other age groups. The value of uric acid was found to be significantly increased in AMI patients with age ≥ 60 years when compared to that of the other two groups. The value of bilirubin did not show any significant difference between the groups.

Table 6 (b) represents the values of albumin, uric acid, bilirubin and ceruloplsmin in normal and in AMI patients according to different time of onset of chest pain. The values of albumin and ceruloplsmin were found to be significantly decreased ($p<0.01$) in all the AMI

patients with different time of onset of chest pain when compared to that of the normal controls. The value of uric acid was found to be significantly increased ($p<0.01$) in AMI patients with different time of onset of chest pain when compared to that of the normal controls. However, the value of bilirubin did not show any significance in AMI patients when compared to that of the normal controls. A comparison between the values of albumin, uric acid, bilirubin and ceruloplasmin in AMI patients with different time of onset of chest pain have shown significantly reduced value of albumin and ceruloplasmin in AMI patients with the time of onset of chest pain from 12 midnight to 6am when compared to that of the other groups. The value of uric acid was found to be significantly increased in AMI patients with time of onset of chest pain from 6pm to 12midnight when compared to that of the other groups. The value of bilirubin was found to be significantly increased in AMI patients with time of onset of chest pain from 6am to 12noon when compared to that of the other groups.

Table 6 (c) represents the values of albumin, uric acid, bilirubin and ceruloplsmin in normal and in AMI patients according to the history of diabetes and hypertension. The values of albumin and ceruloplsmin were found to be significantly decreased ($p<0.01$) in AMI patients in both with and without the history of diabetes and hypertension when compared to that of the normal controls. The value of uric acid was found to be significantly increased ($p<0.01$) in AMI patients in both with and without the history of diabetes and hypertension when compared to that of the normal controls. The value of bilirubin did not show any significant change in AMI patients in both with and without the history of diabetes and hypertension when compared to that of the normal controls. A comparison of the values of these parameters between the AMI patients with and without the history of hypertension have shown significantly decreased value of albumin in AMI patients without the history of hypertension when compared to that of the AMI patients with the history of hypertension. All the other parameters did not show any significant difference between the groups.

Table 6 (d) represents the values of albumin, uric acid, bilirubin and ceruloplasmin in normal and in AMI patients according to the values of cholesterol. The values of albumin and ceruloplsmin were found to be significantly decreased ($p<0.01$) in AMI patients with the cholesterol values >200 mg/dl and <200 mg/dl when compared to that of the normal controls. The value of uric acid was found to be significantly increased ($p<0.01$) in AMI patients with the cholesterol values >200 mg/dl and <200 mg/dl when compared to that of the normal

controls. However, the value of bilirubin did not show significant change in AMI patients with cholesterol values >200 mg/dl and <200 mg/dl when compared to that of the normal controls. A comparison between the values of these parameters in AMI patients with the cholesterol value >200 mg/dl and <200 mg/dl have shown significantly increased value of bilirubin in AMI patients with cholesterol value >200 mg/dl when compared to that of the AMI patients with cholesterol value <200 mg/dl. The other parameters did not show significant alteration between the groups.

Table 6 (e) represents the values of albumin, uric acid, bilirubin and ceruloplasmin in normal and in AMI patients according to the habits of smoking and alcohol intake. The values of albumin and ceruloplsmin were found to be significantly decreased ($p<0.01$) in AMI patients with and without the habits of smoking and alcohol intake when compared to that of the normal healthy subjects. The value of uric acid was found to be significantly increased ($p<0.01$) and the value of bilirubin remained unaltered in AMI patients both with and without the habits of smoking and alcohol intake when compared to that of the normal healthy individuals. A comparison of the values of these parameters in AMI patients both with and without the habit of smoking have shown significantly decreased value of ceruloplsmin in AMI patients with the habit of smoking when compared to that of the AMI patients without the habit of smoking and significantly increased value of uric acid in AMI patients without the habit of smoking when compared to that of the AMI patients with the habit of smoking. A comparison between the AMI patients both with and without the habit of alcohol intake have shown significantly decreased value of albumin and significantly increased value of uric acid in AMI patients without the habit of alcohol intake when compared to that of the AMI patients with the habit of alcohol intake. However, the other parameters did not show significant variation between the groups.

Table 6 (f) represents the values of albumin, uric acid, bilirubin and ceruloplasmin in normal and in AMI patients according to the food habits and family history of AMI. The values of albumin and ceruloplsmin were found to be significantly decreased ($p<0.01$) in AMI patients in both with the habits of vegetarian and nonvegetarian food intake and in both with and without the family history of AMI when compared to that of the normal controls. The value of uric acid was found to be significantly increased in AMI patients with the habits of vegetarian and nonvegetarian food intake and in both with and without the family history

of AMI when compared to that of the normal controls. However, the value of bilirubin did not show any significant change in AMI patients with the habits of vegetarian and nonvegetarian food intake and with and without the family history of AMI. A comparison between the values of these parameters in AMI patients with and without the family history of AMI have shown significantly increased value of uric acid in AMI patients without the family history of AMI when compared to that of the AMI patients with the family history of AMI. The other parameters did not show significant alteration between the groups.

Table 7 represents the values of the markers of oxidative stress in normal, in AMI patients and in different risk groups of AMI patients. The value of SOD was found to be significantly decreased in AMI patients with the habit of alcohol intake. The value of CAT was found to be significantly decreased in AMI patients with a positive family history of AMI and the value of GSH was found to be significantly decreased and the value of lipid peroxidation was significantly increased in AMI females. The values of GPx and GR were found to be significantly decreased in AMI patients with age ≤ 40 years and the value of GST was found to be significantly decreased in AMI patients with time of onset of chest pain from 12 midnight to 6 am.

Table 8 represents the values of albumin, uric acid, bilirubin and ceruloplasmin the so-called antioxidant molecules in normal, in AMI patients and in different risk groups of AMI patients. The values of albumin and ceruloplasmin were found to be significantly decreased in AMI patients with age ≤ 40 years and the value of uric acid was found to be significantly increased in AMI patients with age ≥ 60 years. However, the value of bilirubin did not show any statistical significance between the groups.

4.3.Discussion

The results of the present study clearly shows the decreased values of antioxidant enzymes such as SOD, CAT, GPx, GR and GST in AMI patients when compared to that of the normal controls. The values of antioxidant molecules such as GSH, albumin and ceruloplasmin were also showed decreased values in these patients. However, the values of lipid peroxidation and uric acid were found to be increased in all these AMI patients indicating an increased oxidative stress by free radicals in these patients.

Low SOD levels have been reported in patients following angina pectoris and myocardial infarction (Vukelic, 1997). SOD protects the heart muscle from free radical

damage following ischaemia and serum levels are reportedly low following myocardial infarction and hence it can be used as a marker for the assessment of ischaemic myocardial damage (Sushil, 1995). From the results it is evident that the level of SOD in blood of patients suffering from AMI is very low when compared to the normal controls. The value of catalase was also found to be low in patients with acute myocardial infarction when compared to that of the normal subjects. The decreased activity of catalase in the experimental group could be due to increase in MDA which can cross link with amino group of protein to form intra- and inter-molecular cross links thereby inactivating several membrane bound enzymes such as catalase (Kikugawa *et al*, 1984). The decreased activity of catalase may result in the accumulation of H_2O_2 in the body. Thus the inadequacy of these enzymes lead to the accumulation of free radicals and an increased oxidative stress.

Glutathione, a major non-protein thiol in living organisms, plays a central role in coordinating the body's antioxidant defense processes. Perturbation of glutathione status of a biological system has been reported to lead to serious consequences. Administration of thiol compounds such as glutathione, cysteine and methionine are shown to protect against oxidative stress in humans and animals (Meister, 1989). In the present study we observed a low level of GSH in AMI patients when compared to normal controls. GSH is a substrate for GPx, which removes H_2O_2 and also acts as substrate for dehydroascorbate reductase. We found a low activity of GPx in these patients. Which may be attributed to the unavailability of GSH, the substrate for GPx. The capacity of the glutathione system to cope with free radicals mainly depends upon the activity of peroxidase and glutathione reductase. Glutathione reductase (GR) keeps a high ratio of GSH/GSSG in normal cells. In AMI patients we observed a very low level of glutathione reductase which in turn is linked to decreased concentration of GPx and reduced glutathione. GST plays an essential role in liver by eliminating toxic compounds by conjugating them with glutathione. The substrate variability of GST accounts for the reactivity of this enzyme with a wide range of endogenous, environmental, therapeutic and laboratory substrates (Halliwell and Gutteridge, 1992). We observed a low concentration of GST in all the AMI patients indicating the low activity of glutathione system as a whole in these patients.

Lipid peroxidation is known to play an important role in atherogenesis (Coresh and Kwiterovich, 1996). Increase in serum lipid peroxide level have been reported in diabetics, in

patients with cardiovascular disorders (Gallou *et al*, 1993) and in aging subjects (Uysal *et al*, 1986). In the present study, serum endogenous MDA levels, a measure of lipid peroxidation were found to be higher in patients with acute myocardial infarction as compared to healthy control subjects. It has been proposed that increased endogenous lipid peroxide levels in serum indicate the occurrence of membrane damages provoked by free radical induced diseases (Stringer *et al*, 1989) and that the removal of lipid peroxides in serum before they are taken up by peripheral tissue may improve the prognosis of such diseases (Loeper *et al*, 1991). Hence, these results clearly indicate the role of free radicals causing oxidative damage in AMI patients.

Albumin, uric acid, bilirubin and ceruloplasmin are important antioxidant molecules concerned with the defense against the free radical attack and oxidative stress. We observed the low values of albumin and ceruloplasmin in AMI patients indicating persistent injuries to arteries and increased endothelial damage due to atherosclerosis. The value of uric acid was found to be higher in these patients while the value of bilirubin did not show any alteration.

The values of these antioxidant enzymes, lipid peroxidation, albumin, uric acid and ceruloplasmin showed alterations according to the risk factors taken into account. Irrespective of the age, sex, time of onset of chest pain, history of diabetes and hypertension, the cholesterol values, habits of smoking and alcohol intake, food habits and family history of AMI the values of all these antioxidant enzymes, albumin and ceruloplasmin were found to be decreased and the values of lipid peroxidation and uric acid were found to be increased in all these patients when compared to that of the normal controls.

The values of SOD and catalase were found to be significantly decreased in AMI patients with age ranges from 40-60 years when compared to the other two groups. The values of GPx, GR, albumin and ceruloplasmin were found to be decreased in patients with age $\leq$40 years when compared to that of the other groups and the value of uric acid was found to be significantly increased in AMI patients with age $\geq$60 years. However, GSH, GST, lipid peroxidation and bilirubin did not show significant alteration between the groups. The values of SOD, GPx and GR were found to be significantly decreased in AMI males when compared to that of AMI females. Other parameters did not show significant alteration between the groups. The value of SOD was found to be significantly decreased in AMI patients with time of onset of chest pain from 6 am to 12 noon and the value of uric acid was

found to be significantly increased in AMI patients with time of onset of chest pain from 6pm to 12midnight when compared to other groups. The values of GPx, GR and ceruloplasmin were found to be significantly decreased in AMI patients with time of onset of chest pain from 12 midnight to 6am when compared to that of the other groups and the value of bilirubin was found to be significantly decreased in AMI patients with time of onset of chest pain from 12 noon to 6pm when compared to that of the other groups.

The values of SOD and GR were found to be significantly decreased in AMI patients with the history of diabetes when compared to that of the AMI patients without the history of diabetes. The values of SOD and albumin were found to be significantly decreased in AMI patients without the history of hypertension when compared to AMI patients with the history of hypertension and the value of GR was found to be significantly decreased in AMI patients with the history of hypertension when compared to AMI patients without the history of hypertension. The values of SOD and bilirubin were found to be significantly decreased in AMI patients with cholesterol values <200 mg/dl when compared to that of the AMI patients with cholesterol value >200 mg/dl and the value of GR was found to be significantly decreased in AMI patients with cholesterol value >200mg/dl when compared to AMI patients with cholesterol value <200 mg/dl.

The values of SOD, GPx and GR was found to be significantly decreased in AMI patients with the habits of smoking and alcohol intake when compared to that of AMI patients without the habits of smoking and alcohol intake. The value of uric acid was found to be significantly increased in AMI patients without the habit of smoking and the value of ceruloplasmin was found to be significantly decreased in AMI patients with the habit of smoking. The value of albumin was found to be significantly decreased and the value of uric acid was significantly increased in AMI patients without the habit of alcohol intake. The values of SOD, GPx and GR were found to be significantly decreased in AMI patients with the habit of non vegetarian food intake when compared to AMI patients with the habit of vegetarian food intake. The value of SOD was found to be significantly decreased in AMI patients with the family history of AMI when compared to AMI patients without the family history of AMI. However the values of GSH and GR were found to be significantly decreased and the value of uric acid was found to be significantly increased in AMI patients

without the family history of AMI when compared to AMI patients with the family history of AMI.

All the risk groups with AMI studied, we observed a significantly decreased value of SOD in AMI patients with the habit of alcohol intake and a significantly decreased value of catalase in patients with a positive family history of AMI. The present study indicates the decreased value of GSH and an increased value of lipid peroxidation in AMI females. The values of GPx, GR, albumin and ceruloplasmin were found to be significantly decreased in AMI patients with age ≤ 40 years. The value of GST was found to be significantly decreased in AMI patients with the time of onset of chest pain from 12 midnight to 6 am and the value of uric acid was found to be significantly increased in AMI patients with age ≥ 60 years. However, the value of bilirubin did not show any statistical significance.

From the present study we understand that there is an increased oxidative stress in all the AMI patients irrespective of the risk factors studied, as it is evident from the values of the antioxidant enzymes and molecules, which act as the markers of the oxidative stress. The decreased values of these oxidative stress markers and an increased value of lipid peroxidation products in AMI patients indicate the involvement of free radicals in the pathogenesis of AMI. The important findings of the present study is that eventhouh these parameters are altered in all the risk groups studied, significant change was observed in some of the risk groups such as in female patients, patients with the habit of alcohol intake, patients with a positive family history of AMI and in patients with age ≤ 40 years. Hence, it may be concluded that patients belonging to these risk groups may have a high oxidative damage when compared to other risk groups.

Table : 5

Values of antioxidant enzymes and lipid peroxidation products in normal and in AMI patients.

Parameters	Groups	
	Normal (n=100)	AMI (n=300)
SOD (U/gm.Hb)	1418.99 ± 211.78	753.27 ± 209.73**
CAT (K/gm.Hb)	124.26 ± 12.52	51.28 ± 14.89**
GSH (nmol/ml of blood)	210.64 ± 16.56	138.27 ± 19.05**
GPX (U/gm.Hb)	18.66 ± 1.74	12.29 ± 1.41**
GR (U/gm.Hb)	10.06 ± 0.76	4.70 ± 1.02**
GST(nmol/min/gm.Hb)	60.78 ± 7.73	28.63 ± 5.33**
Lipid Peroxidation products (nmoles/ml of serum)	4.39 ± 0.54	8.01 ± 1.34**

Values are mean ± SD

**p<0.01

Table : 5 (a)

Values of antioxidant enzymes and lipid peroxidation products in normal and in AMI patients according to age and sex

Parameters	Age (years)						Sex			
	≤ 40		40 - 60		≥ 60		Male		Female	
	Normal (n=22)	AMI (n=27)	Normal (n=54)	AMI (n=160)	Normal (n=24)	AMI (n=113)	Normal (n=85)	AMI (n=261)	Normal (n=15)	AMI (n=39)
SOD (U/gm.Hb)	1451.94±153.99	942.07± 78.01^{a**}	1478.25±213.51	628.44± 199.23^{c**}	1255.49±161.49	884.89± 105.93^{b**}	1445.79±105.93	726.45± 222.82^{b**}	1267.18±146.87	878.71± 72.11^{a**}
CAT (K/gm.Hb)	124.41±14.28	57.31± 14.56^{a**}	125.52±11.70	50.71± 13.37^{cb**}	125.32±12.41	51.22±13.45^{b**}	125.32±12.41	51.22±13.45**	118.25±11.48	51.69±11.18**
GSH (nmol/ml of blood)	209.49±13.07	135.53± 21.56**	210.48±19.33	140.11±18.43**	212.08±11.78	136.33±19.03**	210.55±17.14	138.72±19.23**	211.17±12.80	135.28± 17.46**
GPX (U/gm.Hb)	18.62±1.46	11.81± 0.40^{bc**}	18.54±1.61	12.17±1.38^{b**}	18.97±2.15	12.57±1.40^{a**}	18.69±1.71	12.22±1.41^{b**}	18.51±1.88	12.76±1.30^{a**}
GR (U/gm.Hb)	10.00±0.75	3.89±0.54^{c**}	10.11±0.77	4.63±0.98^{b**}	9.99±0.75	4.99±1.03^{a**}	10.06±0.77	4.62±1.01^{b**}	10.03±0.74	5.24±0.88^{a**}
GST (nmol/min/ gm.Hb)	60.65±6.54	27.72±4.49**	60.73±8.35	28.67±5.53**	60.99±7.26	28.79±5.21**	60.84±7.76	28.42±5.29**	60.42±7.55	30.40±5.36**
Lipid Peroxidation(nmoles/ ml of serum)	4.32±0.54	7.82±1.39**	4.44±0.55	7.92±1.33**	4.32±0.52	8.19±1.31**	4.41±0.55	7.97±1.34**	4.24±0.52	8.28±1.30**

Values are mean ± SD

**p < 0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table : 5 (b)

Values of antioxidant enzymes and lipid peroxidation products in normal and in AMI patients according to the time of onset of chest pain.

Parameters	Time of onset of chest pain				
	Normal (n=100)	AMI			
		12midnight to 6am (n=50)	6am to 12noon (n=94)	12noon to 6pm(n=90)	6pm to 12midnight (n=66)
SOD (U/gm.Hb)	1418.99 ± 211.78	$929.16 \pm 68.49^{a**}$	$684.53 \pm 217.46^{cd**}$	$744.39 \pm 223.33^{b**}$	$729.92 \pm 173.77^{bc**}$
CAT (K/gm.Hb)	124.26 ± 12.52	53.51 ± 15.55**	51.13 ± 13.13**	49.88 ± 12.56**	51.71 ± 11.74**
GSH (nmoles/ml of blood)	210.64 ± 16.55	137.41 ± 22.62**	140.05 ± 17.49**	138.90 ± 18.27**	135.52 ± 18.92**
GPX (U/gm.Hb)	18.66 ± 1.74	$12.02 \pm 1.50^{**bcd}$	$12.09 \pm 1.33^{bc**}$	$12.37 \pm 1.41^{ab**}$	$12.65 \pm 1.36^{a**}$
GR (U/gm.Hb)	10.06 ± 0.76	$3.98 \pm 0.59^{d**}$	$4.57 \pm 0.93^{c**}$	$4.99 \pm 1.06^{ab**}$	$5.03 \pm 1.01^{a**}$
GST (nmol/min/gm.Hb)	60.78 ± 7.73	27.55 ± 5.28**	29.00 ± 5.31**	28.43 ± 5.33**	29.20 ± 5.23**
Lipid Peroxidation (nmoles/ml of serum)	4.39 ± 0.54	7.81 ± 1.41**	7.83 ± 1.33**	8.16 ± 1.26**	8.18 ± 1.34**

Values are mean ± SD

**p<0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table : 5 (d)

Values of antioxidant enzymes and lipid peroxidation products in normal and in AMI patients according to the values of cholesterol.

Parameters	Cholestrol (mg/dl)		
	Normal (n=100)	AMI	
		> 200 (n=105)	< 200 (n=195)
SOD (U/gm.Hb)	1418.99 ± 211.78	836.47 ± 159.97[a]**	710.49 ± 220.26[b]**
CAT (K/gm. Hb)	124.26 ± 12.52	51.55 ± 13.34**	50.26 ± 12.36**
GSH(nmoles/ml of blood)	210.64 ± 16.55	138.69 ± 20.57**	138.05 ± 18.19**
GPX (U/gm.Hb)	18.66 ± 1.74	12.11 ± 1.43**	12.38 ± 1.39**
GR (U/gm.Hb)	10.06 ± 0.76	4.12 ± 0.72[b]**	5.02 ± 1.02[a]**
GST (nmol/min/gm.Hb)	60.78 ± 7.73	28.45 ± 5.56**	28.73 ± 5.20**
Lipid Peroxidation(nmoles/ml of serum)	4.39 ± 0.54	7.85 ± 1.38**	8.09 ± 1.30**

Values are mean ± SD

**p < 0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabetd denote heterogenous values.

Table : 5 (e)

Values of antioxidant enzymes and lipid peroxidation products in normal and in AMI patients according to the habits of smoking and alcohol intake.

Parameters	Smoking				Alcohol intake			
	Yes		No		Yes		No	
	Normal (n=62)	AMI (n=222)	Normal (n=38)	AMI (n=78)	Normal (n=20)	AMI (n=160)	Normal (n=80)	AMI (n=140)
SOD(U/gm.Hb)	1476.08 ± 195.99	676.09± 194.62^{b**}	1325.87 ± 203.36	967.19 ± 34.33^{a**}	1467.88 ± 151.41	624.73 ± 200.69^{b**}	1406.78 ± 222.67	895.42 ± 103.88^{a**}
CAT (K/gm.Hb)	124.01 ± 12.76	50.58 ± 13.60**	124.68 ± 12.17	51.28 ± 11.83**	125.46 ± 14.52	51.57 ± 13.96**	123.96 ± 11.95	50.95 ± 12.21**
GSH (nmoles/ml of blood)	210.65 ± 18.68	139.06 ± 19.21**	210.64 ± 12.32	136.03 ± 18.41**	208.67 ± 13.41	139.44 ± 19.05**	211.14 ± 17.20	136.94 ± 18.97**
GPX (U/gm.Hb)	18.61 ± 1.60	12.18 ± 1.41^{b**}	18.74 ± 1.94	12.59 ± 1.37^{a**}	18.54 ± 1.50	12.09 ± 1.40^{b**}	18.69 ± 1.79	12.51 ± 1.39^{a**}
GR (U/gm.Hb)	10.07 ± 0.76	4.56 ± 0.99^{b**}	10.04 ± 0.77	5.02 ± 1.00^{a**}	10.05 ± 0.76	4.44 ± 0.93^{b**}	10.06 ± 0.76	5.00 ± 1.03^{a**}
GST(nmol/min/ gm.Hb)	60.46 ± 7.92	28.48 ± 5.33**	61.29 ± 7.38	29.07 ± 5.31**	60.52 ± 6.01	28.44 ± 5.31**	60.84 ± 8.11	28.85 ± 5.35**
Lipid Peroxidation (nmoles/ml of serum)	4.38 ± 0.55	7.95 ± 1.34**	4.39 ± 0.53	8.18 ± 1.33**	4.32 ± 0.53	7.88 ± 1.36**	4.40 ± 0.55	8.16 ± 1.30**

Values are mean ± SD

**p < 0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table : 5 (f)

Values of antioxidant enzymes and lipid peroxidation products in normal and in AMI patients according to the food habits and a family history of AMI.

Parameters	Food Habits				Family history		
	Vegetarians		Non Vegetarians		Normal (n=100)	AMI	
	Normal (n=16)	AMI (n=27)	Normal (n=84)	AMI (n=273)		+ve (n=82)	-ve (n=218)
SOD (U/gm.Hb)	1270.16 ± 142.67	981.79 ± 55.17^{a**}	1447.35 ± 210.92	726.48 ± 206.34^{b**}	1418.99 ± 211.78	660.71 ± 212.03^{b**}	785.52 ± 200.54^{a**}
CAT (K/gm.Hb)	120.54 ± 10.19	50.86 ± 12.88**	124.97 ± 12.81	51.32 ± 13.19**	124.26 ± 12.52	49.29 ± 13.71**	52.03 ± 12.88**
GSH (nmoles/ml of blood)	211.03 ± 12.41	139.45 ± 15.19**	210.57 ± 17.23	138.15 ± 19.39**	210.64 ± 16.56	141.79 ± 17.74^{a**}	136.94 ± 19.36^{b**}
GPX (U/gm.Hb)	18.62 ± 1.86	12.84 ± 1.23^{a**}	18.67 ± 1.71	12.23 ± 1.41^{b**}	18.66 ± 1.74	12.19 ± 1.33**	12.32 ± 1.44**
GR (U/gm.Hb)	9.98 ± 0.74	5.03 ± 0.84^{a**}	10.07 ± 0.77	4.67 ± 1.03^{b**}	10.06 ± 0.76	5.01 ± 1.04^{a**}	4.58 ± 0.98^{b**}
GST (nmol/min/gm.Hb)	60.54 ± 7.33	27.72 ± 5.19**	60.82 ± 7.80	28.72 ± 5.33**	60.78 ± 7.73	28.79 ± 5.19**	28.57 ± 5.38**
Lipid Peroxidation (nmoles/ml of serum)	4.25 ± 0.50	8.28 ± 1.28**	4.41 ± 0.55	7.99 ± 1.34**	4.39 ± 0.54	7.96 ± 1.29**	8.03 ± 1.36**

Values are mean ± SD

**p<0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table : 6

Values of albumin, uric acid, bilirubin and ceruloplasmin in normal and in AMI patients.

Parameters	Groups	
	Normal (n=100)	AMI (n=300)
Albumin (gm/dl)	4.05 ± 0.88	3.39 ± 1.08**
Uric acid (mg/dl)	4.52 ± 1.07	6.19 ± 0.28**
Bilirubin (mg/dl)	0.62 ± 0.19	0.62 ± 0.27
Ceruloplasmin (mg/dl)	40.30 ± 7.26	17.52 ± 4.13**

Values are mean ± SD

**p<0.01

Table : 6 (a)

Values of albumin, Uric acid, bilirubin and ceruloplasmin in normal and in AMI patients according to the age and sex.

Parameters	Age (years)						Sex			
	≤ 40		40 - 60		≥ 60		Male		Female	
	Normal (n=22)	AMI (n=27)	Normal (n=54)	AMI (n=160)	Normal (n=24)	AMI (n=113)	Normal (n=85)	AMI (n=261)	Normal (n=15)	AMI (n=39)
Albumin (gm/dl)	4.02±0.70	3.04±0.92^{bc}**	4.05±0.89	3.55±1.14^{a}**	4.09±1.00	3.25±0.98^{b}**	4.02±0.87	3.42±1.08**	4.21±0.92	3.18±1.06**
Uric acid (mg/dl)	5.04±1.20	6.23±0.81^{b}**	4.36±0.95	5.98±1.02^{cb}**	4.38±1.05	6.46±0.64^{ab}**	4.56±1.09	6.16±0.93**	4.30±0.91	6.34±0.67**
Bilirubin (mg/dl)	0.61±0.19	0.66±0.43	0.59±0.18	0.62±0.26	0.68±0.19	0.61±0.23	0.61±0.18	0.62±0.28	0.68±0.19	0.65±0.21
Ceruloplas-min (mg/dl)	42.63±9.25	16.21± 3.35^{c}**	39.13±5.52	17.63±4.09^{ab}**	40.81±8.03	17.69±4.30^{a}**	39.96±6.88	17.41±4.08**	42.23±8.89	18.31±4.37**

Values are mean ± SD

**p<0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table: 6 (b)

Values of albumin, Uric acid, bilirubin and ceruloplasmin in normal and in AMI patients according to the time of onset of chest pain.

Parameters	Time of onset of chest pain				
	Normal (n=100)	AMI			
		12midnight to 6am(n=50)	6am to 12noon (n=94)	12noon to 6pm (n=90)	6pm to 12 midnight (n=66)
Albumin(gm/dl)	4.05 ± 0.88	$3.11 \pm 0.99^{bdc**}$	$3.69 \pm 1.24^{a*}$	$3.35 \pm 0.85^{bc**}$	$3.22 \pm 1.07^{c**}$
Uric acid(mg/dl)	4.52 ± 1.07	$6.32 \pm 0.90^{b**}$	$5.98 \pm 1.01^{cd**}$	$6.13 \pm 0.95^{bc**}$	$6.46 \pm 0.57^{ab**}$
Bilirubin(mg/dl)	0.62 ± 0.19	0.65 ± 0.37^{abcd}	0.66 ± 0.26^{ac}	0.57 ± 0.23^{cd}	0.62 ± 0.21^{c}
Ceruloplasmin(mg/dl)	40.30 ± 7.26	$16.77 \pm 3.90^{cbd**}$	$17.77 \pm 4.27^{abc**}$	$17.09 \pm 3.69^{ca**}$	$18.33 \pm 4.48^{a**}$

Values are mean ± SD

**p < 0.01, *p<0.05

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table : 6 (c)

Values of albumin, Uric acid, bilirubin and ceruloplasmin in normal and in AMI patients according to the history of diabetes and hypertension.

Parameters	Normal (n=100)	AMI			
		Diabetes		Hypertenasion	
		Diabetic (n=101)	Non Diabetic (n=199)	Hypertenasion (n=62)	NonHypertenasion (n=238)
Albumin (gm/dl)	4.05 ± 0.88	3.32 ± 1.12**	3.43 ± 1.05**	3.70 ± 1.17[a]**	3.31 ± 1.03[b]**
Uric acid (mg/dl)	4.52 ± 1.07	6.05 ± 0.94**	6.25 ± 0.88**	6.04 ± 1.09**	6.22 ± 0.85**
Bilirubin (mg/dl)	0.62 ± 0.19	0.68 ± 0.32	0.59 ± 0.23	0.61 ± 0.25	0.62 ± 0.28
Ceruloplasmin (mg/dl)	40.30 ± 7.26	17.42 ± 4.37**	17.58 ± 4.01**	17.27 ± 3.73**	17.59 ± 4.23**

Values are mean ± SD

**p < 0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table :6 (d)
Values of albumin, Uric acid, bilirubin and ceruloplasmin in normal and in AMI patients according to the values of cholesterol.

Parameters	Cholestrol (mg/dl)		
	Normal (n=100)	AMI	
		> 200 (n=105)	< 200 (n=195)
Albumin (gm/dl)	4.05 ± 0.88	3.36 ± 1.13**	3.40 ± 1.05**
Uric acid (mg/dl)	4.52 ± 1.07	6.07 ± 0.93**	6.24 ± 0.89**
Bilirubin (mg/dl)	0.62 ± 0.19	0.68 ± 0.32[a]	0.59 ± 0.23[b]
Ceruloplasmin (mg/dl)	40.30 ± 7.26	17.46 ± 4.31**	17.56 ± 4.04**

Values are mean ± SD
**p < 0.01
Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table : 6 (e)

Values of albumin, Uric acid, bilirubin and ceruloplasmin in normal and in AMI patients according to the habits of smoking and alcohol intake.

Parameters	Smoking				Alcohol intake			
	Yes		No		Yes		No	
	Normal (n=62)	AMI (n=222)	Normal (n=38)	AMI (n=78)	Normal (n=20)	AMI (n=160)	Normal (n=80)	AMI (n=140)
Albumin (gm/dl)	4.01 ± 0.80	3.41 ± 1.09	4.12 ± 0.99	3.34 ± 1.06	3.99 ± 0.68	3.53 ± 1.17	4.07 ± 0.93	3.22 ± 0.93
Uric acid (mg/dl)	4.62 ± 1.14	6.08 ± 0.98	4.35 ± 0.93	6.49 ± 0.54	4.93 ± 1.20	6.08 ± 1.00	4.41 ± 1.01	6.31 ± 0.77
Bilirubin (mg/dl)	0.60 ± 0.18	0.62 ± 0.29	0.65 ± 0.19	0.62 ± 0.22	0.61 ± 0.20	0.64 ± 0.30	0.62 ± 0.19	0.59 ± 0.23
Ceruloplasmin (mg/dl)	40.36 ± 7.34	17.23 ± 3.98^{b**}	40.20 ± 7.12	18.36 ± 4.42^{a**}	42.95 ± 9.50	17.49 ± 4.09**	39.64 ± 6.41	17.55 ± 4.19**

Values are mean ± SD

**p<0.01, *p<0.05

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table : 6 (f)

Values of albumin, Uric acid, bilirubin and ceruloplasmin in normal and in AMI patients according to the food habits and a family history of AMI.

Parameters	Food Habits				Family history		
	Vegetarians		Non Vegetarians		Normal (n=100)	AMI	
	Normal (n=16)	AMI (n=27)	Normal (n=84)	AMI (n=273)		+ve (n=82)	-ve (n=218)
Albumin (gm/dl)	4.20 ± 0.93	3.32 ± 1.04**	4.03 ± 0.87	3.39 ± 1.08**	4.05 ± 0.88	3.59 ± 1.15**	3.31 ± 1.04**
Uric acid (mg/dl)	4.35 ± 0.91	6.26 ± 0.77**	4.55 ± 1.10	6.18 ± 0.92**	4.52 ± 1.07	5.91 ± 1.04b**	6.29 ± 0.83[a]**
Bilirubin (mg/dl)	0.67 ± 0.19	0.66 ± 0.22	0.61 ± 0.18	0.62 ± 0.28	0.62 ± 0.19	0.65 ± 0.27	0.61 ± 0.27
Ceruloplasmin (mg/dl)	42.28 ± 8.62	18.35 ± 4.49**	39.93 ± 6.91	17.44 ± 4.09**	40.30 ± 7.26	17.65 ± 4.19**	17.48 ± 4.11**

Values are mean ± SD

**p < 0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table: 7

Comparison of the markers of Oxidative stress in AMI patients in different risk groups.

	Groups	Parameters						
		SOD (U/gm.Hb)	CAT (K/gm.Hb)	GSH (nmol/ml)	GPx (U/l of haemolysate)	GR (nmoles of NADPH consumed/min/gm.Hb)	GST (nmoles of CDNB-GSH formed/min/gm.Hb)	Lipid-peroxidation products (nmoles/ml of Serum)
1	Normal (n=100)	1414.99 ± 211.78	124.26 ±12.52	210.64 ±16.56	18.66± 1.74	10.06 ±0.76	60.78± 7.73	4.39± 0.54
2	AMI (n=300)	753.27 ± 209.73**	51.28±14.89**	138.27± 19.05**	12.29 ±1.41**	4.70 ± 1.02**	28.63 ±5.33**	8.01± 1.34**
3	Age ≤ 40 years (n=22)	942.07 ± 78.01	57.31 ± 14.56	135.53 ± 21.56	11.81 ± 1.40*	3.89 ± 0.54*	27.72 ± 4.49	7.82 ± 1.39
4	Age 40-60 years (n=160)	628.44 ± 199.23	50.71 ± 13.37	140.11 ± 18.43	12.17 ± 1.38	4.63 ± 0.98	28.67 ± 5.53	7.92 ± 1.33
5	Age ≥ 60 years (n=113)	884.89 ± 105.93	51.22 ± 13.45	136.33 ± 19.03	12.57 ± 1.40	4.99 ± 1.03	28.79 ± 5.21	8.19 ± 1.31
6	Male (n=261)	726.45 ± 222.82	51.22 ± 13.45	138.72 ± 19.23	12.22 ± 1.41	4.62 ± 1.01	28.42 ± 5.29	7.97 ± 1.34
7	Female (n=39)	878.71 ± 72.11	51.69 ± 11.18	135.28 ± 17.46*	12.76 ± 1.30	5.24 ± 0.88	30.40 ± 5.36	8.28 ± 1.30*
8	Time of onset of chest pain from 12 midnight to 6 am (n=50)	929.16 ± 68.49	53.51 ± 15.55	137.41 ± 22.62	12.02 ± 1.50	3.98 ± 0.59	27.55 ± 5.28*	7.81 ± 1.41
9	Time of onset of chest pain from 6 am to 12noon (n=94)	684.53 ± 217.46	51.13 ± 13.13	140.05 ± 17.49	12.09 ± 1.33	4.57 ± 0.93	29.00 ± 5.31	7.83 ± 1.33
10	Time of onset of chest pain from 12 noon to 6pm (n=90)	744.39 ± 223.33	49.88 ± 12.56	138.90 ± 18.27	12.37 ± 1.41	4.99 ± 1.06	28.43 ± 5.33	8.16 ± 1.26
11	Time of onset of chest pain from 6pm to 12 midnight (n=66)	729.92 ± 173.77	51.71 ± 11.74	135.52 ± 18.92	12.65 ± 1.36	5.03 ± 1.01	29.20 ± 5.23	8.18 ± 1.34
12	AMI with diabetes (n=101)	651.46 ± 190.03	50.98 ± 12.52	140.14 ± 17.52	12.13 ± 1.43	4.08 ± 0.64	28.33 ± 5.57	7.93 ± 1.30

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13	AMI without diabetes (n=199)	802.42 ± 203.21	51.43 ± 13.49	137.32 ± 19.72	12.37 ± 1.39	5.02 ± 1.02	28.78 ± 5.19	8.05 ± 1.35
14	AMI with hypertension (n=62)	860.40 ± 98.55	50.26 ± 12.36	141.22 ± 15.32	12.12 ± 1.33	3.98 ± 0.60	28.78 ± 5.28	7.87 ± 1.43
15	AMI without hypertension (n=238)	730.83 ± 223.88	51.55 ± 13.34	137.50 ± 19.84	12.33 ± 1.43	4.89 ± 1.02	28.59 ± 5.34	8.05 ± 1.31
16	Cholesterol >200mg/dl (n=105)	836.47 ± 159.97	51.55 ± 13.34	138.69 ± 20.57	12.11 ± 1.43	4.12 ± 0.72	28.45 ± 5.56	7.85 ± 1.38
17	Cholesterol<200 mg /dl (n=195)	710.49 ± 220.26	50.26 ± 12.36	138.05 ± 18.19	12.38 ± 1.39	5.02 ± 1.02	28.73 ± 5.20	8.09 ± 1.30
18	AMI with smoking (n=222)	676.09 ± 194.62	50.58 ± 13.60	139.06 ± 19.21	12.18 ± 1.41	4.56 ± 0.99	28.48 ± 5.33	7.95 ± 1.34
19	AMI without smoking (n=78)	967.19 ± 34.33	51.28 ± 11.83	136.03 ± 18.41	12.59 ± 1.37	5.02 ± 1.00	29.07 ± 5.31	8.18 ± 1.33
20	AMI with alcohol intake (n=160)	624.73 ± 200.69*	51.57 ± 13.96	139.44 ± 19.05	12.09 ± 1.40	4.44 ± 0.93	28.44 ± 5.31	7.88 ± 1.36
21	AMI without alcohol intake (n=140)	895.42 ± 103.88	50.95 ± 12.21	136.94 ± 18.97	12.51 ± 1.39	5.00 ± 1.01	28.85 ± 5.35	8.16 ± 1.30
22	AMI with vegetarian food intake (n=27)	981.79 ± 55.17	50.86 ± 12.88	139.45 ± 15.19	12.84 ± 1.23	5.03 ± 0.84	27.72 ± 5.19	8.28 ± 1.28
23	AMI with nonvegetarian food intake (n=273)	726.48 ± 206.34	51.32 ± 13.19	138.15 ± 19.39	12.23 ± 1.41	4.67 ± 1.03	28.72 ± 5.33	7.99 ± 1.34
24	AMI with +ve family history (n=82)	660.71 ± 212.03	49.29 ± 13.71*	141.79 ± 17.74	12.19 ± 1.33	5.01 ± 1.04	28.79 ± 5.19	7.96 ± 1.29
25	AMI with -ve family history (n=218)	785.52 ± 200.54	52.03 ± 12.88	136.94 ± 19.36	12.32 ± 1.44	4.58 ± 0.98	28.57 ± 5.38	8.03 ± 1.36

Group 2 is compared with group 1

Groups 3,4,5.....are compared with group 2.

**$p < 0.01$, *$p < 0.05$

Table : 8

Comparison of antioxidant molecules in AMI patients in different risk groups

Groups		Parameters			
		Albumin (gm/dl)	Uric acid (mg/dl)	Bilirubin (mg/dl)	Ceruloplasmin (mg/dl)
1	Normal (n=100)	4.05 ± 0.88	4.52 ± 1.07	0.62 ± 0.19	40.30 ± 7.26
2	AMI (n=300)	3.39 ± 1.08	6.19 ± 0.28	0.62 ± 0.27	17.52 ± 4.13
3	Age ≤ 40 years (n=22)	3.04 ± 0.92*	6.23 ± 0.81	0.66 ± 0.43	16.21 ± 3.35*
4	Age 40-60 years (n=160)	3.55 ± 1.14	5.98 ± 1.02	0.62 ± 0.26	17.63 ± 4.09
5	Age ≥ 60 years (n=113)	3.25 ± 0.98	6.46 ± 0.64*	0.61 ± 0.23	17.69 ± 4.30
6	Male (n=261)	3.42 ± 1.08	6.16 ± 0.93	0.62 ± 0.28	17.41 ± 4.08
7	Female (n=39)	3.18 ± 1.06	6.34 ± 0.67	0.65 ± 0.21	18.31 ± 4.37
8	Time of onset of chest pain from 12 midnight to 6 am (n=50)	3.11 ± 0.99	6.32 ± 0.90	0.65 ± 0.37	16.77 ± 3.90
9	Time of onset of chest pain from 6 am to 12noon (n=94)	3.69 ± 1.24	5.98 ± 1.01	0.66 ± 0.26	17.77 ± 4.27
10	Time of onset of chest pain from 12 noon to 6pm (n=90)	3.35 ± 0.85	6.13 ± 0.95	0.57 ± 0.23	17.09 ± 3.69
11	Time of onset of chest pain from 6pm to 12 midnight (n=66)	3.22 ± 1.07	6.46 ± 0.57	0.62 ± 0.21	18.33 ± 4.48
12	AMI with diabetes (101)	3.32 ± 1.12	6.05 ± 0.94	0.68 ± 0.32	17.42 ± 4.37
13	AMI without diabetes (199)	3.43 ± 1.03	6.25 ± 0.88	0.59 ± 0.23	17.58 ± 4.01

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14	AMI with hypertension (n=62)	3.70 ± 1.17	6.04 ± 1.09	0.61 ± 0.25	17.27 ± 3.73
15	AMI without hypertension (n=238)	3.31 ± 1.03	6.22 ± 0.85	0.62 ± 0.28	17.59 ± 4.23
16	Cholesterol >200mg/dl (n=105)	3.36 ± 1.13	6.07 ± 0.93	0.68 ± 0.32	17.46 ± 4.31
17	Cholesterol<200 mg /d (n=195)	3.40 ± 1.05	6.24 ± 0.89	0.59 ± 0.23	17.56 ± 4.04
18	AMI with smoking (n=222)	3.41 ± 1.09	6.08 ± 0.98	0.62 ± 0.29	17.23 ± 3.98
19	AMI without smoking (n=78)	3.34 ± 1.06	6.49 ± 0.54	0.62 ± 0.22	18.36 ± 4.42
20	AMI with alcohol intake (n=160)	3.53 ± 1.17	6.08 ± 1.00	0.64 ± 0.30	17.49 ± 4.09
21	AMI without alcohol intake (n=140)	3.22 ± 0.93	6.31 ± 0.77	0.59 ± 0.23	17.55 ± 4.19
22	AMI with vegetarian food intake (n=27)	3.32 ± 1.04	6.26 ± 0.77	0.66 ± 0.22	18.35 ± 4.49
23	AMI with nonvegetarian food intake (n=273)	3.39 ± 1.08	6.18 ± 0.92	0.62 ± 0.28	17.44 ± 4.09
24	AMI with +ve family history (n=82)	3.59 ± 1.15	5.91 ± 1.04	0.65 ± 0.27	17.65 ± 4.19
25	AMI with -ve family history (n=218)	3.31 ± 1.04	6.29 ± 0.83	0.61 ± 0.27	17.48 ± 4.11

Group 2 is compared with group 1
Groups 3,4,5.....are compared with Group 2
**p < 0.01, *p < 0.05

EVALUATION OF THE ROLE OF MATRIX METALLOPROTEINASES AND OXIDATIVE STRESS IN THE PATHOGENESIS AND COURSE OF ACUTE MYOCARDIAL INFARCTION

THESIS SUBMITTED TO THE UNIVERSITY OF CALICUT FOR THE DEGREE OF

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CHAPTER 5

MICRONUTRIENTS AND TRACE ELEMENTS IN ACUTE MYOCARDIAL INFARCTION

5.0.Introduction

Prospective studies have demonstrated reduced risk of coronary artery disease in subjects with a greater intake of vitamin E - tocopherol (Rimm *et al*, 1993) and vitaminC-ascorbic acid (Enstrom *et al*, 1992), because these antioxidant vitamins inhibit oxidation of low density lipoprotein (LDL), a critical event in the pathogenesis of CAD. Investigators have speculated that these vitamins reduce coronary artery disease by limiting the development or progression of atherosclerotic lesions. Vitamin E is an essential micronutrient and is capable of removing the oxygen-derived free radicals by virtue of its antioxidant property. The endothelial dysfunction and the increased platelet aggregation are present as the key events of atherosclerosis and its complications. Vitamin E is found to prevent the endothelial dysfunction as well as the increased platelet aggregation by virtue of the formation of tocopherol radicals. Further, these tocopherol radicals are responsible for the prevention of LDL oxidation by means of removing the free radicals (Tom Saldeen *et al*, 1999).

Ascorbic acid is known to be the most effective water-soluble antioxidant in plasma (Frei *et al*, 1989). In addition to limiting the LDL oxidation, this vitamin may further oppose acute coronary events by direct effects on vascular cell function. Such effects include increased resistance to the cytotoxic effects of oxidized LDL, decreased production of reactive oxygen species and increased effective production of nitric oxide (Diaz *et al*, 1997). Ascorbic acid treatment acutely improves endothelial nitric oxide action in patients with coronary artery disease and decreased monocyte adhesiveness to endothelial cells in cigarette smokers. Ascorbic acid may also increase prostacyclin production and lower the blood pressure (Weber *et al*, 1996 and Beetens and Herman, 1983).

The role of essential micronutrient metals in lipid metabolism has gained particular attention recently. The metabolism of zinc (Zn) is significantly altered in patients with cardiovascular disease as evidenced by abnormally low plasma or serum concentrations of zinc. Zinc is an integral component of various metallo-enzymes including the matrix metalloproteinases (MMPs), and along with other metals, can activate a wide variety of enzymes. It would seem to follow logically that the syndrome of zinc deficiency is due to a decrease in the activity of essential zinc containing enzymes. Zinc functions to stabilize membranes, perhaps by decreasing lipid peroxidation of these structures (Sukavani *et al*,

1998). Zinc also inhibits lysyl oxidase *in vitro*. The salutary effect of zinc in wound healing may be related to the membrane stabilizing effect. Thus, cell damage would be decreased. Recent studies have shown a relationship between zinc status and the metabolism of cholesterol and lipoproteins (Subramanyam, 1998). However, studies on the role of zinc in AMI is scanty and hence the importance of the investigation of zinc in the serum of AMI patients.

Magnesium (Mg), another trace element is essential for the action of a variety of enzymes involved in cellular metabolism, especially in neurochemical transmission and muscular activity. It has been shown that magnesium depletion modifies coronary blood flow, blood clotting and atherogenesis (Sheehter *et al*, 1995). Several studies have reported low serum levels of magnesium after myocardial infarction and depleted magnesium contents in the infarcted / ischaemic cardiac tissue (Rasmossen and Aurup, 1986). It has also been postulated that this cellular loss of magnesium may be the mechanism in the development of arrhythmias following myocardial infarction (Teo, 1995).

There is a strong relationship between iron levels and cardiovascular disease. Experiments with animal models during the past decade have revealed a strong relationship between iron and lipid metabolism. Iron is supposed to be involved in the process of lipid peroxidation. Active iron molecules modify LDL to interact with oxidized LDL receptors found in the macrophages. Exposure of membrane lipids to oxygen radicals in the presence of iron salts stimulates the process of lipid peroxidation (Halliwell and Gutterdige, 1992). Atherosclerotic lesions have recently been shown to be rich in both iron and copper and were found to induce lipid peroxidation that was inhibited by iron ion chelator desferrioxamine (Smith *et al*, 1992). Transferrin accounts for about 90% of the serum iron binding capacity (TIBC), and is a major serum antioxidant and thus might have a protective role in the development of CAD.

Increased iron levels are associated with hypertension and excess risk of heart attack (Salonen *et al*, 1992). It has also been found recently that NIDDM patients had higher levels of plasma thiobarbituric acid (TBA) reactivity and lipid peroxides than normal individuals (Collier *et al*, 1992). Thus diabetes mellitus may, in part, result from oxidative stress catalyzed by transition metals (Wolf, 1987). Sheltering up to one-third of the total body iron, ferritin appears as the major iron storage protein. Because the concentration of ferritin in the

serum is directly proportional to the level of body iron store, its measurement has been shown to be the most valuable diagnostic aid in the assessment of the iron status of individuals. However, the usefulness and the clinical applicability of ferritin measurement is somewhat hampered by the fact that several pathological conditions including CAD may increase or lower its concentration.

A detailed study regarding the involvement of trace elements and the essential micronutrients in AMI is very rare. Hence, the present study was undertaken to evaluate the role of these antioxidant vitamins (vitamin E and vitamin C), which serves as essential micronutrients and the trace elements such as zinc, magnesium and iron along with TIBC, % transferrin saturation, ferritin and ceruloplasmin in AMI patients.

5.1.Materials and Methods

Vitamin E was estimated by the method of Baker and Frank. Vitamin C was estimated by the method of Donald, Zn was estimated by the method of Akita and Sumiko and Mg was done by using the method of Yong. Iron and TIBC in the serum were determined by the method outlined by Varley. %Transferrin saturation was measured by using the formula given by R.Gupta. Ferritin in the serum was done by using ELISA. The detailed procedures used for the assays are given in chapter2.

5.2.Results

Table 9 represents the values of micronutrients and trace elements in normal and in AMI patients. The values of vitamin E, vitamin C, Zn and Mg were found to be decreased significantly ($p<0.01$) in the serum of the AMI patients as compared to those of the normal controls.

Table 9 (a) depicts the values of micronutrients and trace elements in normal and in AMI patients according to the age and sex. Irrespective of the age and sex the values of all these parameters were significantly decreased ($p<0.01$) in all age groups when compared to that of their respective controls. Among the different age groups studied the value of vitamin C was found to be significantly decreased in AMI patients with age $\leq$40 years when compared to the other two groups and the value of Mg was found to be significantly decreased in AMI patients in the age group 40-60 years, when compared to that of the other two groups. Vitamin E and Zn did not show any significant variation among the groups. The values of vitamin C and Mg were found to be significantly decreased in AMI males when

compared to that of AMI females. However, the values of vitamin E and Zn did not show significant changes between the groups.

Table 9 (b) represents the values of micronutrients and trace elements in normal and in AMI patients according to the time of onset of chest pain. The values of vitamin E, vitamin C, Zn and Mg were found to be significantly decreased ($p<0.01$) in all the AMI patients with different time of onset of chest pain. The values of vitamin E and vitamin C were found to be significantly decreased in AMI patients with onset of chest pain from 12 midnight to 6 am when compared to the other three groups. The value of Mg was found to be significantly decreased in AMI patients with the onset of chest pain from 6 am to 12 noon when compared to the other three groups. However, the value of Zn did not show any significant variation among the groups with different time of onset of chest pain.

Table 9 (c) represents the values of micronutrients and trace elements in normal and in AMI patients with the history of diabetes and hypertension. The values of these micronutrients and trace elements were found to be decreased significantly ($p<0.01$) in AMI patients both with and without the history of diabetes and hypertension when compared to that of the normal healthy individuals. A comparison of AMI patients with and without the history of diabetes have shown the significantly decreased values of vitamin E, vitamin C and Mg in AMI patients with the history of diabetes when compared to AMI patients without the history of diabetes. However, the values of Zn did not show any significant difference between these two groups. A comparison between the AMI patients with and without the history of hypertension have shown significantly decreased values of Zn and Mg in AMI patients with the history of hypertension when compared to that of the AMI patients without the history of hypertension. Vitamin E and vitamin C did not show significant variation between these groups.

Table 9 (d) represents the values of micronutrients and trace elements in normal and in AMI patients according to the values of cholesterol. The values of vitamin E, vitamin C, Zn and Mg were found to be significantly decreased ($p<0.01$) in AMI patients with cholesterol value >200 mg/dl and <200 mg/dl. A comparison between the values of these micronutrients and trace elements in AMI patients with cholesterol value >200 mg/dl and <200 mg/dl have shown significantly decreased values of vitamin E, Zn and Mg in AMI patients with cholesterol value >200 mg/dl when compared to AMI patients with cholesterol

value <200 mg/dl. The value of vitamin C did not show any statistical significance between these two groups.

Table 9 (e) represents the values of micronutrients and trace elements in normal and in AMI patients according to the habit of smoking and alcohol intake. The values of vitamin E, vitamin C, Zn and Mg were found to be significantly decreased ($p<0.01$) in AMI patients both with and without the habits of smoking and alcohol intake when compared to their respective controls. A comparison of AMI patients with and without the habit of smoking have shown significantly decreased values of vitamin C and Mg in AMI patients with the habit of smoking when compared to that of the AMI patients without the habit of smoking. Vitamin E and Zn did not show any statistical significance between the groups. A comparison between the AMI patients with and without the habit of alcohol intake have shown significantly decreased value of Mg in AMI patients with the habit of alcohol intake when compared to AMI patients without the habit of alcohol intake. The values of vitamin E, vitamin C and Zn did not show any statistical significane between these two groups.

Table 9 (f) represents the values of micronutrients and trace elements in normal and in AMI patients according to the food habits and family history of AMI. The values of all these micronutrients and trace elements were found to be significantly decreased ($p<0.01$) in AMI patients with the habit of both vegetarian and nonvegetarian food intake and both with and without the family history of AMI. A comparison between the AMI patients with vegetarian and nonvegetarian food intake have shown significantly decreased values of vitamin E and Mg in AMI patients with the habit of nonvegetarian food intake when compared to that of the AMI patients with the habit of vegetarian food intake. The values of vitamin C and Zn did not show any statistical significance between these groups. A comparison between the AMI patients with and without the family history of AMI have shown the significantly decreased value of vitamin E in AMI patients without the family history of AMI when compared to AMI patients with the family history of AMI. The values of vitamin C, Zn and Mg were not found to be significantly different between these groups.

Table 10 represents the values of iron, TIBC, %transferrin saturation and ferritin in normal and in AMI patients. The values of iron, %transferrin saturation and ferritin were found to be significantly increased ($p<0.01$) in AMI patients when compared to that of the

normal controls. However, the value of TIBC was found to be significantly decreased ($p<0.01$) in AMI patients when compared to that of the normal controls.

Table 10 (a) represents the values of iron, TIBC, %transferrin saturation and ferritin in normal and in AMI patients according to the age and sex. The values of iron, %transferrin saturation and ferritin were found to be significantly elevated ($p<0.01$) in AMI patients with different age groups and sex when compared to that of the normal controls. The value of TIBC was found to be significantly decreased ($p<0.01$) in AMI patients with different age groups and sex when compared to that of the normal controls.

Table 10 (b) represents the values of iron, TIBC, %transferrin saturation and ferritin in normal and in AMI patients according to the time of onset of chest pain. The values of iron, %transferrin saturation and ferritin were found to be significantly increased ($p<0.01$) in AMI patients with different time of onset of chest pain when compared to that of the normal controls. The value of TIBC was found to be significantly decreased ($p<0.01$) in AMI patients with different time onset of chest pain when compared to that of the normal controls. A comparison between AMI patients with different time of onset of chest pain have shown significantly increased value of ferritin in AMI patients with time of onset of chest pain from 12 midnight to 6 am when compared to the other three groups. The values of iron, TIBC and %transferrin saturation did not show statistically significant difference between the groups.

Table 10 (c) represents the values of iron, TIBC, %transferrin saturation and ferritin in normal and in AMI patients both with and without the history of diabetes and hypertension. The values of iron, %transferrin saturation and ferritin were found to be significantly increased ($p<0.01$) in AMI patients with and without the history of diabetes and hypertension when compared to that of the normal controls. The value of TIBC was found to be significantly decreased ($p<0.01$) in AMI patients both with and without the history of diabetics and hypertension when compared to that of the normal controls.

Table 10 (d) represents the values of iron, TIBC, %transferrin saturation and ferritin in normal and in AMI patients according to the values of cholesterol. The values of iron, %transferrin saturation and ferritin were found to be significantly increased ($p<0.01$) in AMI patients with cholesterol values >200 mg/dl and <200 mg/dl when compared to that of the normal controls. The value of TIBC was found to be significantly decreased ($p<0.01$) in AMI

patients with cholesterol values >200 mg/dl and <200 mg/dl when compared to that of the normal controls.

Table 10 (e) represents the values of iron, TIBC, %transferrin saturation and ferritin in normal and in AMI patients according to the habit of smoking and alcohol intake. The values of iron, %transferrin saturation and ferritin were found to be significantly increased ($p<0.01$) in AMI patients with and without the habits of smoking and alcohol intake when compared to that of the normal controls. However, the value of TIBC was found to be significantly decreased ($p<0.01$) in AMI patients with and without the habits of smoking and alcohol intake when compared to that of the normal controls.

Table 10 (f) represents the values of iron, TIBC, %transferrin saturation and ferritin in normal and in AMI patients according to the food habits and family history of AMI. The values of iron, %transferrin saturation and ferritin were found to be significantly increased ($p<0.01$) in AMI patients with the habits of vegetarian and nonvegetarian food intake and both with and without the family history of AMI when compared to that of the normal controls. The value of TIBC was found to be significantly decreased ($p<0.01$) in AMI patients with the habits of vegetarian and nonvegetarian food intake and with and without the family history of AMI when compared to that of the normal controls. The comparison between the AMI patients with and without the family history of AMI have shown significant value of %transferrin saturation in AMI patients without the family history of AMI when compared to that of the AMI patients with the family history of AMI. However, the other parameters did not show significant alteration between the groups.

Table 11 represents the values of vitamin E, vitamin C, Zn, Mg, iron, TIBC, %transferrin saturation and ferritin in normal, AMI patients and in AMI patients with various risk groups taken for the study. Among the various risk groups studied the value of vitamin E was found to be significantly decreased in patients with cholesterol value >200mg/dl. The values of vitamin C and TIBC were found to be significantly decreased in AMI patients with age $\leq$ 40 years. The value of Zn was significantly decreased in AMI patients with the history of hypertension and the value of Mg was found to be significantly decreased in AMI patients with age ranges from 40-60 years. The values of iron and %transferrin saturation were found to be significantly increased in AMI patients with a positive family history of AMI and the

value of ferritin was found to be significantly increased in AMI patients with the time of onset of chest pain from 12 noon to 6 pm.

5.3.Discussion

The role of micronutrients and trace elements in the pathogenesis of CAD have recently been reported, hence studies related to this aspect are very rare. However, the significant role of antioxidant vitamins such as vitamin E and vitamin C, the two very important micronutrients in the pathogenesis of AMI have been described. Vitamin E which provides protection against endothelial injury have been associated with atherosclerosis by preserving endothelium derived nitric oxide (NO) activity (Keaney *et al*, 1996). Being a chain breaking antioxidant, vitamin E inhibits or delays arterial thrombogenesis (Mehta *et al*, 1999). Vitamin C, a water soluble antioxidant vitamin, have been reported to improve the endothelial function, thus reducing the risk of CAD. The antioxidant activity of vitamin C is not restricted to extracellular fluids. It is actively transported into cells and may play a role in the regulation of intracellular redox state and antioxidant defenses (Meister, 1994), possibly via regulation of intracellular thiol species such as glutathione.

In the present study the values of vitamin E and vitamin C were found to be decreased in AMI patients when compared to those of the normal healthy individuals. Irrespective of the risk factors the values of these vitamins were found to be decreased in all the AMI patients either due to a low supplementation or due to an impaired metabolism. Further, the value of vitamin C was found to be less in AMI patients with age $\leq$40 years when compared to other age groups. In AMI males the value of vitamin C was found to be less than that in AMI females. According to the time of onset of chest pain the values of vitamin E and vitamin C were found to be less in patients with time of onset of chest pain from 12 midnight to 6 am when compared to that of other groups. The values of vitamin E and vitamin C were found to be less in AMI patients with the history of diabetes and hypertension, indicating that the values of these vitamins may be less in diabetic and hypertensive patients. Further, the value of vitamin E was found to be less in AMI patients with cholesterol value >200 mg/dl, in patients with nonvegetarian food intake and in patients without the family history of AMI. Vitamin C was found to be less in patients with the habit of smoking. All these results together indicate the impairment of the endothelial function in these AMI patients due to a reduction in the amount of these antioxidant vitamins in the body.

Zn and Mg, the two essential trace elements required for the normal functioning of the body is thought to counteract the oxidative injury induced by free radicals or ROS (Simmi and Veena, 2000). Zn is an integral component of various metallo-enzymes and along with other metals can activate a wide variety of enzymes. Further, Zn functions to stabilize the membranes, perhaps by decreasing the lipid peroxidation of these structures. The salutary effect of Zn in wound healing may be related to the membrane stabilizing effect. Thus, cell damage would be decreased (Sukavani *et al*, 1998). Hypomagnesaemia has been reported in patients with coronary artery disease (Mohan and Jain, 1994). Magnesium is an obligatory cofactor in the enzyme reactions of GSH synthesis and in all biosynthetic enzyme reactions involving ATP and Mg deficiency has been reported to inhibit biosynthesis of GSH (Mills *et al*, 1986). Furthermore, Mg deficiency also leads to reduced levels of endogenous antioxidant defenses (vitamin E and vitamin C), which may limit free radical detoxification (Mills *et al*, 1986).

In the present study we have observed decrease in serum Zn and Mg values in all the AMI patients when compared to those of the normal controls. The value of Mg was found to be less in AMI patients in the age group 40-60 years and in AMI males when compared to those of the AMI females. The value was found to be significantly lower in patients with the onset of chest pain from 6 am to 12 noon, in patients with the history of diabetes and hypertension, patients with a cholesterol value >200 mg/dl and in patients with the habit of nonvegetarian food intake. The value of Zn was found to be less in patients with history of hypertension and cholesterol value >200 mg/dl. These results indicate that the values of Zn and Mg are decreased in all AMI patients and it could potentiate the oxidative injury to myocardium by free radicals.

The presence of iron in cytochromes, catalase, hydroxylase, peroxidases, saturases, lipoxygenases and cyclooxygenases suggest that iron has an important role in various metabolic events related to lipids, such as the oxidative degradation of fatty acids and the synthesis of unsaturated fatty acids, plasminogens and prostaglandins. However, studies in this area are limited. Oxidation of LDL-cholesterol is catalyzed by iron present in atherosclerotic gruel (Gupta *et al*, 2000). Serum deficient in iron has minimal oxidative capacity that increases with iron repletion. Several studies have been conducted in developed countries to assess the association of iron with coronary heart disease or AMI (Sharma *et al*,

2000). Iron besides promoting lipid peroxidation could increase the risk of AMI through the elevation of blood haematocrit and blood hemoglobin levels. This in turn increases the viscosity of blood and has a direct thrombogenic effect (Sharma *et al*, 2000). Ferritin is the storage form of iron and high levels of ferritin are always associated with CAD (Salonen *et al*, 1992). The concentration of ferritin in the serum is directly proportional to the levels of body iron stores. The body iron status is in turn related to the concentration of the ferritin, its storage form and transferrin, the transport form. The total iron binding capacity (TIBC) is also considered to be an indicator to know the iron status of the body.

The results of the present study have shown increased values of iron, %transferrin saturation and ferritin in AMI patients when compared to that of the normal healthy individuals. The TIBC value was found to be decreased. The value of ferritin was found to be more in patients with time of onset of chest pain from 12 noon to 6 pm and the value of % transferrin saturation was found to be more in AMI patients with a family history of AMI. However, all the other parameters did not show much alteration between the groups. So the present study indicate the increased levels of iron, %transferrin saturation and ferritin and decreased values of TIBC in AMI patients.

Among the various risk groups studied the value of vitamin E was found to be significantly decreased in patients with cholesterol value >200mg/dl. The values of vitamin C and TIBC were found to be significantly decreased in AMI patients with age $\leq$ 40 years and the value of Zn was significantly decreased in AMI patients with the history of hypertension. The value of Mg was found to be significantly decreased in AMI patients with age ranges from 40-60 years and the values of iron and %transferrin saturation were found to be significantly increased in AMI patients with a positive family history of AMI. The value of ferritin was found to be significantly increased in AMI patients with the time of onset of chest pain from 12 noon to 6 pm.

The results of the present study show decreased values of miicronutrients such as vitamin E, vitamin C, Zn and Mg and increased values of iron, %transferrin saturation, ferritin and decreased values of TIBC in all AMI patients as compared to that of the normal controls. Eventhough the values of these micronutrients and trace elements were found to be significantly different between various risk groups with AMI, a significant change in these parameters were seen in patients with cholesterol value >200 mg/dl, in patients with

hypertension, patients with a positive family history of AMI, in patients with age ≤ 40 years and in patients with time of onset of chest pain from 12 noon to 6pm. Hence, it may be concluded that the extent of AMI is more in patients belong to these risk groups as compared to all other risk groups studied due to a change in the levels of these micronutrients and trace elements.

Table: 9

Values of micronutrients and trace elements in normal and in AMI patients.

Parameters	Groups	
	Normal (n=100)	AMI (n=300)
Vitamin E (mg/dl)	5.94 ± 0.82	2.89 ± 0.75**
Vitamin C (mg/dl)	1.19 ± 0.22	0.24 ± 0.13**
Zn (mgm / dl)	88.82 ± 12.04	48.71 ± 8.71**
Mg. (mg/dl)	2.31 ± 0.30	0.73 ± 0.36**

Values are mean ±SD

**p<0.01

Table : 9 (a)

Values of micronutrients and trace elements in normal and in AMI patients according to the age and sex.

Parameters	Age (years)						Sex			
	≤ 40		40 - 60		≥ 60		Male		Female	
	Normal(n=22)	AMI(n=27)	Normal(n=54)	AMI(n=160)	Normal(n=24)	AMI(n=113)	Normal(n=85)	AMI(n=261)	Normal(n=15)	AMI(n=39)
Vitamin E (mg/dl)	5.80 ± 0.86	2.56 ± 0.11**	6.03 ± 0.80	2.98 ± 0.78**	5.86 ± 0.79	2.84 ± 0.55**	5.95 ± 0.82	2.89 ± 0.78**	5.88 ± 0.80	2.84 ± 0.56**
Vitamin C (mg/dl)	1.15 ± 0.24	0.19 ± 0.13^{bc}**	1.21 ± 0.22	0.23 ± 0.14^{b}**	1.19 ± 0.21	0.27 ± 0.12^{a}**	1.19 ± 0.23	0.23 ± 0.13^{b}**	1.20 ± 0.22	0.29 ± 0.12^{a}**
Zn (mgm / dl)	89.47±12.08	51.00 ± 8.42**	88.62±11.74	48.08±9.01**	88.69±12.64	49.04±8.31**	89.15±11.95	48.57±8.84**	87.00±12.36	49.64±8.01**
Mg. (mg/dl)	2.19 ± 0.27	0.72 ± 0.29^{b}**	2.36 ± 0.29	0.63 ± 0.31^{bc}**	2.29 ± 0.30	0.87 ± 0.39^{ba}**	2.30 ± 0.30	0.71±0.35^{b}**	2.36 ± 0.29	0.90±0.37^{a}**

Values are mean ±SD

**p<0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values

Table : 9 (b)

Values of micronutrients and trace elements in normal and in AMI patients according to the time of onset of chest pain.

Parameters	Time of onset of chest pain				
	Normal (n=100)	AMI			
		12midnight to 6am (n=50)	6am to 12noon (n=94)	12noon to 6pm (n=90)	6pm to 12 midnight (n=66)
Vitamin E (mg/dl)	5.94 ± 0.82	$2.51 \pm 0.88^{bcd**}$	$2.79 \pm 0.67^{bcd**}$	$3.25 \pm 0.75^{a**}$	$2.82 \pm 0.55^{cdb**}$
Vitamin C (mg/dl)	1.19 ± 0.22	$0.21 \pm 0.14^{cbd**}$	$0.24 \pm 0.16^{b**}$	$0.22 \pm 0.11^{bc**}$	$0.29 \pm 0.11^{a**}$
Zn (mgm / dl)	88.82 ± 12.04	49.13 ± 7.93**	47.66 ± 8.01**	48.60 ± 10.39**	50.03 ± 7.54**
Mg. (mg/dl)	2.31 ± 0.30	$0.67 \pm 0.26^{c**}$	$0.65 \pm 0.35^{cd**}$	$0.71 \pm 0.35^{bcd**}$	$0.92 \pm 0.38^{a**}$

Values are mean ±SD

**p<0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table : 9 (c)

Values of micronutrients and trace elements in normal and in AMI patients with the history of diabetes and hypertension.

Parameters	Normal (n=100)	AMI			
		Diabetes		Hypertension	
		Diabetic (n=101)	Nondiabetic (n=199)	Hypertensive (n=62)	Non Hypertensive (n=238)
Vitamin E (mg/dl)	5.94 ± 0.82	2.51 ± 0.74^{b**}	3.08 ± 0.69^{a**}	2.62 ± 0.56**	2.95 ± 0.78**
Vitamin C (mg/dl)	1.19 ± 0.22	0.21 ± 0.15^{b**}	0.25 ± 0.12^{a**}	0.22 ± 0.16**	0.24 ± 0.13**
Zn (mgm / dl)	88.82 ± 12.04	47.65 ± 8.24**	49.24 ± 8.88**	46.55 ± 8.48^{b**}	49.27 ± 8.71^{a**}
Mg. (mg/dl)	2.31 ± 0.30	0.65 ± 0.28^{b**}	0.77 ± 0.38^{a**}	0.65 ± 0.32^{b**}	0.75 ± 0.37^{a**}

Values are mean ±SD

**p<0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table: 9 (d)

Values of micronutrients and trace elements in normal and in AMI patients according to the values of cholesterol.

Parameters	Cholesterol (mg/dl)		
	Normal (n=100)	AMI	
		>200 (n=105)	<200 (n=195)
Vitamin E (mg/dl)	5.94 ± 0.82	2.51 ± 0.72[b]**	3.09 ± 0.69[a]**
Vitamin C (mg/dl)	1.19 ± 0.22	0.21 ± 0.16**	0.25 ± 0.12**
Zn (mgm / dl)	88.82 ± 12.04	47.22 ± 8.36[b]**	49.51 ± 8.77[a]**
Mg. (mg/dl)	2.31 ± 0.30	0.64 ± 0.28[b]**	0.78 ± 0.38[a]**

Values are mean ±SD

**p<0.01

Inter group comparisons were done by one way ANOVA. Same alphabetd denote homogenous values and different alphabets denote heterogenous values.

Table : 9 (e)

Values of micronutrients and trace elements in normal and in AMI patients according to the habits of smoking and alcohol intake.

Parameters	Smoking				Alcohol intake			
	Yes		No		Yes		No	
	Normal (n=62)	AMI (n=222)	Normal (n=38)	AMI (n=78)	Normal (n=20)	AMI (n=160)	Normal (n=80)	AMI (n=140)
Vitamin E (mg/dl)	5.90 ± 0.81	2.91 ± 0.82**	6.00 ± 0.81	2.82 ± 0.54**	5.85 ± 0.89	2.99 ± 0.80**	5.96 ± 0.79	2.80 ± 0.69**
Vitamin C (mg/dl)	1.18 ± 0.23	0.22 ± 0.14[b]**	1.20 ± 0.21	0.28 ± 0.12[a]**	1.15 ± 0.25	0.22 ± 0.15**	1.20 ± 0.22	0.25 ± 0.11**
Zn (mgm / dl)	89.17 ± 11.40	48.42 ± 9.03**	88.26 ± 13.03	49.52 ± 7.81**	89.24 ± 12.64	48.70 ± 8.66**	88.71 ± 11.91	48.72 ± 8.81**
Mg. (mg/dl)	2.28 ± 0.29	0.68 ± 0.33[b]**	2.36 ± 0.30	0.88 ± 0.39[a]**	2.17 ± 0.27	0.66 ± 0.32[b]**	2.35 ± 0.29	0.81 ± 0.39[a]**

Values are mean ±SD

**p<0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table: 9 (f)

Values of micronutrients and trace elements in normal and in AMI patients according to the food habits and family history of AMI.

Parameters	Food habits				Family history		
	Vegetarians		Non Vegetarians		Normal (n=100)	AMI	
	Normal (n=16)	AMI (n=27)	Normal (n=84)	AMI (n=273)		+ ve (n=82)	- ve (n=218)
Vitamin E (mg/dl)	5.84 ± 0.78	3.88 ± 0.60[a]**	5.96 ± 0.82	2.79 ± 0.69[b]**	5.94 ± 0.82	3.36 ± 0.73[a]**	2.71 ± 0.69[b]**
Vitamin C (mg/dl)	1.22 ± 0.22	0.21 ± 0.11**	1.19 ± 0.23	0.24 ± 0.14**	1.19 ± 0.22	0.25 ± 0.15**	0.23 ± 0.13**
Zn (mgm / dl)	87.34 ± 12.04	46.32 ± 9.43**	89.11 ± 12.06	48.94 ± 8.60**	88.82 ± 12.04	47.85 ± 8.66**	49.03 ± 8.71**
Mg. (mg/dl)	2.39 ± 0.31	1.02 ± 0.32[a]**	2.29 ± 0.29	0.70 ± 0.35[b]**	2.31 ± 0.30	0.74 ± 0.35**	0.73 ± 0.36**

Values are mean±SD

**p<0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table: 10

Values of Iron, TIBC, % transferrin saturation and Ferritin in normal and in AMI patients.

Parameters	Groups	
	Normal (n=100)	AMI (n=300)
Iron (mgm / dl)	99.67 ± 13.51	154.96 ± 23.08**
TIBC (mgm / dl)	324.74 ± 15.84	256.21 ± 24.16**
%Transferrin saturation	30.68 ± 4.35	59.87 ± 4.00**
Ferritin (ngm / ml)	65.78 ± 12.78	131.33 ± 24.76**

Values are mean ±SD

**p<0.01

Table : 10 (a)

Values of micronutrients and trace elements in normal and in AMI patients according to the age and sex.

Parameters	Age (years)						Sex			
	≤ 40		40 - 60		≥ 60		Male		Female	
	Normal(n=22)	AMI(n=27)	Normal(n=54)	AMI(n=160)	Normal(n=24)	AMI(n=113)	Normal(n=85)	AMI(n=261)	Normal(n=15)	AMI(n=39)
Iron (mgm / dl)	99.16±11.34	151.57± 24.57**	100.71±13.62	155.83 ± 23.17**	97.80±14.79	154.52 ± 22.45**	100.07±12.93	155.67 ± 23.23**	97.39±16.19	150.20 ± 21.34**
TIBC (mgm / dl)	315.72±15.91	251.56 ± 24.57**	329.52±14.96	257.32 ± 24.10**	322.25±13.30	255.74 ± 24.01**	325.19±16.03	256.74 ± 24.22**	322.20±14.42	252.64 ± 23.45**
%Transferrin saturation	31.12 ± 3.46	59.85 ± 3.92**	30.58 ± 4.47	60.37 ± 4.16**	30.51 ± 4.76	60.22 ± 3.78**	30.62 ± 4.35	60.36 ± 4.09**	31.05 ± 4.33	59.62 ± 3.30**
Ferritin (ngm / ml)	67.55 ± 8.85	128.98 ± 40.17**	69.81±10.44	131.89 ± 22.98**	55.08±14.48	131.09 ± 22.19**	67.88±11.74	131.76 ± 25.37**	53.86±11.87	128.42 ± 20.01**

Values are mean±SD

**p<0.01

Table : 10 (b)

Values of Iron, TIBC, % transferrin saturation and Ferritin in normal and in AMI patients according to the time of onset of chest pain.

Parameters	Time of onset chest of chest pain				
	Normal (n=100)	AMI			
		12midnight to 6am (n=50)	6am to 12noon (n=94)	12noon to 6pm (n=90)	6pm to 12 midnight (n=66)
Iron (mgm / dl)	99.67 ± 13.51	154.84 ± 26.36**	156.11 ± 22.52**	154.28 ± 22.11**	154.31 ± 22.40**
TIBC (mgm / dl)	324.74 ± 15.84	254.54 ± 26.66**	258.28 ± 24.04**	256.24 ± 23.01**	254.47 ± 23.68**
%Transferrin saturation	30.68 ± 4.35	60.27 ± 4.28**	60.16 ± 4.33**	60.48 ± 3.83**	60.11 ± 3.48**
Ferritin (ngm / ml)	65.78 ± 12.78	$128.33 \pm 35.52^{d**}$	$129.82 \pm 21.94^{bd**}$	$136.29 \pm 22.00^{ad**}$	$129.73 \pm 20.88^{abcd**}$

Values are mean ±SD

**p<0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table : 10 (c)

Value of Iron, TIBC, % transferrin saturation and Ferritin in normal and in AMI patients with the history of diabetes and hypertension.

Parameters	Normal (n=100)	AMI			
		Diabetes		Hypertension	
		Diabetic (n=101)	Nondiabetic (n=199)	Hypertensive (n=62)	Non Hypertensive (n=238)
Iron (mgm / dl)	99.67 ± 13.51	154.80 ± 24.47**	155.03 ± 22.33**	155.19 ± 25.07**	154.89 ± 22.51**
TIBC (mgm / dl)	324.74 ± 15.84	255.92 ± 23.06**	256.78 ± 26.17**	255.89 ± 24.43**	256.29 ± 24.10**
%Transferrin saturation	30.68 ± 4.35	59.88 ± 4.57**	60.46 ± 3.67**	60.06 ± 3.87**	60.32 ± 4.04**
Ferritin (ngm / ml)	65.78 ± 12.78	127.25 ± 29.42**	133.39 ± 21.73**	128.76 ± 23.15**	132.00 ± 25.11**

Values are mean ±SD

**p<0.01

Table: 10 (d)

Values of Iron, TIBC, % transferrin saturation and Ferritin in normal and in AMI patients according to the values of cholesterol.

Parameters	Cholesterol (mg/dl)		
	Normal (n=100)	AMI	
		>200 (n=105)	<200 (n=195)
Iron (mgm / dl)	99.67 ± 13.51	155.02 ± 24.89**	154.92 ± 22.02**
TIBC (mgm / dl)	324.74 ± 15.84	257.38 ± 25.98**	255.57 ± 23.11**
%Transferrin saturation	30.68 ± 4.35	60.01 ± 4.54**	60.41 ± 3.68**
Ferritin (ngm / ml)	65.78 ± 12.78	127.58 ± 29.10**	133.34 ± 21.79**

Values are mean ±SD

**p<0.01

Table : 10(e)

Value of Iron, TIBC, % transferrin saturation and Ferritin in normal and in AMI patients according to the habits of smoking and alcohol intake.

Parameters	Smoking				Alcohol intake			
	Yes		No		Yes		No	
	Normal(n=62)	AMI(n=222)	Normal(n=38)	AMI(n=78)	Normal(n=20)	AMI(n=160)	Normal(n=80)	AMI(n=140)
Iron (mgm / dl)	100.99 ± 11.78	155.58 ± 23.21**	97.51 ± 15.62	153.15 ± 22.53**	98.53 ± 11.53	156.18 ± 23.83**	99.95 ± 13.92	153.56 ± 22.09**
TIBC (mgm / dl)	324.95 ± 16.76	256.77 ± 24.26**	324.41 ± 14.17	254.61 ± 23.81**	315.83 ± 16.64	257.22 ± 24.47**	326.97 ± 14.79	255.06 ± 23.76**
%Transferrin saturation	31.01 ± 3.84	60.39 ± 4.10**	30.14 ± 5.03	59.91 ± 3.69**	30.98 ± 3.51	60.30 ± 4.20**	30.61 ± 4.53	60.22 ± 3.76**
Ferritin (ngm / ml)	68.40 ± 10.69	132.13 ± 25.90**	61.50 ± 14.59	129.06 ± 21.00**	66.80 ± 8.81	130.17 ± 26.62**	65.53 ± 13.56	132.65 ± 22.36**

Values are mean±SD

**p<0.01

Table : 10 (f)

Values of Iron, TIBC, % transferrin saturation and Ferritin in normal and in AMI patients according to rhe food habits and family history of AMI.

Parameters	Food habits				Family history		
	Vegetarians		Non Vegetarians		Normal (n=100)	AMI	
	Normal (n=16)	AMI (n=27)	Normal (n=84)	AMI (n=273)		+ ve (n=82)	- ve (n=218)
Iron (mgm / dl)	97.57 ± 15.68	152.68 ± 22.49**	100.07 ± 13.01	155.18 ± 23.11**	99.67 ± 13.51	156.88 ± 20.97**	154.23 ± 20.97**
TIBC (mgm / dl)	322.94 ± 14.24	254.36 ± 24.81**	325.09 ± 16.09	256.39 ± 24.08**	324.74 ± 15.84	257.04 ± 23.97**	255.89 ± 24.22**
%Transferrin saturation	30.38 ± 4.93	59.73 ± 3.32**	30.74 ± 4.22	60.32 ± 4.06**	30.68 ± 4.35	61.12 ± 3.69^{a}**	59.94 ± 4.07^{b}**
Ferritin (ngm / ml)	54.18 ± 11.53	131.46 ± 18.56**	67.99 ± 11.77	131.32 ± 25.27**	65.78 ± 12.78	134.58 ± 21.42**	130.11 ± 25.79**

Values are mean±SD

**p<0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table: 11

Comparison of trace elements and micronutrients in AMI patients in different risk groups.

Groups		Parameters							
		Vitamin E (mg/dl)	Vitamin C (mg/dl)	Zn(ugm/dl)	Mg (mg/dl)	Iron (ugm/dl)	TIBC (ugm/dl)	%Transferrin Saturation	Ferritin (ngm/dl)
1	Normal (n=100)	5.94 ± 0.82	1.19 ± 0.22	88.82 ± 12.04	2.31 ± 0.30	99.67 ± 13.51	324.74 ± 15.84	30.68 ± 4.35	65.78 ± 12.78
2	AMI (n=300)	2.89 ± 0.75**	0.24 ± 0.13**	48.71 ± 8.71**	0.73 ± 0.36**	154.96 ± 23.08**	256.21 ± 24.16**	59.87 ± 4.00**	131.33±24.76**
3	Age ≤40 years (n=22)	2.56 ± 0.11	0.19 ± 0.13*	51.00 ± 8.42	0.72 ± 0.29	151.57 ± 24.57	251.56 ± 24.57*	59.85 ± 3.92	128.98 ± 40.17
4	Age 40-60 years (n=160)	2.98 ± 0.79	0.23 ± 0.14	48.08 ± 9.03	0.63 ± 0.31*	155.83 ± 23.17	257.32 ± 24.10	60.37 ± 4.16	131.89 ± 22.98
5	Age ≥ 60 years (n=113)	2.84 ± 0.55	0.27 ± 0.12	49.04 ± 8.31	0.87 ± 0.39	154.52 ± 22.45	255.74 ± 24.01	60.22 ± 3.78	131.09 ± 22.19
6	Male (n=261)	2.89 ± 0.78	0.23 ± 0.13	48.57 ± 8.84	0.71 ± 0.35	155.67 ± 23.23	256.74 ± 24.22	60.36 ± 4.09	131.76 ± 25.37
7	Female (n=39)	2.84 ± 0.56	0.29 ± 0.12	49.64 ± 8.01	0.90 ± 0.37	150.20 ± 21.34	252.64 ± 23.45	59.62 ± 3.30	128.42 ± 20.01
8	Time of onset of chest pain from 12 midnight to 6 am (n=50)	2.51 ± 0.88	0.21 ± 0.14	49.13 ± 7.93	0.67 ± 0.26	154.84 ± 26.36	254.54 ± 26.66	60.27 ± 4.28	128.33 ± 35.52
9	Time of onset of chest pain from 6 am to 12noon (n=94)	2.79 ± 0.67	0.24 ± 0.16	47.66 ± 8.01	0.65 ± 0.35	156.11 ± 22.52	258.28 ± 24.04	60.16 ± 4.33	129.82 ± 21.94
10	Time of onset of chest pain from 12 noon to 6pm (n=90)	3.25 ± 0.75	0.22 ± 0.11	48.60 ± 10.39	0.71 ± 0.35	154.28 ± 22.11	256.24 ± 23.01	60.48 ± 3.83	136.29±22.00*
11	Time of onset of chest pain from 6pm to 12 midnight (n=66)	2.82 ± 0.55	0.29 ± 0.11	50.03 ± 7.54	0.92 ± 0.38	154.31 ± 22.40	254.47 ± 23.68	60.11 ± 3.48	129.73 ± 20.88
12	AMI with diabetes (n=101)	2.51 ± 0.74	0.21 ± 0.15	47.65 ± 8.24	0.65 ± 0.28	154.80 ± 24.47	255.92 ± 23.06	59.88 ± 4.57	127.52 ± 29.42
13	AMI without diabetes (n=199)	3.08 ± 0.69	0.25 ± 0.12	49.24 ± 8.88	0.77 ± 0.38	155.03 ± 22.33	256.78 ± 26.17	60.46 ± 3.67	133.39 ± 21.73

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14	AMI with hypertension (n=62)	2.62 ± 0.56	0.22 ± 0.16	46.55 ± 8.48*	0.65 ± 0.32	155.19 ± 25.07	255.89 ± 24.43	60.06 ± 3.87	128.76 ± 23.15
15	AMI without hypertension (n=238)	2.95 ± 0.78	0.24 ± 0.13	49.27 ± 8.71	0.75 ± 0.37	154.89 ± 22.51	256.29 ± 24.10	60.32 ± 4.04	132.00 ± 25.11
16	Cholesterol >200mg/dl (n=105)	2.51 ± 0.72*	0.21 ± 0.16	47.22 ± 8.36	0.64 ± 0.28	155.02 ± 24.89	257.38 ± 25.98	60.01 ± 4.54	127.58 ± 29.10
17	Cholesterol<200 mg /dl (n=195)	3.09 ± 0.69	0.25 ± 0.12	49.51 ± 8.77	0.78 ± 0.38	154.92 ± 22.02	255.57 ± 23.11	60.41 ± 3.68	133.34 ± 21.79
18	AMI with smoking (n=222)	2.91 ± 0.82	0.22 ± 0.14	48.42 ± 9.03	0.68 ± 0.33	155.58 ± 23.21	256.77 ± 24.26	60.39 ± 4.10	132.13 ± 25.90
19	AMI without smoking (n=78)	2.82 ± 0.54	0.28 ± 0.12	49.52 ± 7.81	0.88 ± 0.39	153.15 ± 22.53	254.61 ± 23.81	59.91 ± 3.69	129.06 ± 21.00
20	AMI with alcohol intake (n=160)	2.99 ± 0.80	0.22 ± 0.15	48.70 ± 8.66	0.66 ± 0.32	156.18 ± 23.83	257.22 ± 24.47	60.30 ± 4.20	130.17 ± 26.62
21	AMI without alcohol intake (n=140)	2.80 ± 0.69	0.25 ± 0.11	48.72 ± 8.81	0.81 ± 0.39	153.56 ± 22.09	255.06 ± 23.76	60.22 ± 3.76	132.65 ± 22.36
22	AMI with vegetarian food intake (n=27)	3.88 ± 0.60	0.21 ± 0.11	46.32 ± 9.43	1.02 ± 0.32	152.68 ± 22.49	254.36 ± 24.81	59.73 ± 3.32	131.46 ± 18.56
23	AMI with nonvegetarian food intake (n=273)	2.79 ± 0.69	0.24 ± 0.14	48.94 ± 8.60	0.70 ± 0.35	155.18 ± 23.11	256.39 ± 24.08	60.32 ± 4.06	131.32 ± 25.27
24	AMI with +ve family history (n=82)	3.36 ± 0.73	0.25 ± 0.15	47.85 ± 8.66	0.74 ± 0.35	156.88 ± 20.97*	257.04 ± 23.97	61.12 ± 3.69*	134.58 ± 21.42
25	AMI with -ve family history (n=218)	2.71 ± 0.69	0.23 ± 0.13	49.03 ± 8.71	0.73 ± 0.36	154.23 ± 20.97	255.89 ± 24.22	59.94 ± 4.07	130.11 ± 25.79

Group 2 is compared with group 1

Groups 3,4,5..... are compared with group 2.

**p < 0.01, *p < 0.05

EVALUATION OF THE ROLE OF MATRIX METALLOPROTEINASES AND OXIDATIVE STRESS IN THE PATHOGENESIS AND COURSE OF ACUTE MYOCARDIAL INFARCTION

THESIS SUBMITTED TO THE UNIVERSITY OF CALICUT FOR THE DEGREE OF

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CHAPTER 6

EVALUATION OF THE ROLE OF MATRIX METALLOPROTEINASES IN THE PATHOGENESIS AND COURSE OF ACUTE MYOCARDIAL INFARCTION

6.0.Introduction

Proteolytic machinery was initially thought to be devoted primarily to the recognition and elimination of unassembled or misfolded proteins. However, recent progress in this area has uncovered additional roles for regulated proteolysis in a variety of important biological processes. In particular, the developmental significance of the modulation of the extracellular microenvironment by metalloproteinases has become apparent (Werb, 1997).

Matrix metalloproteinases (MMPs) are the extrinsic regulators of each of the functions during the normal development of a cell (Vu and Werb, 2000). The MMP family currently consists of ~24 members characterized in humans, rodents and amphibians. Initially classified as the zinc dependent proteinases capable of digesting the various structural components of the extracellular matrix (ECM), their specific proteolytic targets have since expanded to many other extracellular proteins. These substrates include an array of other proteinases, proteinase inhibitors, clotting factors, chemotactic molecules, latent growth factors, growth factor binding proteins, cell surface receptors and cell-cell and cell-matrix adhesion molecules (Woessner and Nagase, 2000).

There are two closely related metalloproteinase families : matrix metalloproteinases (MMPs) and metalloproteinase-disintegrins (ADAMs). MMPs require Zn^{2+} binding for their proteolytic activity. ADAMs are the transmembrane proteins that contain disintegrin and metalloproteinase domains, indicative of cell adhesion and proteinase activities. Tissue inhibitors of metalloproteinases (TIMPs) are a family of secreted proteins that selectively, but reversibly, inhibit metalloproteinases (Sternlicht and Werb, 2001).

Regulation of MMP function occurs at multiple levels. MMP mRNA expression is under tight, cell type-dependent control, with expression of individual MMPs associated with specific inflammatory, connective tissue or epithelial cell types. MMP transcripts are generally expressed at low levels, but these levels rise rapidly when tissues undergo remodeling, such as in inflammation, wound healing, cardiovascular diseases and cancer. MMPs are synthesized as latent enzymes that can be stored in inflammatory cell granules but are more often secreted and found anchored to the cell surface or tethered to other proteins on the cell surface or within the ECM. Latent MMPs are proteolytically activated in multiple

steps resulting in the release of propeptide domains. Once active, MMPs are subject to inhibition by a family of endogenous tissue inhibitors as well as by α_2-macroglobulin, a plasma inhibitor.

MMPs have been linked with the pathophysiology of cardiovascular diseases. It has been reported that a gene mediated increased proteolysis in the arterial wall may act as a susceptibility factor for the development of CAD in most of the patients. The complications of CAD including acute myocardial infarction is thought to be caused by the increased proteolysis of the fibrous cap of the plaque by MMPs. Earlier studies have proved that the MMP2 and MMP9 have been linked with plaque rupture, the most complicated event in many forms of the acute coronary syndromes including acute myocardial infarction. The enhanced activities of MMP2 and MMP9 in the fibrous cap make the collagenous material to disintegrate and release the contents of the plaque into the surroundings. Through the blood stream these contents will move and block the branches of the small arteries, thus causing an infarction. Sometimes these infarctions will be very severe leading to the sudden death of the patient. Other forms of the CAD such as coronary aneurism (CA) as well as unstable angina (UA) have also been linked with other MMPs such as MMP3 and MMP12 along with MMP2 and MMP9.

The present study is mainly focused to evaluate the amount of MMP2 and MMP9 in the serum of patients with AMI. Since the fibrous cap of the plaque is rich of collagen, the substrate for these two MMPs, we have selected these two MMPs for the assay. Further, we have divided the patients according to the risk factors, to study whether these MMPs are significantly altered in any of these risk groups or only in certain risk groups. Hence, we can categorize these patients according to the alteration in MMPs and probably can be used as markers for the prediction of AMI.

6.1.Materials and Methods

MMP2 and MMP9 in the serum of both normal as well as in AMI patients were assayed by using the sandwich enzyme immunoassay kit developed by Chemicon International, California. The detailed procedure used for the assays is given in Chapter 2.

6.2.Results

Table 12 represents the values of MMP2 and MMP9 in the serum of both normal as well as AMI patients. When compared to the normal individuals the values of both MMP2 and MMP9 were found to be increased considerably ($p<0.01$) in AMI patients.

The values of MMP2 and MMP9 in normal as well as in AMI patients according to the categorization of age and sex are given in table 12 (a). In all the three age groups ($\leq$40 years, 40-60 years and $\geq$60 years) with AMI, both MMP2 and MMP9 were found to be increased ($p<0.01$) when compared to their normal controls. Among the three age groups (intra groups) MMP9 showed a statistically significant elevation in $\leq$40 years age group when compared to the other two groups. However, MMP2 did not show any significant alteration within these groups. MMP2 and MMP9 were found to be elevated both in males and females with AMI when compared to their normal controls ($p<0.01$). When AMI males were compared with AMI females, MMP9 was found to be more in AMI males than in females.

Table 12 (b) represents the values of MMP2 and MMP9 in normal as well as in AMI patients based on the time of onset of chest pain. When compared to normal all the four groups showed statistically significant elevation in MMP2 and MMP9 ($p<0.01$). Within the group the patients with an onset of chest pain at 12 midnight to 6 am showed an elevation in the value of MMP9 when compared to the other three groups and MMP2 showed a statistically significant elevation in patients with the onset of chest pain at 6pm to 12 midnight.

Table 12 (c) represents the values of MMPs in normal as well as in AMI patients with diabetes and hypertension. Both MMPs were increased in diabetic as well as in nondiabetic, and hypertensive and nonhypertensive AMI patients when compared to their normal controls respectively. MMP9 values are significantly elevated in hypertensive AMI patients when compared to nonhypertensive AMI patients, where as MMP2 did not show any significant difference between the groups. Both MMP2 and MMP9 did not show any significant change in diabetic AMI patients when compared to nondiabetic AMI patients.

Table 12 (d) shows the values of both MMP2 and MMP9 in AMI patients both with the cholesterol value >200 mg/dl and < 200 mg/dl. The values of both MMP2 and MMP9 were found to be statistically ($p<0.01$) elevated in both these groups when compared to the normal controls. The comparison of AMI patients with cholesterol value >200 mg/dl with cholesterol value <200 mg/dl have showed the elevated value of MMP9 in AMI patients with cholesterol value >200 mg/dl. However, the value of MMP2 did not show any significant difference between these two groups.

Table 12 (e) represents the values of MMPs in normal as well as in AMI patients with the habit of smoking and intake of alcohol. Here both the values of MMP2 and MMP9 were found to be increased significantly ($p<0.01$) in AMI patients both with and without the habit of smoking as well as intake of alcohol when compared to their normal controls. A comparison of the AMI patients with the habit of smoking with AMI patients without the habit of smoking have showed the elevated value of MMP9 in AMI patients with the habit of smoking. MMP2 was not significantly differing in either of these groups. The values of MMP2 and MMP9 did not show any significant difference in alcoholic AMI patients when compared to nonalcoholic patients.

Table 12 (f) represents the values of MMP2 and MMP9 in normal as well as in AMI patients who differ in food habits as well as with and without the history of AMI. When compared to the normal the vegetarian AMI patients and the nonvegetarian AMI patients showed elevated values of both MMP2 and MMP9 ($p<0.01$). However the comparison of nonvegetarian AMI patients with the vegetarian AMI patients have showed a significant value in AMI nonvegetarians for MMP9 only. MMP2 did not show any significant difference between these groups. AMI patients with and without the family history of AMI showed elevated values of both MMP2 and MMP9 when compared with normal controls ($p<0.01$). A comparison between AMI patients with and without the history of AMI have showed the significant value of MMP9 in AMI patients without the history of AMI. MMP2 did not show any significant difference.

Table 13 represents the values of MMP2 and MMP9 in normal, in AMI patients, and in AMI patients with various risk groups taken for the study. Among the different risk groups

studied the value of MMP2 was found to be significantly higher in AMI females and the value of MMP9 was found to be significantly higher in AMI patients with age ≤40 years as coompared to that of the whole AMI patients.

6.3.Discussion

The results presented in this chapter show that values of both MMP2 and MMP9 are elevated in patients with acute myocardial infarction. These MMPs play an important role in ECM remodeling during all phases of atherosclerosis. The ECM constitutes the bulk of most advanced atherosclerotic plaques. Therefore, ECM metabolism in atherosclerotic lesions is considered to favor the overall net accumulation rather than degradation of matrix components (Hisashi Kai *et al*, 1998). However, focal accumulation of cells that overexpress activated forms of MMPs may promote local destruction of ECM in atheroma, leading to plaque destabilization and rupture (Dolleny *et al*, 1995). The increased plaque rupture may be due to the increased amount of these MMPs, which ultimately leads to AMI.

Irrespective of the risk factors, both MMP2 and MMP9 were found to be elevated in all the AMI patients when compared to normal healthy individuals. However, an important finding is that the values of MMP9 was found to be altered in some of the risk groups such as male gender, time of onset of chest pain, hypertension, cholesterol value >200 mg/dl, smoking, food habits and a positive family history. Induction of MMP9 expression as well as interstitial collagenase and stromelysin has been shown in both vascular smooth muscle cells (VSMCs) and accumulating macrophages in atherosclerotic plaques, particularly in the shoulder and core of plaques prone to rupture. MMP9 degrades type IV collagen constituting the major structural component of the basement membrane and ECM. Further, type IV collagen is abundant in the atherosclerotic fibrous cap.

Our study clearly supports the role of MMP2 and MMP9 in the pathogenesis of acute myocardial infarction irrespective of the risk factors. However, the amount of MMP9 was significantly altered in some of the risk groups taken to evaluate the severity of the disease. MMP9 was found to be significantly altered in ≤40 years age groups of patients, where as MMP2 did not show any significant alteration in these groups. Further MMP9 was found to be elevated in AMI males, patients with the onset of chest pain from 12 midnight to 6 am,

AMI patients with the history of hypertension, patients with a cholesterol value of >200 mg/dl, patients with the habit of smoking, patients with the habit of non-vegetarian food intake and without a family history of AMI. The MMP2 did not show any alteration in these groups except in patients with an onset of chest pain from 6pm to 12 midnight. The significant alteration of MMP9 in these subgroups when compared to that of MMP2 may be due to the increased expression of MMP9. Further, the substrate of MMP9 (type IV collagen) is more in the atherosclerotic plaque that can also contribute to the increased activity of MMP9. The value of MMP2 was found to be more in AMI females among all the risk groups studied and the value of MMP9 was found to be more in patients with age ≤40 years among all the risk groups. These observations raised the possibility that these MMPs are strongly associated with the molecular mechanism of the onset and development of ACS, especially AMI.

The important findings of the present study is that the value of MMP9 was found to be more in AMI patients with age ≤40 years as compared to all other risk groups with AMI. Hence, it may be concluded that the extent of plaque rupture may be more in patients with age ≤40 years. Between the two metalloproteinases, MMP9 showed a higher elevation as compared to MMP2, hence, MMP9 may be used as a diagnostic marker for the early detection of AMI.

Table: 12

Values of MMP2 and MMP9 in normal and in AMI patients

Parameters	Groups	
	Normal (n = 100)	AMI (n = 300)
MMP2 (ng/ml)	468.34 ± 46.74	899.84 ± 140.02 * *
MMP9 (ng/ml)	30.17 ± 6.59	96.32 ± 23.89 * *

Values are mean ± SD

**p<0.01

Table: 12 (a)

Values of MMP2 and MMP9 in normal and in AMI patients according to the age and sex.

Parameters	Age (years)						Sex			
	≤ 40		40 - 60		≥ 60		Male		Female	
	Normal(n=22)	AMI (n=27)	Normal(n=54)	AMI (n=160)	Normal(n=24)	AMI (n=113)	Normal (n=85)	AMI (n=261)	Normal (n=15)	AMI (n=39)
MMP2 (ng/ml)	497.68±48.22	908.07±136.38**	460.41±44.57	893.35±151.85**	459.29±38.58	907.06±121.78	471.26±47.70	895.08±143.99**	451.80±36.75	931.69±104.40
MMP9 (ng/ml)	31.18±6.59	120.21±20.21^{a**}	30.54±7.18	95.05±25.34^{b**}	28.38±6.07	92.41±18.83^{bc**}	30.57±6.74	98.29±24.50^{a**}	27.85±5.11	83.14±13.19^{b**}

Values are mean ± SD

**p<0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table : 12 (b)

Values of MMP2 and MMP9 in normal and in AMI patients according to the time of onset of chest pain.

Parameters	Time of Onset of chest pain				
	Normal (n=100)	AMI			
		12midnight to 6am(n=50)	6am to 12 noon (n=94)	12noon to 6pm(n=90)	6pm to 12midnight (n=66)
MMP2 (ng/ml)	468.34 ± 46.74	$922.92 \pm 174.71^{ab**}$	$868.04 \pm 148.58^{cd**}$	$898.38 \pm 111.32^{abc**}$	$929.65 \pm 122.14^{a**}$
MMP9 (ng/ml)	30.17 ± 6.59	$113.19^{a**} \pm 25.11$	$91.57 \pm 25.15^{cd**}$	$91.91 \pm 18.39^{c**}$	$96.31 \pm 21.91^{b**}$

Values are mean ± SD

**p<0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabetd denote heterogenous values.

Table: 12 (c)

Values of MMP2 and MMP in normal and in AMI patients according to the history of Diabetes and Hypertension.

Parameters	Normal (n=100)	AMI			
		Diabetes		Hypertension	
		Diabetic (n=101)	Nondiabetic (n=199)	Hypertensive (n=62)	Non hypertension (n=238)
MMP2 (ng/ml)	468.34 ± 46.74	876.80 ± 175.17**	911.54 ± 116.51**	875.88 ± 154.62**	906.08 ± 135.26**
MMP9 (ng/ml)	30.17 ± 6.59	97.89 ± 25.41**	95.52 ± 23.04**	104.65 ± 28.27[a**]	94.15 ± 22.10[b**]

Values are mean ± SD

**p<0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table: 12 (c)

Values of MMP2 and MMP in normal and in AMI patients according to the history of Diabetes and Hypertension.

Parameters	Normal (n=100)	AMI			
		Diabetes		Hypertension	
		Diabetic (n=101)	Nondiabetic (n=199)	Hypertensive (n=62)	Non hypertension (n=238)
MMP2 (ng/ml)	468.34 ± 46.74	876.80 ± 175.17**	911.54 ± 116.51**	875.88 ± 154.62**	906.08 ± 135.26**
MMP9 (ng/ml)	30.17 ± 6.59	97.89 ± 25.41**	95.52 ± 23.04**	104.65 ± 28.27[a**]	94.15 ± 22.10[b**]

Values are mean ± SD

**p<0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

14	AMI with hypertension (n=62)	875.88 ± 154.64	104.65 ± 28.27
15	AMI without hypertension (n=238)	906.08 ± 135.26	94.15 ± 22.10
16	Cholesterol >200mg/dl (n=105)	879.36 ± 172.70	118.14 ± 21.91
17	Cholesterol<200 mg /dl (n=195)	910.87 ± 117.28	84.57 ± 15.01
18	AMI with smoking (n=222)	891.28 ± 144.63	99.60 ± 25.76
19	AMI without smoking (n=78)	924.21 ± 122.76	86.98 ± 13.75
20	AMI with alcohol intake (n=160)	894.04 ± 155.89	96.70 ± 13.74
21	AMI without alcohol intake (n=140)	906.47 ± 118.97	95.88 ± 22.06
22	AMI with vegetarian food intake (n=27)	922.25 ± 97.58	84.01 ± 14.17
23	AMI with nonvegetarian food intake (n=273)	897.63 ± 143.34	97.54 ± 24.28
24	AMI with +ve family history (n=82)	904.62 ± 130.86	85.87 ± 21.23
25	AMI with -ve family history (n=218)	898.05 ± 143.27	100.25 ± 23.65

Group 2 is compared with group 1
Groups 3,4,5.....are compared with group 2.

Table : 12 (d)

Values of MMP2 and MMP9 in normal and in AMI patients according to the value of Cholesterol.

Parameters	Cholesterol (mg/dl)		
	Normal (n=100)	AMI	
		> 200 (n=105)	< 200 (n=195)
MMP2 (ng/ml)	468.34 ± 46.74	879.36 ± 172.70**	910.87 ± 117.28**
MMP9 (ng/ml)	30.17 ± 6.59	118.14 ± 21.91[a**]	84.57 ± 15.01[b**]

Values are mean ± SD

**p<0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table : 12 (e)

Values of MMP2 and MMP9 in normal and in AMI patients with the habits of Smoking and Alcohol intake.

Parameters	Smoking				Alcohol intake			
	Yes		No		Yes		No	
	Normal (n=62)	AMI (n=222)	Normal (n= 38)	AMI (n=78)	Normal (n= 20)	AMI (n=160)	Normal (n= 80)	AMI (n=140)
MMP2 (ng/ml)	473.64 ± 51.76	891.28 ± 144.63**	459.68 ± 35.45	924.21 ± 122.76**	505.15 ± 44.01	894.04±155.89**	459.13 ± 42.71	906.47 ±118.97**
MMP9 (ng/ml)	30.96 ± 6.48	99.60 ± 25.76[a**]	28.86 ± 6.57	86.98 ± 13.75[b**]	31.88 ± 6.57	96.70 ± 13.74**	29.74 ± 6.91	95.88 ± 22.06**

Values are mean±SD

**p<0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table : 12 (f)

Values of MMP2 and MMP9 in normal and in AMI patients according to the food habits and family history of AMI.

Parameters	Food habits				Family history		
	Vegetarians		Non Vegetarians		Normal (n=100)	AMI	
	Normal (n=16)	AMI (n=27)	Normal (n=84)	AMI (n=273)		+ve (n=82)	-ve (n=218)
MMP2 (ng/ml)	454.81 ± 37.45	922.25 ± 97.58**	470.91 ± 47.88	897.63 ± 143.34**	468.34 ± 46.74	904.62 ± 130.86**	898.05 ± 143.27**
MMP9 (ng/ml)	27.46 ± 5.17	84.01 ± 14.17^{b**}	30.68 ± 6.71	97.54 ± 24.28^{a**}	30.17 ± 6.59	85.87 ± 21.23^{b**}	100.25 ± 23.65^{a**}

Values are mean±SD

**p<0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogenous values.

Table: 13

Comparison of MMP2 and MMP9 in AMI patients in different risk groups

Groups		Parameters	
		MMP2 (ng/ml)	MMP9 (ng/ml)
1	Normal (n=100)	468.34 ± 46.74	30.17 ± 6.59
2	AMI (n=300)	899.84 ± 140.02**	96.32 ± 23.89**
3	Age ≤ 40 years (n=22)	908.07 ± 136.38	120.21 ± 20.21**
4	Age 40-60 years (n=160)	893.35 ± 151.85	95.05 ± 25.34
5	Age ≥ 60 years (n=113)	907.06 ± 121.78	92.41 ± 18.83
6	Male (n=261)	895.08 ± 143.99	98.29 ± 24.50
7	Female (n=39)	931.69 ± 104.40**	83.14 ± 13.19
8	Time of onse tof chest pain from 12 midnight to 6 am (n=50)	922.92 ± 174.71	113.19 ± 25.11
9	Time of onset of chest pain from 6 am to 12noon (n=94)	868.04 ± 148.58	91.57 ± 25.15
10	Time of onset of chest pain from 12 noon to 6pm (n=90)	898.38 ± 111.32	91.91 ± 18.39
11	Time of onset of chest pain from 6pm to 12 midnight (n=66)	929.65 ± 122.14	96.31 ± 21.91
12	AMI with diabetes (n=101)	876.80 ± 175.17	97.89 ± 25.41
13	AMI without diabetes (n=199)	911.54 ± 116.51	95.52 ± 23.04

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14	AMI with hypertension (n=62)	875.88 ± 154.64	104.65 ± 28.27
15	AMI without hypertension (n=238)	906.08 ± 135.26	94.15 ± 22.10
16	Cholesterol >200mg/dl (n=105)	879.36 ± 172.70	118.14 ± 21.91
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Group 2 is compared with group 1
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CHAPTER 7

SUMMARY AND CONCLUSIONS

Summary and Conclusion

An increasing trend has recently been observed in the incidence of acute coronary syndromes in Indian population. Acute myocardial infarction, a major manifestation of acute coronary syndrome is faster emerging as a major cause of death in developing countries, especially India. Mechanisms involved in the formation and rupture of atheromatous plaque is not fully established. The most important mechanism underlying the sudden acute myocardial infarction is erosion or uneven thinning and rupture of the fibrous cap. The risk of plaque erosion or rupture appears to depend critically on the cellular and extracellular composition of plaque. Several pathologic mechanisms are involved in the process of plaque rupture, including inflammation, rheological factors, circumferential wall stress and vasoconstriction as well as destabilizing changes in fibrous plaque.

The degradation of the extracellular matrix is done by the proteolytic enzymes such as matrix metalloproteinases. These are enzymes essential for the vascular remodeling. The fibrous cap of the atherosclerotic plaque is made up of collagen, the substrate for metalloproteinases. These metalloproteinases are also called collagenases and two of these - matrix metalloproteinase-2 (MMP2) and matrix metalloproteinase-9 (MMP9), are responsible for the degradation of the fibrous cap of the atherosclerotic plaque and the release of its contents into the blood stream. These contents can block the small blood vessels supplying the heart and can lead to infarction.

The biological molecules such as nucleic acids, proteins and lipids are subjected to oxidative damage by reactive oxygen species (ROS). The major contents of the plasma membranes are the proteins and lipids. The oxidative damage of these molecules will change the normal fluidity and integrity of the plasma membrane, which can lead to various diseases. Several mechanisms are responsible for the protection of the vascular cells from potential cytologic damage caused by free radicals, the main being scavenger enzymes of ROS-superoxide dismutase, catalase and glutathione peroxidase. It has now become apparent that antioxidants may favourably influence coronary artery disease through alternative mechanisms, including improvement of endothelial function, inhibition of platelet aggregability and a decrease in the risk of plaque rupture.

Although, there is a high prevalence of coronary artery disease and causative risk factors, not much data is available linking the oxidative stress and the levels of matrix metalloproteinases and the incidence of myocardial infarction in Indian population. Hence, it is considered worthwhile to study the role of scavenger enzymes-superoxide dismutase, catalase, glutathione peroxidase and the levels of matrix metalloproteinases in AMI patients with a view to assess the role of these parameters as a risk predictor of AMI and also as a guide in the management of patients after myocardial infarction.

The present study included 300 AMI patients and 100 normal healthy individuals. The AMI was diagnosed by ECG changes and by assaying the cardiac markers. The patients were classified based on different risk factors. The blood was collected from each patient and used for the biochemical analysis. The routine biochemical investigations along with lipid profiles and markers of oxidative stress such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione and lipid peroxidation were estimated. The trace elements and micronutrients were also estimated in the serum of both AMI and normal individuals. MMP2 and MMP9 were estimated by immunological methods. The major aim of the study is to evaluate the role of these parameters in AMI patients when compared to those of the normals and to study their variation in different risk groups. The important findings obtained from the study are as follows.

1.The present study clearly indicates the elevated values of total WBC count, ESR, creatinine, random blood glucose, CK and CK-MB in all the AMI patients when compared to those of the normal controls. The lipd profile parameters such as cholesterol, TG and LDL-chol was also found to be significantly elevated in AMI patients and HDL-chol was found to be significantly decreased when compared to that of the normal controls.

2.The markers of oxidative stress such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, albumin and ceruloplasmin were found to be decreased in AMI patients. But the values of lipid peroxidation and uric acid were found to be increased in AMI patients. The change observed in thses markers indicate an increased oxidative stress in all the AMI patients and the extent of the oxidative stress was

found to be more in female patients, in patients with the habit of alcohol intake, patients with a positve family history of AMI and in patients with age ≤40 years.

3. The results of the present study show decreased values of micronutrients such as vitamin E, vitamin C, Zn and Mg in AMI patients and increased values of iron, %transferrin saturation and ferritin and decreased values of TIBC as compared to that of the normal controls. Eventhough the values of these micronutrients and trace elements were found to be significantly different in various risk groups with AMI, a significant change in these parameters were observed in patients with cholesterol value > 200 mg/dl, in patients with hypertension, with a positive family history of AMI, with age ≤ 40 years and in patients with time of onset of chest pain from 12 noon to 6 pm.

4. The study shows elevated values of MMP2 and MMP9 in all the AMI patients. The important findings of the present study is that the value of MMP2 was found to be more in AMI females and the value of MMP9 was found to be more in AMI patients with age ≤40 years as compared to all other risk groups with AMI. Hence, it may be concluded that the extent of plaque rupture may be more in female patients and in patients with age ≤40 years. Between these two metalloproteinases MMP9 showed a higher elevation as compared to MMP2, hence, MMP9 may be used as a diagnostic marker for the early detection of AMI.

EVALUATION OF THE ROLE OF MATRIX METALLOPROTEINASES AND OXIDATIVE STRESS IN THE PATHOGENESIS AND COURSE OF ACUTE MYOCARDIAL INFARCTION

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EVALUATION OF THE ROLE OF MATRIX METALLOPROTEINASES AND OXIDATIVE STRESS IN THE PATHOGENESIS AND COURSE OF ACUTE MYOCARDIAL INFARCTION

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ABBREVIATIONS

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ACS	Acute Coronary Syndromes
AMI	Acute Myocardial Infarction
CAD	Coronary Artery Disease
CHD	Coronary Heart Disease
UA	Unstable Angina
CVD	Cardio Vascular Disease
LVH	Left Ventricular Hypertrophy
BMI	Body Mass Index
WHR	Waist-Hip Ratio
BP	Blood Pressure
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
TG	Triglycerides
DNA	Deoxyribo Nucleic Acid
MMPs	Matrix Metalloproteinases
MT-MMPs	Membrane Type Matrix Metalloproteinases
TIMP	Tissue Inhibitor of Metaloproteinase
ROS	Reactive Oxygen Species
ECG	Electro Cardio Gram
CK	Creatine Kinase
CKMB	Creatine Kinase Isoenzyme
SOD	Superoxide Dismutase
CAT	Catalae
GSH	Reduced Glutathone
GSSG	Oxidised Glutathione
GR	Glutathione Reductase
GPx	Glutathione Peroxidase
Lp(a)	Lipoprotein (a)
Apo(a)	Apolipoprotein (a)
PUFA	Poly Unsaturated Fatty Acid
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
HSPs	Heat Shock Proteins
IL	Inter leukin
TNF	Tumor Necrosis Factor
IFN	Interferon
ECM	Extra Cellular Matrix
ADAMs	A Disintegrin and Metalloproteinase
ADAMTS	A Disintegrin and Metalloproteinase Thrombospondin
TACE	TNF-Alpha Converting Enzyme
TS	Thrombospondin
GPI	Glycophosphatidyl Inositol
RECK	Reversion-inducing Cysteine rich protein with Kazal motifs

VEGF	Vascular Endothelial Growth Factor
KGF	Keratinocyte Growth Factor
FGF	Fibroblast Growth Factor
EGF	Epidermal Growth Factor
NGF	Neuronal Growth Factor
PDGF	Platelet Derived Growth Factor
TGF	Tumor Growth Factor
IGF	Insulin like Growth Factor
EMMPRIN	Extracellular Matrix Metalloproteinase Inducer
SNPs	Single-Nucleotide Polymorphisms
PAR-1	Proteinase-Activated Receptor-1
mRNA	Messenger Ribonucleic Acid
IGF-BP	Insulin like Growth Factor Binding Protein
TS2	Thrombospondin 2
LRP	Lipoprotein Receptor related Protein
BMP-1	Bone Morphogenetic Protein-1
EGFR	Epidermal Growth Factor Receptor
TBARS	Thiobarbituric acid Reactive Substances