STUDY OF HIGH SENSITIVE C-REACTIVE PROTEIN AND SERUM URIC ACID IN CORONARY ARTERY DISEASES – A CROSS SECTIONAL STUDY

BY

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DISSERTATION SUBMITTED TO

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH,

TAMAKA, KOLAR, KARNATAKA

IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF MEDICINE
IN
BIOCHEMISTRY

UNDER THE GUIDANCE OF

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APRIL 2013

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ACKNOWLEDGEMENT

The trails and tribulations of making this dissertation were far outweighed by the pleasure and stimulation I received from working on it. This was largely due to those wonderful people who helped me, taught me and advised me. I thank them, each and every one, for the knowledge they imparted to me and for the time they so graciously gave me.

No words can express my gratitude to ***Dr.Krishnamurthy N****, Professor& HOD, Department of Biochemistry. He showed and maintained a constant and most active interest during the entire period of this work. It was a pleasure to be associated with his persuasive and stimulating personality.*

I wish to express my sincere thanks to ***Dr.Raveesha A****, Professor, Department of Medicine, for his constant support during the period of this study.*

I sincerely thank our beloved Principal, ***Dr.M.B Sanikop*** *and Director Post-graduate studies* ***Dr.S.R Prasad,*** *Sri Devaraj Urs Medical College, Kolar for allowing me to utilize the facilities at this institution.*

I express my humble, sincere indebtedness to ***Dr.Ashakiran S,*** *Professor,* ***Dr.Shashidhar K.N.****,* ***Dr.Dayanand C.D****., Associate Professors, Department of Biochemistry, who have been a source of inspiration from their exemplary discipline, rare intellect and immense experience. I am very fortunate to obtain benefit of their vast experience, valuable guidance, sincere suggestion, keen interest and constant encouragement at each step throughout my post-graduate career.*

I owe my deep sense of gratitude to ***Dr.Anand kumar D.R.****, Professor, Department of Cardiology, Narayana Hruduyalaya for his invaluable suggestions, constant encouragement and support throughout the study.*

I am grateful to ***Dr.Sumathi ME****,* ***Mrs.Deena Mendez****,* ***Mrs.Mamatha Kunder*** *and* ***Mrs.Shymali Chatterjee,*** *Assistant Professor Department of Biochemistry, for their invaluable guidance, kind support, co-operation and timely advice.*

I render my hearty thanks to ***Dr.Deepthi Kiran****, Assistant Professor, Department of Community Medicine, SDUMC and* ***Dr.Sumit Garg****, PG in the Department of Physiology, and* ***Dr.Achal Shetty,*** *PG in Department of Community Medicine for helping me in statistical analysis.*

I wish to express my deepest gratitude to ***Dr.Prabhavathi K****, Assistant*

professor, PES Institute of Medical Sciences, Kuppam, for her constant support and guidance.

I acknowledge with thanks the unflagging co-operation and succour rendered by my colleagues ***Dr. Nisha R, Dr. Ganesh G, Dr. Esha Mati, Dr. Harish R****, upon whose complacency and co-operation this work is build. My association with them has developed into everlasting friendship, which I cherish highly.*

I would like to acknowledge the help rendered by all the ***technicians*** *in the Clinical Biochemistry Laboratory, all the nursing staff in Narayana Hruduyalay RLJH&RC, Kolar in conducting this study.*

I express my sincere heartfelt gratitude to all the ***subjects*** *for giving their willingness to undergo the required procedure and enabling me to carry out this study.*

I thank all ***non teaching staff*** *of the department for their kind co-operation.*

Above all I am proud of my parents ***Dr. Channappa T, Mrs. Pushpavati,*** *my husband* ***Dr. Rajashekhar****, my adorable son* ***Aarush*** *and* ***my in-laws, Brother and Sisters*** *for their constant encouragement, love & attention which have made me accomplish this work. They have formed the basis of this work without which it would not have seen the light of the day.*

In the end, I thank The ***Almighty*** *for the blessing for the fulfillment of this work.*

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DR NANDINI TAKKALAKI

DEDICATED WITH REVERENCE

TO

MY FAMILY

FOR THEIR SELFLESSNESS AND

INSPIRATION THAT MOTIVATES ME IN

ALL MY ENDAVEOURS

LIST OF ABBREVIATIONS

CAD - Coronary Artery Disease

CHD - Coronary Heart Disease

CRP - C-Reactive Protein

CK-MB - Creatine Kinase

DM - Diabetes Mellitus

ICAM - Intra Cellular Adhesion Molecule

ECG - Electrocardiography

HDL - High Density Lipoprotein

Hs- CRP- High Sensitive C-Reactive Protein

HTN - Hypertension

Ig - Immunoglobulin

IL-1 - Interleukin-1

LDH - Lactate Dehydrogenase

LDL - Low Density Lipoprotein

MI - Myocardial Infarction

MMP - Matrix Metalloproteinases

NO - Nitric Oxide

SOD - Super Oxide Dismutase

TNF - Tissue necrosis factor

UA - Uric Acid

UA - Unstable Angina

VCAM - Vascular Cell Adhesion Molecule

XO - Xanthine Oxidase.

ABSTRACT:

BACKGROUND:

Coronary artery disease (CAD) is the leading cause of mortality and morbidity in the world. Other than traditional risk factors such as diabetes mellitus, hypertension, dyslipidemia and old age which are prevalent in CHD, there are some emerging risk factors that are involved in the pathogenesis of these cardiovascular diseases, which includes hsCRP and serum uric acid.

OBJECTIVES:

1. To estimate the serum uric acid and hsCRP in CAD cases and compare with controls.
2. To see the correlation between each of these parameters.

MATERIALS AND METHODS:

Study included a total of 60 subjects, of which 30 were diagnosed cases of CHD and 30 were healthy controls. Blood was collected, serum separated to estimate glucose, CK-MB, serum Uric acid and high sensitive CRP.

RESULTS:

There was an increased level of highly sensitive CRP in CHD cases compared to controls. These patients also had increased levels of serum uric acid when compared to controls. There was no correlation between CK-MB with hs-CRP and uric acid levels.

CONCLUSION:

The hyperuricemia seen in CHD patients is due to the endothelial dysfunction and oxidative stress and elevated high sensitive CRP levels due to atherosclerosis because of ongoing subclinical inflammation in CHD patients. These biochemical alterations may increase the risk of mortality and morbidity in these cases and may help in predicting the outcome.

Key words:

Highly sensitive CRP; Coronary Heart Disease; Diabetes Mellitus; Hypertension; Serum Uric Acid.

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INTRODUCTION

INTRODUCTION

Coronary heart disease is the most common, acute and chronic illness and is the leading cause of mortality and morbidity in the industrialized world. The cardiovascular epidemic is a worldwide phenomenon that accounts for almost 50% of all deaths in industrialized nations. In India 2.03 million deaths have occured due to CAD by 2010 and prevalence of CAD is reported to be 2-3 times higher in urban population as compared to rural population, prevalence in urban 96.7% per 1000 population and 27.1% per 1000 population in rural.[1]

Atherosclerosis the underlying cause of most CHD[2], is a process that starts early in life and progresses slowly and silently for decades. The clinical manifestations usually occur in the form of myocardial infarction, stroke, angina or sudden death. Cholesterol screening has been used as a tool to identify individuals who are at risk of developing future coronary events. Although this approach has been useful, it fails to identify some individuals who develop MI, who have either normal or only moderately increased serum cholesterol concentrations.

Laboratory and clinical evidence has demonstrated that atherosclerosis is not simply a disease of lipid deposition. Rather, systemic inflammation also plays a pivotal role in atherothrombotic inception and progression[3,4]. Mononuclear cells, macrophages and T lymphocytes are prominent in atheromatous plaques in the arterial wall[5,6]. Furthermore, the shoulder region of a plaque, the most vulnerable site for rupture in acute coronary syndromes is heavily infiltrated with inflammatory cells.[7]

C-reactive protein is an acute phase protein that appears in circulation in response to inflammatory cytokines and high-sensitivity C-reactive protein (hs-CRP) – is a quantitative analysis of very low levels of c-reactive protein, that may provide a

novel method for detecting individuals at high risk of plaque rupture, as inflammation plays a major role in atherothrombosis. Cytokines, which cause the de novo hepatic production of acute phase reactants such as C-reactive protein[8], have been shown to increase in acute coronary syndromes even in the absence of myocardial necrosis.[9] Therefore CRP has been examined as a surrogate marker of other inflammatory mediators such as interleukin-6 and tumor necrosis factors-alpha to better understand the inflammatory component of atherosclerosis. Following a systematic review of the association between inflammatory markers and coronary heart diseases, the American Heart association developed a scientific statement that recommends hs CRP as a sensitive assay for the prediction of vascular disease, compared to traditional assays for circulating C-reactive protein levels.[10]

Uric acid is the product of purine metabolism, which is degraded in most mammals by the hepatic enzyme xanthine oxidase and is freely excreted in urine. Uric acid may also be used as an indicator of increased oxidative stress. Xanthine oxidase is a critical enzyme in the degradation of purine to uric acid has been shown to be an important source of superoxide free radicals. The activity of xanthine oxidase increases during ischemia and intensifies during reperfusion in coronary endothelial cells. The base line increase in serum uric acid levels indicates coronary heart disease events. Each 50 micro mol /L increase in the base line serum uric acid concentration was associated with 14% increase in cardiovascular mortality.[11]

Hence this study was taken up to know the association between serum uric acid and highly sensitive C - reactive protein in coronary heart disease.

AIMS

&

OBJECTIVES

AIMS AND OBJECTIVES

1. To estimate the levels of serum uric acid and hs CRP in coronary artery disease (CAD) cases with hypertension or DM.
2. To see the association of serum uric acid and hs CRP levels in CAD cases with HTN or DM.
3. To see the correlation of serum uric acid and hs CRP levels with CK MB in CAD.

REVIEW

OF

LITERATURE

REVIEW OF LITERATURE

CORONARY ARTERY DISEASE:

Coronary artery diseases are considered to be the major public health concerns throughout the world, including India. Despite significant improvement in the diagnosis, treatment and prevention, CAD remains the most common, acute, and chronic illness, which is the leading cause of mortality and morbidity in the world.

The Burden of Coronary Heart Disease in Asian Indians[12]:

Asian Indians have considerably higher prevalence of premature coronary artery disease (CAD) and standardized mortality rates for CAD compared with Europeans, Chinese and Malaysians. A recent report from the Study of Health Assessment and Risk in Ethnic Groups (SHARE) indicates a significantly higher risk of cardiovascular events among South Asians compared with Europeans and Chinese. Within the Indian subcontinent, a dramatic increase in the prevalence of CAD has been predicted in the next 20 years due to rapid changes in demography and lifestyle consequent to economic development.

Earlier studies on Asian Indians, mostly in migrant populations, have reported on the high prevalence of CAD and its occurrence at a young age (premature CAD). A study on the prevalence of CAD in an urban South Indian population showed that the prevalence of CAD in urban Indians is now approaching the figures reported in migrant Indians, which range between 7% to 17%. The overall figure of 11% of CAD in the population represents approximately a 10-fold increase in the prevalence of CAD in urban India during the last 40 years.

INDIAN SCENARIO[13]:

Coronary Heart Disease is expected to be the single most important cause of death in India by the year 2015. India is now in the middle of a CAD epidemic with urban Indians having CAD rates similar to overseas Indians, which is 4-fold higher than Americans. Whereas the CAD rates halved in the West in the past 30 years, the rates doubled in India with no signs of a downturn yet. The average age of first myocardial infarction (MI) has decreased by 20 years in India. The pooled estimates from studies carried out in 1990s up to 2002 shows the prevalence rate of CHD in urban areas as 6.4% and 2.5% in rural areas. In urban areas the pooled estimate was 6.1% for males and 6.7% for females. In rural areas the estimate was 2.1% for males and 2.7% for females. According to medical certification of cause of death data, 25.1% of total deaths in urban areas are attributable to diseases of circulatory system. Therefore, it was assumed that mortality rate due to CHD in rural areas are expected to be half of CHD specific mortality rates in urban areas.

DEFINITION:

Coronary Artery Disease is the narrowing of the coronary blood vessels, which leads to inadequate supply of blood and oxygen to a portion of myocardium, occurs when a combination of fatty material, calcium and scar tissue (plaque) builds up in these vessels, which leads to hardening of arteries. The most common cause by far is atherosclerosis.

ATHEROSCLEROSIS[14]:

NON-MODIFIABLE RISK FACTORS

- Increasing age: Men aged 45 and older have increased risk, as do women aged 55 and older.
- Family history of premature congenital heart disease.

MODIFIABLE RISK FACTORS

- Physical inactivity
- Obesity: Women with waist measurements of more than 35 inches have an increased risk, as do men with waist measurements of more than 40 inches. People with body mass index (BMI) values of 25 or higher.
- Hypertension (BP>140/90mmHg).
- Diabetes mellitus
- Cigarette smoking
- High blood cholesterol:

1. Total blood cholesterol levels of 200 mg/dl or higher.
2. Low density lipoprotein cholesterol (LDL) levels of 160 mg/dl (with no other atherosclerosis risk factors)
3. Low density lipoprotein cholesterol (LDL) levels of 100 mg/dl or higher (with heart disease or diabetes).
4. High density lipoprotein cholesterol (HDL) levels of less than 40 mg/dl.
5. Triglyceride levels above 150 mg/dL

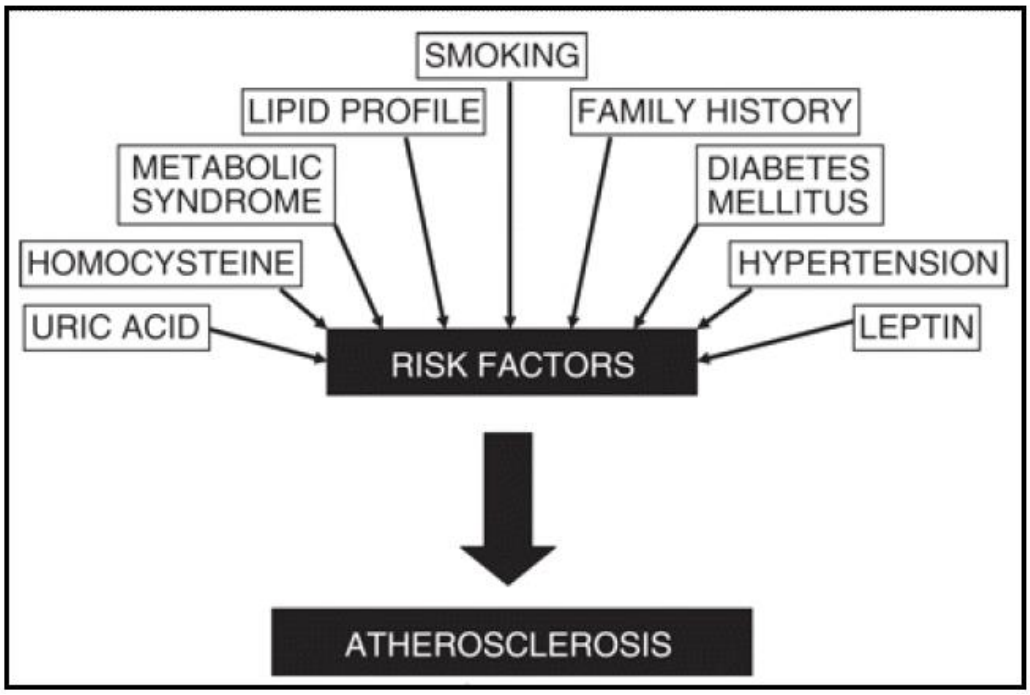


FIGURE 1: RISK FACTORS FOR ATHEROSCLEROSIS.

EMERGING RISK FACTORS:

Newer risk factors are as follows,

- Apolipoprotein B
- Apolipoprotein A-1
- Triglycerides
- Triglyceride rich remnant lipoproteins
- Oxidized LDL
- Lipoprotein (a)
- Homocysteine
- Impaired fasting glucose
- Subclinical atherosclerosis
- hs-CRP (high sensitive C-reactive protein).

PATHOGENESIS OF ATHEROSCLEROSIS[15]:

Atherosclerosis the underlying cause of most CHD, is a process that starts early in life and progresses slowly and silently for decades. Growth of atherosclerotic plaques occurs discontinuously, with periods of relative quiescence punctuated by periods of rapid evolution. After a generally prolonged silent period, it clinically manifests. The clinical manifestations usually occur in the form of myocardial infarction, stable and unstable angina, stroke or sudden death.

ATHEROSCLEROTIC PLAQUE FORMATION [16]:

The process responsible for the formation of the atherosclerotic plaque starts with the accumulation of lipoprotein particles (LDL) in the intima; these lipoproteins undergo modifications that include oxidation and glycation. Oxidative stress, including products found in modified lipoproteins, can induce local cytokine generation. The cytokines induces increased expression of adhesion molecules for leukocytes that cause their attachment and chemo-attractant molecules that direct their migration into the intima. Blood monocytes, entering the artery wall in response to chemo-attractant cytokines, such as monocyte chemo-attractant protein 1, encounter stimuli such as macrophage colony stimulating factor that can augment the expression of scavenger receptors. Scavenger receptors mediate the uptake of modified lipoprotein particles and promote the development of foam cells. Macrophage foam cells are a source of mediators, including other cytokines and effector molecules, such as hypochlorus acid, superoxide anion and matrix metalloproteinases. Smooth muscle cells in the intima divide and other smooth muscle cells migrate into the intima from the media. Smooth muscle cells can then divide and elaborate extracellular matrix, promoting extracellular matrix accumulation in the growing atherosclerotic plaque. In this manner, the fatty streak can evolve into a fibro-fatty lesion. In later stages,

calcification can occur and fibrosis continues, sometimes accompanied by smooth muscle cell death, including apoptosis, yielding a relatively acellular fibrous capsule surrounding a lipid-rich core that may also contain dying or dead cells and their debris.

Proliferation of smooth muscle cells, matrix synthesis and lipid accumulation may narrow the arterial lumen gradually and lead to myocardial ischemia and anginal pain but survival is good if thrombotic complications can be prevented. As a result, the crucial question is why some plaques remain thrombus-resistant and innocuous while others, after years of indolent growth, become thrombus-prone and life-threatening. In this light, plaque vulnerability and thrombogenicity have emerged as being more important than plaque size and stenosis severity.

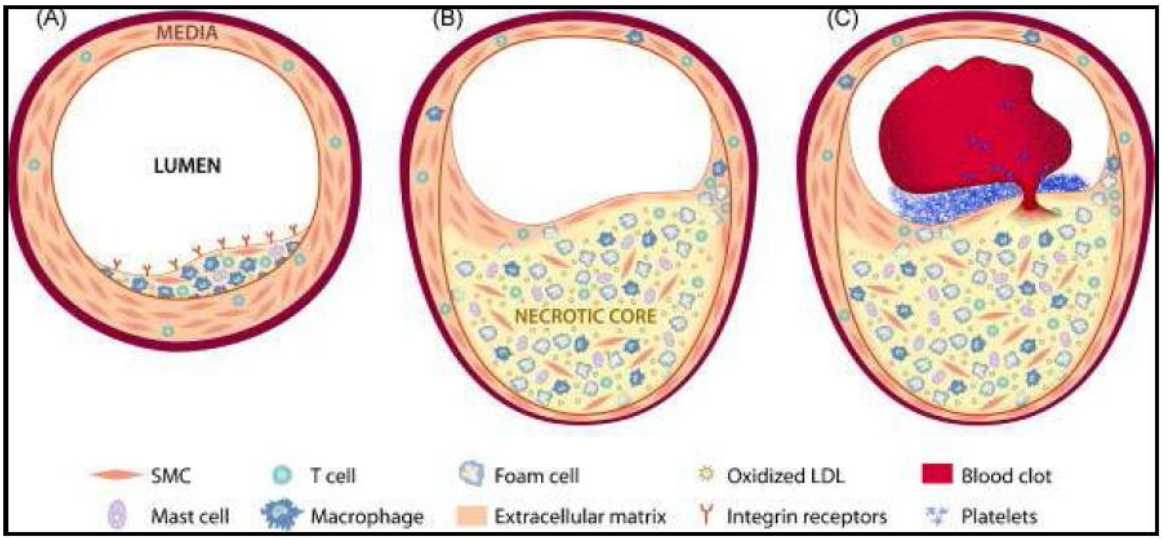


FIGURE 2: ATHEROSCLEROTIC PLAQUE FORMATION

VASCULAR REMODELLING:

Vascular remodelling is defined as any enduring change in the size and/or composition of an adult blood vessel by allowing adaptation and repair. On the other

hand, inappropriate remodelling, including its absence, underlies the pathogenesis of major cardiovascular diseases, such as atherosclerosis and restenosis. Since degradation of the extracellular matrix scaffold enables reshaping of tissue, participation of specialized enzymes called matrix metalloproteinases (MMPs) has become the object of intense recent interest in relation to physiological ("good") and pathological ("bad") vascular remodeling.

Experimental evidence acquired in vitro and in vivo suggests that the major drivers of vascular remodelling, hemodynamics, injury, inflammation and oxidative stress, regulate MMP expression and activity. Alternatively, non-specific MMP inhibition seems to oppose remodeling, as suggested by the inhibition of intimal thickening and outward arterial remodeling. An emerging concept is that MMP-related genetic variations may contribute to heterogeneity in the presentation and natural history of atherosclerosis. The hypothesis that MMPs contribute to weakening of atherosclerotic plaques is especially attractive for the potential development of therapeutic interventions aimed at preventing plaque disruption, a major cause of acute cardiovascular events.

Arterial remodelling was not a uniform response to lesion progression. As plaques are mostly eccentric in this location, i.e., did not occupy the whole circumference, compromise of the lumen area may not occur to the same extent as for the diameter. The term "remodeling" is used in de novo atherosclerosis, restenosis and transplant vasculopathy equally.

HEMODYNAMIC STIMULI AND REMODELLING:

In normal arteries, remodelling is a homeostatic response to changes in the flow and circumferential stretch to restore normal shear stress and wall tension

respectively. Outward remodeling in response to increased flow is largely dependent on shear-responsive endothelial production of nitric oxide and the gelatinase matrix metalloproteinases (MMPs) MMP-2 and MMP-9. Nitric oxide appears to be central in this process because it can induce metalloproteinases, inhibit proliferation and promote apoptosis of smooth muscle cells. In contrast, in low-flow states, accentuated production of mitogenic and fibrogenic growth factors, such as platelet-derived growth factor and transforming growth factor-ß, probably mediates inward remodeling by increasing smooth muscle cell proliferation and collagen deposition/cross-linking, whereas metalloproteinase induction helps to reorganize vessel structure.

INFLAMMATION, SCARRING AND REMODELLING[17]:

Inflammatory cells likely play a major role in atherosclerotic remodelling because of their production of metalloproteinases. Recruitment of monocyte/macrophages by cell adhesion molecules, such as intra cellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM), is shear sensitive and partly explains the predominance of macrophages and T cells in the upstream side of focal lesions and in vessels in which outward remodelling is more prevalent. Hyperlipidemia also increases inflammatory cell infiltration into atherosclerotic lesions and promotes their expression of MMPs. Much of the metalloproteinase expression in plaques originates from macrophage foam cells, is readily reduced by lipid lowering or by reduction in lipid oxidation and may underlie the apparent stimulatory effect of hypercholesterolemia on outward remodelling response, which in extreme hypercholesterolemia may result in vessel ectasia. Ultra structural changes in the internal elastic lamina induced by hypercholesterolemia are similar to those observed in high flow and suggest a common metalloproteinase-dependent

mechanism of outward remodelling. Elevated local metalloproteinase activity induced by hypercholesterolemia may explain why some eccentric plaques appear to initiate remodelling in the vessel wall directly beneath the plaque and why medial thinning underlying a plaque is directly proportional to plaque burden.

REMODELING AND PLAQUE RUPTURE[18]:

Plaque rupture underlies most unstable angina, myocardial infarction and sudden death from coronary artery disease. Many angiographic studies have demonstrated that most lesions responsible for myocardial infarction were minimally occlusive before rupture, consistent with the fact that many patients had no prior history of ischemia. Patients who died with plaque rupture without prior history of coronary disease showed large lesions, suggesting that considerable outward remodelling had prevented angiographic stenosis despite significant histological stenosis. Prevention of the development of a flow-limiting stenosis by outward remodelling unfortunately also removes the stimulus for the development of collaterals that could prevent myocardial infarction when plaque rupture with thrombotic occlusion occurs.

Several lines of evidence from recent observational studies have suggested that the process of outward remodelling may be associated with plaque rupture. Initially, it was noted that the remodelling response correlates with mechanical characteristics and clinical presentation of the plaque. In patients presenting for angioplasty, calcified plaques are associated with inadequate outward or with inward remodelling, whereas soft plaques exhibit better compensatory enlargement. Lesions responsible for unstable syndromes have larger, softer plaques with more outward remodelling than those in stable angina, which are more fibrous and calcified.

The most devastating sign of CHD is abrupt, unexpected cardiac arrest. Symptoms usually occur during exercise or activity because the heart muscles increased demand for nutrients and oxygen is not being met by the blocked coronary blood vessels.

Most common symptoms include, chest pain, shortness of breath, palpitations, dizziness, light headedness or fainting, weakness, irregular heartbeat on exertion, which may be relieved by rest, and also jaw pain, back pain or arm pain especially on left side, either during exertion or at rest.[19]

Silent ischemia is a condition in which no symptoms occur even though an electrocardiogram and or other tests show evidence of ischemia. Arteries may be blocked 50% or more without causing any symptoms.

DIAGNOSIS:

ELECTROCARDIOGRAM[20]:

ECG remains the key test in the diagnosis of acute and chronic coronary syndromes. The earliest and most consistent ECG finding during acute ischemia is elevation of ST segment.

QRS changes: Abnormal Q waves can sometimes be associated with subendocardial infarcts and transmural infarcts can occur without Q waves.

LABORATORY DIAGNOSIS OF MYOCARDIAL INFARCTION[21]:

A number of laboratory tests are available. None is completely sensitive and specific for myocardial infarction, particularly in the hours following onset of

symptoms. Timing is important, as are correlation with patient symptoms, electrocardiograms and angiographic studies.

The following tests are available as markers:

CREATINE KINASE - TOTAL:

The total CK is a simple and inexpensive test that is readily available using many laboratory instruments. However, an elevation in total CK is not specific for myocardial injury, because most CK is located in skeletal muscle and elevations are possible from a variety of non-cardiac conditions.

CREATINE KINASE - MB FRACTION:

Creatine kinase can be further subdivided into three isoenzymes: MM, MB, and BB. The MM fraction is present in both cardiac and skeletal muscle, but the MB fraction is much more specific for cardiac muscle: about 15 to 40% of CK in cardiac muscle is MB, while less than 2% in skeletal muscle is MB. The BB fraction (found in brain, bowel and bladder) is not routinely measured.

Thus, CK-MB is a very good marker for acute myocardial injury, because of its excellent specificity and it rises in serum within 2 to 8 hours of onset of acute myocardial infarction. Serial measurements every 2 to 4 hours for a period of 9 to 12 hours after the patient is first seen will provide a pattern to determine whether the CKMB is rising, indicative of myocardial injury. The CK-MB is also useful for diagnosis of reinfarction or extensive of an MI because it begins to fall after a day, dissipating in 1 to 3 days, so subsequent elevations are indicative of another event.

CREATINE KINASE -MB ISOFORMS:

The CK-MB fraction exists in two isoforms called 1 and 2 identified by electrophoretic methodology. The ratio of isoform 2 to 1 can provide information about myocardial injury. An isoform ratio of 1.5 or greater is an excellent indicator for early acute myocardial infarction. CK-MB isoform 2 demonstrates elevation even before CK-MB by laboratory testing. However, the disadvantage of this method is that it is a labor intensive method since electrophoresis is required and large numbers of samples cannot be run simultaneously nor continuously. False positive results with congestive heart failure and other conditions can occur.

TROPONINS:

Troponin I and T are structural components of cardiac muscle. They are released into the blood stream with myocardial injury. They are highly specific for myocardial injury, more so than CK-MB and help to exclude elevations of CK with skeletal muscle trauma. Troponins will begin to increase following MI within 3 to 12 hours, about the same time frame as CK-MB. However, the rate of rise for early infarction may not be as dramatic as for CK-MB.

Troponins will remain elevated longer than CK-MB up to 5 to 9 days for troponin I and up to 2 weeks for troponin T. This makes troponins a superior marker for diagnosing myocardial infarction in the recent past, better than lactate dehydrogenase (LDH). However, this continued elevation has the disadvantage of making it more difficult to diagnose reinfarction or extension of infarction in a patient who has already suffered an initial MI. Troponin T lacks some specificity because elevations can appear with skeletal myopathies and with renal failure.

MYOGLOBIN:

Myoglobin is a protein found in skeletal and cardiac muscle which binds oxygen. It is a very sensitive indicator of muscle injury. The rise in myoglobin can help to determine the size of an infarction. A negative myoglobin can help to rule out myocardial infarction. It is elevated even before CK-MB. However, it is not specific for cardiac muscle and can be elevated with any form of injury to skeletal muscle.

LACTATE DEHYDROGENASE:

The LDH has been supplanted by other tests. It begins to rise in 12 to 24 hours following MI and peaks in 2 to 3 days, gradually dissipating in 5 to 14 days. Measurement of LDH isoenzymes is necessary for greater specificity for cardiac injury. There are 5 isoenzymes (1 through 5). Ordinarily, isoenzyme 2 is greater than 1, but with myocardial injury, this pattern is "flipped" and 1 is higher than 2. LDH-5 from liver may be increased with centrilobular necrosis from passive congestion with congestive heart failure following ischemic myocardial injury.[22, 23, 24]

IMAGING STUDIES:

Chest radiography, Echocardiography, MRI, Technetium-99m sestamibi scan, Thallium scanning.

C- REACTIVE PROTEIN [25, 26, 27, 28]:

PREVIEW:

Up to half of all events associated with cardiovascular disease are reported to occur in apparently healthy individuals who have few or none of the traditional risk factors, including dyslipidemia, as a result, attention has increasingly turned to the role of other factors such as inflammation in the development of atherosclerosis and CVD. These efforts have led to the search for inflammatory biomarkers to improve the detection of coronary and cardiovascular risk among seemingly healthy individuals. Prominent among the possible candidates for a clinically useful biomarker of CVD risk is C-reactive protein (CRP) as measured by a high-sensitivity (hs) assay.

Although several promising new risk factors have been identified, C-reactive protein (CRP) has been proposed as one of the most useful new potential additions to CVD risk screening. High-sensitivity CRP (hs-CRP) assays can identify patients at risk of first MI even with low-to-moderate risk lipid levels.[29, 30]

HISTORY:

C-reactive protein is the first protein to be discovered which behaves as an acute phase reactant. It has been named for its calcium-dependent interaction with the somatic C-polysaccharide of pneumococci.

The discovery of C-reactive protein was reported in 1930 by Tillet and Francis. They were investigating serological reactions in pneumonia with various extracts of pneumococci and observed that a non-type-specific somatic polysaccharide fraction, which they designated fraction C, was precipitated by the

sera of acutely ill patients. After the crisis, the capacity of the patient's sera to precipitate C-polysaccharide rapidly disappeared and the C-reactive material was not found in the sera from normal healthy individuals.[31]

Avery OT and his collaborators (1941) characterized the C-reactive material as a protein which required calcium ions for its reaction with C-polysaccharide and introduced the term "acute phase" to refer to serum from patients acutely ill with infectious disease and containing the C-reactive protein.[32, 33]

In the 1940s and 1950s, CRP was one of the most frequently requested clinical laboratory tests for initial evaluation of patients with acute inflammation of any origin but because of the costs involved in its measurement, its lack of quantifiability (at that time) and the ease of measuring the erythrocyte sedimentation rate, CRP determinations fell out of favour[34]. In recent years, however, with the development of highly sensitive quantitative tests for CRP, it is being used much more commonly.

STRUCTURE:

C - reactive protein belongs to the pentraxin family of calcium-dependent ligand-binding plasma proteins, which are part of lectin fold super family of calcium dependent ligand binding and lectin proteins. The human CRP molecule is a homologous molecule with a molecular weight of 1,15,000 to 1,40,000. It is composed of five identical non-glycosylated polypeptide subunits, of molecular weight of 23,027; each containing 206 amino acid residues. The protomers are noncovalently associated in an annular configuration with cyclic pentameric symmetry.[35]

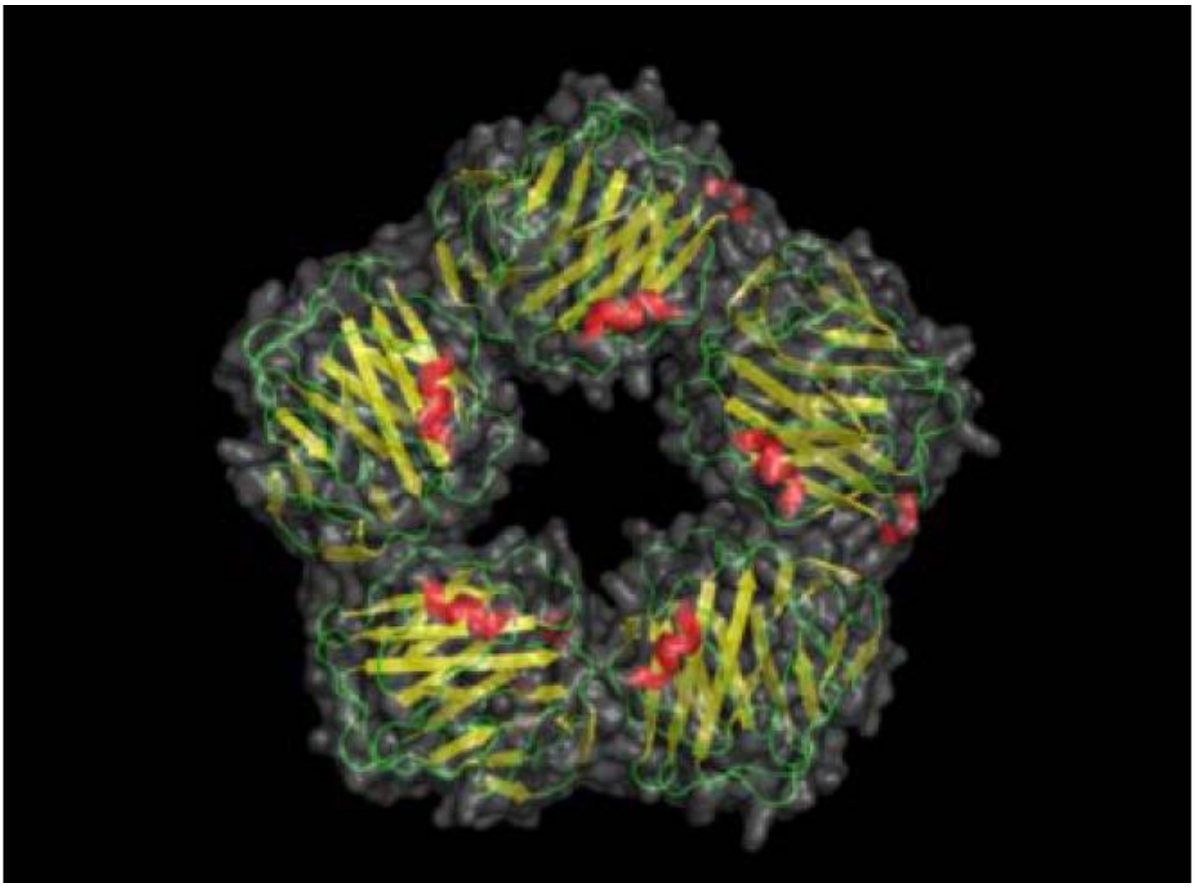


FIGURE 3: THREE-DIMENSIONAL STRUCTURE OF HUMAN C-REACTIVE PROTEIN.

GENE AND GENE EXPRESSION:

Gene for CRP is expressed on chromosome 1. It consists of a coding sequence for a single peptide of 18 residues and the first two amino acids of the native protein followed by an intron of 278bp and then coding sequence for the remaining 204 residues.

FUNCTIONS OF CRP:

The function of CRP is felt to be related to its role in the innate immune system. Similar to immunoglobulin IgG, it activates complement, binds to Fc

receptors and acts as an opsonin for various pathogens. Interaction of CRP with Fc receptors leads to the generation of proinflammatory cytokines that enhance inflammatory response. Unlike IgG, which specifically recognizes distinct antigenic epitopes, CRP recognizes altered self and foreign molecules based on pattern recognition. Thus CRP is thought to act as a surveillance molecule for altered self and certain pathogens. This recognition provides an early defence and leads to a proinflammatory signal and activation of the humoral, adaptive immune system.

Thus a number of functions have been ascribed to CRP, including initiation of opsonisation and phagocytosis and activation of complement, neutrophils and monocyte-macrophage. Collectively these properties imply an important role for CRP in the recognition of microbial organisms and as an immune modulator in the host defence. CRP may also be important in the recognition of necrotic tissues. CRP binds to apoptotic cells, protects the cells from assembly of the terminal complement components and sustains an anti-inflammatory innate immune response.[36,37]

C-REACTIVE PROTEIN RESPONSE IN DISEASES [38, 39]:

Major CRP acute phase response

Infections: Bacterial, Systemic / Severe Fungal, Mycobacterial, Viral.

Allergic Complications of Infection: Rheumatic Fever, Erythema - Nodosum.

Inflammatory disease: Rheumatoid arthritis, Juvenile chronic arthritis, Ankylosing spondylitis, Psoriatic arthritis, Systemic vasculitis, Polymyalgia rheumatica, Reiter's disease, Crohn's disease, Familial Mediterranean Fever.

Necrosis: Myocardial infarction, Tumor Embolisation, Acute pancreatitis.

Trauma: Surgery, Burns, Fractures.

Malignancy: Lymphoma, Carcinoma, Sarcoma.

Modest or absent CRP acute-phase response

• Systemic lupus erythematosus

• Scleroderma

• Dermatomyositis

• Ulcerative colitis

• Leukemia

• Graft-versus-host disease.

IDENTIFICATION OF C-REACTIVE PROTEIN VARIANTS AND ASSOCIATION WITH SERUM LEVELS:

C-reactive protein is a marker for inflammatory processes. More recent research demonstrates that an elevated C-reactive protein level is a risk factor for diabetes, hypertension and cardiovascular disease and that genetic variation within the gene coding for the protein may be associated with levels circulating in the blood stream.

The study participants came from the National Health and Nutrition Examination Survey (NHANES). DNA analyses were conducted on 7,159 participants. Genotyping was performed on all samples for nine single nucleotide polymorphisms in the C-reactive protein gene found previously. Several of the genetic

variations for increased and decreased C-reactive protein levels were more or less prevalent in different racial groups making up the study participants.

These findings are important because they confirm that serum levels of C-reactive protein are genetically influenced. The genetic variations identified in this study could be used to identify people at risk for cardiovascular and other serious diseases or to target people for more aggressive interventions to prevent heart disease from occurring.[40]

LABORATORY METHODS OF MEASURING CRP:

CONVENTIONAL C-REACTIVE PROTEIN:

Conventional CRP assays include qualitative, semi-quantitative and quantitative assays, with indications for use for evaluation of infection, tissue injury and inflammatory disorders. These assays provide information for the diagnosis, therapy and monitoring of inflammatory diseases. CRP is one of the cytokine induced "acute-phase" proteins whose blood levels rise during a general, unspecific response to infections and non-infectious inflammatory processes.

For conventional CRP assays, test values are typically considered to be clinically significant at levels above 10 mg/L. In apparently healthy person's blood CRP levels are below 5 mg/L, while in various conditions this threshold is often exceeded within four to eight hours after an acute inflammatory event, with CRP values reaching approximately 20 to 500 mg/L. CRP is a more sensitive and more reliable indicator of acute inflammatory processes than the erythrocyte sedimentation rate (ESR) and leukocyte count. Blood CRP levels rise more rapidly than erythrocyte

sedimentation rate and after the disease has subsided CRP values rapidly fall and reach the reference interval often days before ESR has returned to normal.[41-43]

CARDIAC C-REACTIVE PROTEIN (cCRP):

Cardiac CRP assays are indicated for use as an aid in the identification and stratification of individuals at risk for future cardiovascular disease. When used in conjunction with traditional clinical laboratory evaluation of acute coronary syndromes, cCRP may be useful as an independent marker of prognosis for recurrent events in patients with stable coronary disease or acute coronary syndrome. Cardiac CRP assays, like hsCRP assays, have measurement ranges that extend below the measurement range typical of most conventional CRP assays.[44]

HIGH SENSITIVITY CRP (hsCRP):

It is no way different from conventional CRP and hence is not a different analyte. The "high sensitivity" refers simply to the lower detection limit of the assay procedures being used. The actual CRP analyte, the plasma protein that is being measured, is the same regardless of the assay range. Hence by applying these, CRP levels are detected even in the normal range with individuals in the upper level of the normal (ULN) having adverse future cardiovascular events being healthy prior to that.

Centre for Disease Control and Prevention/American Heart Association (CDC/ AHA) recommendations for the use of hsCRP in Clinical and Public Health Practice[44]:

I. POPULATION SCIENCE:

(1) The entire adult population should not be screened for hs-CRP for purpose of cardiovascular risk assessment (Class III, level of evidence C).

II. CLINICAL PRACTICE:

1. Measurement of hs-CRP is an independent marker of risk and in those judged at intermediate risk by global risk assessment (10-20% risk of CHD per 10 years), at the discretion of the physician, may help direct further evaluation and therapy in the primary prevention of CVD. The benefit of such therapy based on this strategy remains uncertain (Class IIa, level of evidence B).

2. Measurement of hs-CRP is an independent marker of risk and may be used at the discretion of the physician as part of a global coronary risk assessment in adults without known CVD. The benefits of this strategy remain uncertain (Class IIb, level of evidence C).

3. hs-CRP levels may be useful in motivating patients to improve lifestyle behaviours. The benefits of this strategy remain uncertain (Class IIb, level of evidence C).

4. Patients with persistently unexplained marked elevation of hs-CRP (>10 mg/L) after repeated testing should be evaluated for noncardiovascular etiologies (Class IIa, level of evidence B).

5. Other inflammatory markers (cytokines, other acute phase reactants) should not be measured for the determination of coronary risk in addition to hs-CRP (Class III, level of evidence C).

LABORATORY TESTING:

1. Of current inflammatory markers identified, hs-CRP has the analyte and assay characteristics most conclusive to use in practice (Class IIA, level of evidence B)
2. hs-CRP assays are based on either,
    - Immune nephalometric method.
    - Immune turbidimetric method.
    - Immune luminometric method.
    - Micro plate enzyme immune assay.
    - Sandwich immune assay method.

3. hs-CRP levels, using standardizing assays, categorize patients as follows:

Relative risk category: Average hs-CRP levels

Low : <1 mg/L

Average: 1.0 to 3.0 mg/L

High: >3 mg/L (Class IIa, level of evidence B)

4. hs-CRP results should be expressed as mg/L only (Class I, level of evidence C).

In the Physician's Health Study, men with greater than 2.11 mg/L had about 3 times the risk of suffering a heart attack compared with individuals with CRP less than 0.55 mg/L (the lowest quartile of scores).[45,46]

COMPARISON OF C-REACTIVE PROTEIN TO STANDARD AND OTHER NOVEL RISK FACTORS:

Despite the fact that other measures of inflammation can predict CVD (e.g., interleukin-6, intercellular adhesion molecule-1, macrophage inhibitory cytokine-1) either a short half-life or difficult, expensive and non-standardized assays limit their use. Fibrinogen, a promising biomarker of both inflammation and thrombosis, suffers from poorly standardized assays despite its relatively good population-based data.

White cell count and erythrocyte sedimentation rate has proven less reliable in clinical settings. CRP has several advantages as a risk assessment tool. It is very stable, with little difference in values between fresh or frozen plasma due to its pentraxin structure and its long plasma half-life of up to 20 hours. In addition, hsCRP assays have been standardized in many commercial laboratories. Perhaps most important is that the relative magnitude of hsCRP predictive ability appears to outweigh that of other novel risk factors, including homocysteine and lipoprotein (a). The predictive ability of hsCRP may even surpass that of low-density lipoprotein and/or total: high-density lipoprotein cholesterol ratios. Finally, adding hsCRP to total: high-density lipoprotein cholesterol ratio significantly improves the predictive ability of both tests.[47-49]

ATHEROSCLEROSIS, INFLAMMATION, AND C-REACTIVE PROTEIN[50-55]:

Research over the past decade has led to the current understanding of atherosclerosis as an inflammatory disease that occurs in response to endothelial dysfunction. The earliest identifiable lesion is the fatty streak, an inflammatory lesion that consists of monocyte-derived macrophages (foam cells) and T lymphocytes. As a fatty streak progresses to an intermediate and advanced lesion, it forms a fibrous plaque, a process that involves a complex interaction between the endothelium, inflammatory cytokines and numerous blood elements.

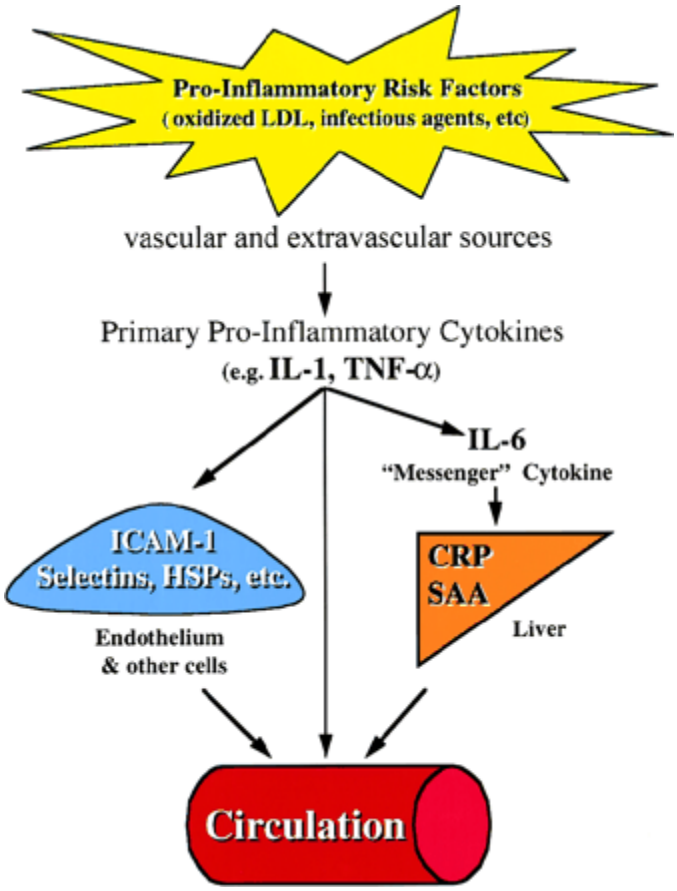


FIGURE 4: CRP IN INFLAMMATION

C-reactive protein, an acute-phase reactant synthesized in the liver in response to the cytokine interleukin-6, is also a factor in the development of atherosclerotic plaque. Although CRP was initially believed to be only a marker of vascular inflammation, recent research indicates that it also plays an active role in atherogenesis. It is detectable in the early stages of plaque development and is believed to be involved throughout the atherogenic process, facilitating everything from the initial recruitment of leukocytes to the arterial wall to the eventual rupture of the plaque.

Smooth muscle cells of the human coronary arteries may also produce CRP as a local response to inflammatory cytokines. They further noted that this locally produced CRP may participate in the atherogenic process. Loss of the pentameric symmetry of CRP can result in a modified or monomeric CRP, which may be the major CRP promoter of the proinflammatory response in the coronary arteries.

Rupture of atheromatous plaque is thought to be the mechanism for acute myocardial infarction and acute coronary syndrome. The most common site of plaque rupture appears to be the shoulder region where inflammatory cells are most prominent. Thus the release of acute phase reactants as a response to inflammation has been proposed as a potential marker of an unstable atheromatous plaque and underlying atherosclerosis.

C-REACTIVE PROTEIN AND CARDIOVASCULAR RISK PREDICTION:

The role of elevated high-sensitivity (hs) C-reactive protein (CRP) as a risk marker for cardiovascular diseases, including coronary heart disease (CHD), stroke and peripheral arterial disease is well established through consistent results from a number of prospective studies. But CRP also conveys important prognostic

information in the setting of the acute coronary syndrome. Subjects presenting with unstable angina or non ST-elevation myocardial infarction and increased levels of hs-CRP are candidates for a variety of adverse events like recurrent angina, ST elevation MI or coronary death. This holds true short term for in hospital complications but also long-term over years as has recently convincingly been shown by data from the Fast Revascularization during InStability in Coronary artery disease (FRISC) trial. Even in the presence of the results of troponin measurements, hs-CRP adds relevant prognostic information. Moreover, persistent elevation of hs-CRP levels after optimal treatment of unstable angina according to current strategies, measured at the time of hospital discharge, is predictive of recurrent events. Thus from the clinical point of view, hs-CRP testing represents a valuable additional diagnostic tool.[56-60]

The ability of hsCRP levels to predict future cardiovascular events has been examined in several cohorts in North America and Europe. Observations are fairly consistent that hsCRP provides predictive value for vascular and total mortality, over and above that provided by more traditional risk factors, such as elevated total cholesterol or LDL-C, lipoprotein (a), homocysteine, or the TC: HDL-C ratio, as well as other markers of inflammation. In these trials, rates of cardiovascular events tend to increase with increasing quartile or quintile of measured hsCRP, with about a two to threefold increase in the highest stratum compared with the lowest.

Studies have shown a positive association between CRP and coronary artery disease. In a survey of 388 British men aged 50-69, the prevalence of coronary artery disease increased 1.5 fold for each doubling of CRP level.[61, 62, 63]

In a recently published prospective study comprising 28 000 women, Ridker et al showed that C-reactive protein (CRP) is a better predictor of the risk of

cardiovascular events than low-density lipoprotein (LDL) cholesterol. The implication of this and many other supporting studies is profound and will change the way we screen and manage our patients with atherosclerosis and its associated clinical syndromes.[64, 65]

hs-CRP >8 mg/L observed in an apparently healthy person should be repeated 2-3 weeks later to rule out a recent undetected infection or injury.[66]

C-REACTIVE PROTEIN AND MYOCARDIAL INFARCTION:

Tissue necrosis is a potent acute-phase stimulus and following myocardial infarction, there is a major CRP response, the magnitude of which reflects the extent of myocardial necrosis. Furthermore, the peak CRP values at around 48 hours after the onset powerfully predict outcome after myocardial infarction, this peak value is associated with outcome, both early and late. Ventricular rupture occurs only in patients with peak serum CRP levels >200 mg/litre and high CRP levels predict mortality over the next 6 months from all causes related to myocardial infarction. Importantly, CRP is co-deposited with activated complement within all acute myocardial infarcts and compelling experimental evidence now suggests that the CRP response not only reflects tissue damage in this context but may also contribute significantly to the severity of ischemic myocardial injury.[67-70]

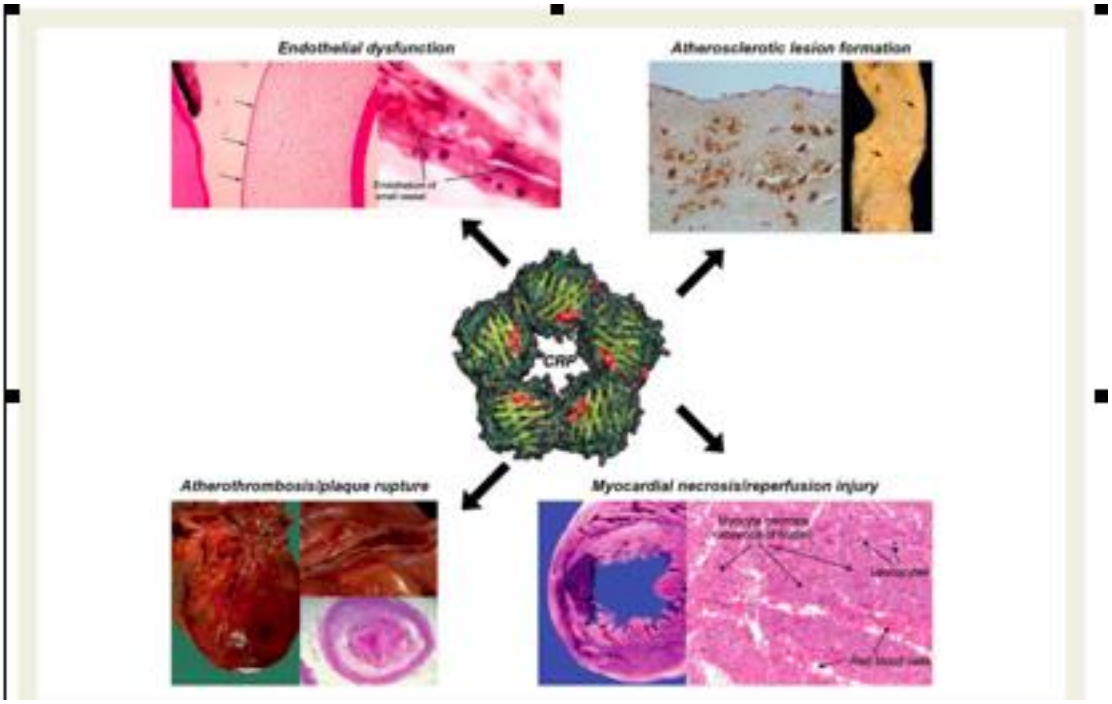


FIGURE 5: EFFECTS OF CRP ON HEART.

Voulgari et al[71] measured CRP in 17 patients with MI. CRP was elevated in all patients. A raised serum CRP level was found on admission in four patients before a rise in creatinine kinase MB isoenzyme. The Peak CRP level was reached on the third post-infarct day. They found that serial monitoring of serum CRP in parallel with cardiac proteins of short half life (CK-MB) and long half life (tropomyosin) provides maximal information for diagnosis and for detecting post infarct complications.

Berk et al[72] measured C-reactive protein in 37 patients with unstable angina, 30 patients with nonischemic illness and 32 patients with stable coronary artery disease. CRP levels were significantly elevated (normal <0.6 mg/dl) in 90% of the unstable angina group compared to 20% in patients with nonischemic illness and 13% of stable angina group. The average CRP values were significantly different ($p < 0.001$) for the unstable angina group (2.2 + 2.9 mg/dl) compared to the patients with non ischemic illness (0.9 + 0.7 mg/dl) and stable angina (0.7 + 0.2 mg/dl) groups. There was a trend for unstable angina patients with ischemic ST-T wave

abnormalities to have higher CRP values (2.6 + 3.4) than those without electrocardiographic changes (1.3 + 0.9, p=0.1). They concluded that an inflammatory component in "active" angina may contribute to the susceptibility of these patients to vasospasm and thrombosis.

It is well recognized that myocardial damage promotes the synthesis of CRP and the level of this CRP has been reported to be associated with poor prognosis after acute myocardial infarction (AMI). However, CRP is primarily synthesized and secreted rapidly in liver 6 h after an acute inflammatory stimulus. Thus, serum levels of CRP within 6 h after the onset of AMI are suggested to offer valuable information with respect to cell biology activity on ruptured plaque without being affected by the effects of myocardial necrosis after AMI.

CRP levels increase dramatically in patients with myocardial infarction beginning 6 hours after the onset of ischemia and peaking at approximately 50 hours. CRP values after acute myocardial infarction predict outcome, including death and heart failure.[73]

Myocardial necrosis following AMI induces free radical generation and triggers the inflammatory cascade. Reperfusion therapy may also lead to further intensification of the inflammatory reaction, with the recruitment of neutrophils into the reperfused myocardium Although this inflammatory response is important in the healing process, it can also extend myocardial injury and anti-inflammatory strategies have been successful in reducing infarct size in animal models.[74]

Hyperglycemia is associated with increased mortality following AMI. Acute hyperglycemia is proinflammatory, inducing the release of cytokines by an oxidative mechanism, resulting in neutrophil-mediated injury of the reperfused myocardium.

On the other hand, insulin exerts anti-inflammatory effects independent of glycaemia, and this can have important implications following myocardial ischemia. Insulin not only suppresses the expression of nuclear factor B in endothelial cells, but also inhibits plasminogen activator inhibitor-1, thus facilitating clot dissolution following AMI. In an intensive care unit study, maintaining normoglycemia with insulin infusion inhibited CRP levels and improved survival. In another study of AMI patients, insulin infusion attenuated the rise of CRP and enhanced fibrinolysis.[75-81]

C-REACTIVE PROTEIN AND ANGIOPLASTY:

Percutaneous coronary intervention, which includes percutaneous transluminal coronary angioplasty and coronary stenting, has continued expanding its role in the management of coronary atherosclerosis. The pathogenesis of restenosis is multifactorial and includes smooth muscle cell proliferation and migration, extracellular matrix production, organization of thrombus, elastic recoil and negative remodelling. Stent implantation, in particular, precipitates arterial intimal cellular proliferation and extracellular matrix synthesis mediated largely by inflammatory processes. Because of these considerable contributions of inflammation, preprocedural measurement of the inflammatory marker CRP has been proposed as a method to identify patients at higher risk of restenosis.

It was also found that preprocedural CRP elevation (>0.3 mg /dl) in patients undergoing coronary angioplasty predicted early complications and late clinical restenosis. So in general, patients with stable or unstable angina and CRP levels greater than 0.3 mg /dl before undergoing coronary angioplasty or bypass surgery should be considered at high risk for ischemic complications.

Furthermore, in patients with significant CRP elevation immediately after coronary intervention, an attempt should be made to measure CRP approximately 72 hours after the procedure to identify an even higher risk group. Those with postprocedural CRP levels greater than 0.5 mg/dl should be followed closely for ischemic events and restenosis.[82, 83]

C-REACTIVE PROTEIN AND ANGINA PECTORIS:

CRP levels correlate with the clinical severity of CAD and with coronary events in both the acute and sub acute phases of myocardial ischemia. Patients who are hospitalized for the treatment of unstable angina and have CRP concentrations above 0.3 mg/dL have significantly more ischemic episodes in the hospital than patients with lower CRP levels.

CRP concentrations are significantly lower in patients with stable angina pectoris than in those with unstable angina pectoris or an acute coronary syndrome. Patients with chronic stable angina who have stable, low CRP levels over time have fewer subsequent cardiovascular events during follow up. On the other hand, in patients with unstable angina pectoris, elevated CRP levels are strong predictors of plaque instability.[84]

Bazzino and associates evaluated the prognostic value of the stress test and CRP concentration after medical stabilization of unstable angina. They showed that elevated levels of CRP (>1.5 mg/dL) were found more often in patients who had died or who had had an MI at 90 days after an acute coronary event. When compared with stress testing, CRP levels demonstrated a greater sensitivity (88% versus 47%) and specificity (81% versus 70%). Moreover, an elevated CRP level at the time of hospital discharge appears to be a more sensitive and specific test marker for increased risk

than a positive stress test. Higher CRP levels are strong predictors of recurrent events, whereas low CRP levels suggest a good outcome.[85]

C-REACTIVE PROTEIN AND OBESITY:

Since human fat cells, particularly those that form around the abdomen, release the pro-inflammatory cytokine interleukin 6 and this induces low grade systemic inflammation. It has been proposed that persons with excess body fat are likely to have higher levels of CRP.

Elevated CRP levels were present in 27.6% of the population. Both overweight and obese persons were more likely to have elevated CRP levels than their normal-weight counterparts. Waist-to-hip ratio was positively associated with both elevated and clinically raised CRP levels, independent of body size. Restricting the analyses to young adults aged between 17-39 years and excluding smokers, persons with inflammatory disease, cardiovascular disease or diabetes mellitus and estrogen users did not change the main findings. These findings suggest a state of low-grade systemic inflammation in overweight and obese person.

The implications are that being fat is partly an inflammatory disorder and body fat promotes inflammation. This may be part of the reason why being overweight increases the risk of diabetes, heart disease and other disorders. CRP levels are generally elevated in overweight children as well as adults.[86]

C-REACTIVE PROTEIN AND HYPERTENSION:

The possible predictive value of serum CRP for the development of hypertension was evaluated in an analysis from the Women's Health Study of over 20,000 female health professionals in the United States with a baseline blood pressure

<140/90 mmHg and no history of hypertension. Serum CRP was measured at baseline and the women followed for a median of 7.8 years; hypertension developed in 11.5 percent. There was a progressive increase in the rate of developing hypertension with increasing values of serum CRP. This observation suggests a role for inflammation in the pathogenesis of hypertension. The association could be related in part to an association between serum CRP and the metabolic syndrome. It is also possible that CRP may directly contribute by reducing nitric oxide synthesis in endothelial cells, leading to increased vascular resistance.[87, 88, 89]

C-REACTIVE PROTEIN AND HEART FAILURE:

Recently, inflammatory markers have been implicated as predictors of heart failure. The inflammatory marker that presently seems most suitable to assess inflammation is CRP. Elevated CRP levels have been associated with an adverse prognosis in patients with heart failure and elevated CRP levels have shown to be predictive of the development of heart failure in high-risk participants. In the Cardiovascular Health Study, increased CRP was an independent predictor of heart failure, the association persisting after adjustment for clinically prevalent as well as subclinical atherosclerotic disease. The Framingham Heart Study demonstrated that participants with CRP serum levels of ≥5 mg/L experienced a significantly increased risk of heart failure, even after adjustment for prevalent cardiovascular disease and the occurrence of myocardial infarction during follow-up.[90-97]

REDUCTION OF C-REACTIVE PROTEIN:

In one large study aspirin was found effective in reducing both CRP and adverse cardiovascular events, especially for men with the highest CRP levels. This observation in fact suggests that aspirin may have benefits over and above its well

known anti-platelet effect, which involves interference with thrombus formation by platelets and is the rationale for its widespread promotion and use in preventive medicine, in spite of the small but significant risk of adverse gastrointestinal bleeding and hemorrhagic stroke. Unfortunately, the effect of aspirin on CRP levels is uncertain, since the data from available studies are in fact inconsistent. In addition, the current U.S. consensus on the use of aspirin for primary prevention of cardiovascular events concludes that the balance of benefit vs. harm is most favourable in patients at high risk of CHD.[98,99]

URIC ACID:

It is the final breakdown product of purine metabolism in humans. It is a weak acid with pKa of 5.75 and 10.3. It is more soluble in urine than in water, possibly because of the presence of urea, proteins and mucopolysaccharides.

HISTORY:

In 1776 a Swedish chemist *Scheele*[100] isolated it from a urinary tract stone. In 1797, a British chemist *Wallaston* detected uric acid in a tophus which was removed from his own ear[101]. About 50 years later *Alfred Baring Garrod*, a British physician showed by chemical isolation that uric acid was abnormally high in gouty patients[102].

Raised serum uric acid has been reported to be associated with an increased risk of coronary heart disease and is commonly encountered with essential hypertension, even untreated hypertension and type 2diabetes, which are in turn associated with coronary heart disease.[103]

It is now known that raised serum uric acid increases the risk of hypertension and type 2 diabetes independently of known risk factors such as age, obesity, alcohol consumption and physical activity.[104]

CHEMISTRY:

The chemical nature of uric acid was shown by Fischer.

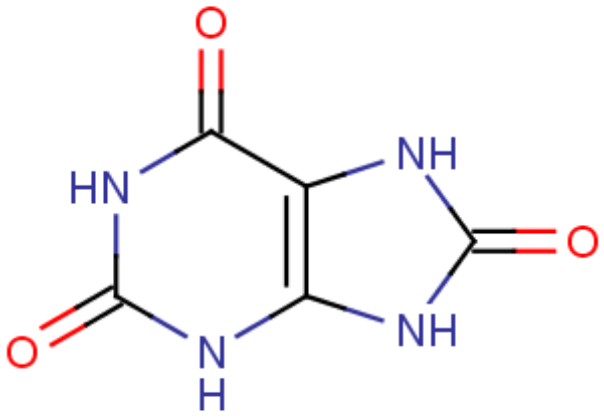


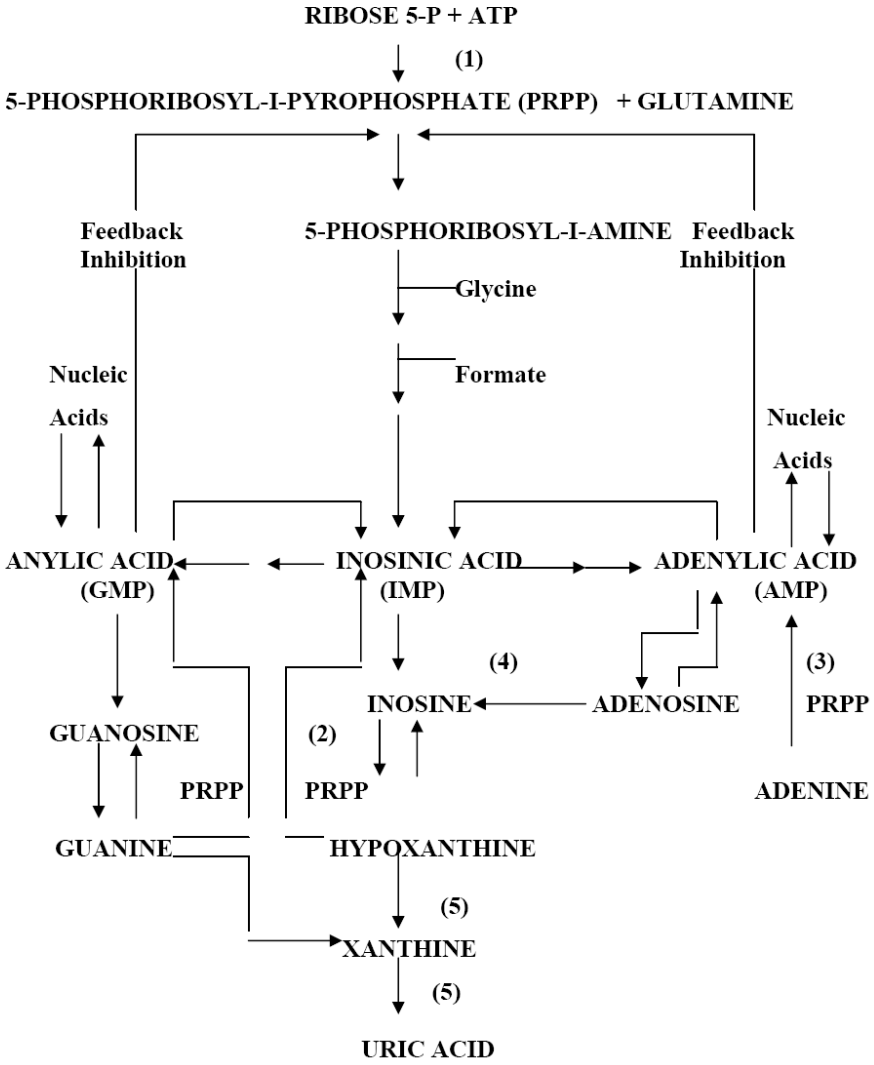
FIGURE 6: STRUCTURE OF URIC ACID.

METABOLISM[105]:

Uric acid is derived from catabolism of,

- Ingested nucleoproteins
- Endogenous nucleoproteins
- Endogenous purine nucleotides.

Uric acid is a breakdown product of purine metabolism. It is produced in liver and excreted through kidney. Uric acid is completely filtered through glomerulus almost fully reabsorbed at proximal convoluted tubule and actively secreted along the tubule.



1. PRPP synthetase.
2. Hypoxanthine guanine phosphoribosyl transferase.
3. Adenine phosphoribosyl transferase.

FIGURE 7: METABOLISM OF PURINE.

While the purine pathway is regulated in a complex manner, the intracellular concentration of 5-phospho-ribosyl-I-pyrophosphate (PRPP) appears to be a major determinant of the rate of synthesis of uric acid in humans. Generally when the concentration of 5-phospho-ribosyl-I-pyrophosphate in the cell is high, uric acid synthesis is elevated, when the concentration of 5-phospho-ribosyl-I-pyrophosphate is reduced the synthesis of uric acid is also reduced.

NORMAL VALUES:

Usually, the urate pool size of an adult male is about 1200 mg, and 700 mg urate is produced daily[106]. The production is balanced by the excretion of 500 mg of urate into the urine and 200 mg into the small intestine. Any imbalance in the two, results in hyperuricemia or hypouricemia.

The normal serum values vary with age and sex. Most children have serum urate concentrations of 180-240 μmol/L i.e., 3 to 4 mg/dl. Levels start rising during puberty in males, but remain low in females till menopause. The gender variation could probably be due to higher excretion in females.

Mean serum urate values of adult men and premenopausal women are 5.3 and 4.7 mg/dl respectively. After menopause, values for women increase to approximate those at men. Among adults, concentrations vary with height, body weight, blood pressure and renal function as well as alcohol intake.

EXCRETION OF URIC ACID:

Of the 700-1000mg of uric acid produced in normal man on a normal diet, about 2/3rd to 3/4th is excreted by the kidney. The remainder is eliminated via small intestine, where intestinal bacteria act on uric acid secreted into the lumen and break it down to more soluble products. On the other hand, renal excretion is the major regulator of plasma urate; decreases or increases in renal clearance are rapidly reflected by inverse changes in the plasma concentration.

CLINICAL SIGNIFICANCE OF URIC ACID:

Hyperuricemia:

Hyperuricemia may be defined as a plasma (or serum) urate concentration >420 umol/L (7.0 mg/dL).

CAUSES OF HYPERURICEMIA [107,108]:

Hyperuricemia may be classified as *primary* or *secondary* depending on whether the cause is innate or is the result of an acquired disorder.

PRIMARY CAUSES:

Gout: Total body content of uric acid may increase to 18000 – 30000 mg.

Clinical conditions in which uric acid is increased are,

- Primary Gout: Ribose Phosphate phosphorylase is deficient.
- Secondary Gout: Due to increased purine catabolism seen in leukemia, prolonged fasting, polycythemia.
- Renal Gout: Uric Acid transport System is affected, resulting in failure of excretion.
- Secondary Renal Gout: Generalized renal failure.

SECONDARY CAUSES:

- Renal failure
- Ketoacidosis

- Lactate Excess
- Pre eclampsic toxemia with increased lactic acid
- Diuretics
- Diet with high purine like meat, viscera, leguminous vegetable and yeast.

Positive relationship of hyperuricemia to hyperlipidemia is seen in following conditions:

• Obesity

• Atherosclerosis

• Diabetes Mellitus

• Hypertension

• Exercise

• Achievement oriented behaviours

INCREASED SERUM URIC ACID IN HYPERTENSION[109]:

The mechanisms underlying the increase in serum uric acid (SUA) and its potential prognostic implications in patients with essential hypertension are still not completely known. Uric acid, a final product of purine metabolism, 5% of it is bound to plasma proteins and is freely filtered at the glomerulus as a function of renal blood flow, is 99% reabsorbed in the proximal tubule, secreted by the distal tubule and subjected to considerable post secretory reabsorption. Fractional secretion of uric acid is about 7% to 10%. A direct association exists between SUA and renal vascular resistance in subjects with essential hypertension.

Uric acid is also commonly associated with hypertension. It is present in 25% of untreated hypertensive subjects, in 50% of subjects taking diuretics and in >75% of subjects with malignant hypertension. The increase in serum uric acid in hypertension may be due to the decrease in renal blood flow that accompanies the hypertensive state, since a low renal blood flow will stimulate urate reabsorption. Hypertension also results in microvascular disease and this can lead to local tissue ischemia.[110]

In addition to the release of lactate that blocks urate secretion in the proximal tubule, ischemia also results in increased uric acid synthesis. With ischemia, ATP is degraded to adenine and xanthine and there is also increased generation of xanthine oxidase. The increased availability of substrate (xanthine) and enzyme (xanthine oxidase) results in increased uric acid generation as well as oxidant (O_2^-) formation. The finding that ischemia results in an increase in uric acid levels may also account for why uric acid is increased in preeclampsia and congestive heart failure.[111]

Other factors may also contribute to why uric acid is associated with hypertension, including alcohol abuse, lead intoxication, obesity and insulin resistance and diuretic use.

The observation that an elevated uric acid is associated with subjects at cardiovascular risk may account for why hyperuricemia predicts the development of cardiovascular disease in the general population, in subjects with hypertension and in subjects with pre-existing cardiovascular disease.

PATHOGENESIS:

Uric acid induces renal vasoconstriction mediated by endothelial dysfunction with reduced nitric acid level and activation of Renin Angiotensin System, this induces hypertension.

Patients with HTN and hyperuricemia have a 3 to 5 fold increased risk of experiencing CAD. In untreated patients with essential hypertension raised uric acid is powerful risks marks for subsequent cardiovascular disease. Uric Acid stimulates vascular smooth muscle cell proliferation mediated by stimulation of mitogen activated protein kinase, cycloxygenase 2, platelet derived growth factor. So elevated uric acid predicts severity of heart failure and need for heart transplantation in patients with chronic heart failure.[110]

Serum uric acid was directly related to BMI, creatinine, triglyceride, LDL cholesterol, components of metabolic syndrome and inversely proportional to HDL cholesterol. There is significant association of serum uric acid with preclinical target organ damage namely LVH, carotid atherosclerosis, microalbuminuria, in a untreated essential hypertensive patients regardless of other cardiovascular risk factors.

In vitro, free uric acid was found to increase vascular smooth muscle cells, monocyte chemo attractant protein I((MCP I), increased mRNA protein expression occurring as early as 3 hr after uric acid incubation.

In addition, uric acid activated the transcription factors kB, activator protein I, mitogen activated protein kinase signalling molecule ERK p44/42 and p38 and increased cycloxygenase 2 mRNA expression.

The NHANES 1 Epidemiologic follow study showed raised uric acid level is independently associated with risk of cardio vascular mortality.

URIC ACIS IS INCREASED IN GROUPS AT CARDIOVASCULAR RISK [109]:

GROUP	MECHANISM
Postmenopausal women and men	Estrogen is uricosuric
Renal disease	Decrease in GFR increases uric acid levels
Diuretics	Volume contraction promotes urate reabsorption
Obesity/ insulin resistance	Insulin increases sodium reabsorption and is tightly linked to urate reabsorption.
Hypertension	Urate reabsorption increased in setting of increased renal vascular resistance; microvascular disease predisposes to tissue ischemia that leads to increased urate generation and reduced excretion.
Alcohol use	Increase urate generation, decrease urate excretion.

TABLE 1 : GROUPS AT RISK OF CVD WITH HYPERURICEMIA

URIC ACID AS A MARKER OF SUBCLINICAL ISCHEMIA[112]:

Adenosine is synthesized and released by cardiac and vascular myocytes. Binding to specific adenosine receptors causes relaxation of vascular smooth muscle and arteriolar vasodilatation. Under conditions of hypoxia and tissue ischaemia, vascular adenosine synthesis and release are upregulated, causing significantly increased circulating concentrations. Cardiac and visceral ischemia promotes generation of adenosine, which may serve as an important regulatory mechanism for restoring blood flow and limiting the ischaemia.[113]

Adenosine synthesized locally by vascular smooth muscle in cardiac tissue is rapidly degraded by the endothelium to uric acid, which undergoes rapid efflux to the vascular lumen due to low intracellular pH and negative membrane potential. Xanthine oxidase activity and uric acid synthesis are increased *in vivo* under ischemic conditions and therefore elevated serum uric acid may act as a marker of underlying tissue ischemia. In the human coronary circulation, hypoxia, caused by transient coronary artery occlusion, leads to an increase in the local circulating concentration of uric acid.[114]

Study of tourniquet-induced lower limb exsanguination in patients undergoing surgery shows a five-fold increase in systemic vascular xanthine oxidase activity during reperfusion and a significant elevation of serum uric acid, which persists for atleast 2 h.[115]

These findings are also consistent with the inverse relation between baseline serum uric acid concentration and maximal lower limb blood flow in patients with cardiac failure, where higher concentrations could predict subclinical ischaemia[116]. In conclusion therefore, elevated serum uric acid may be a marker of local or systemic tissue ischemia and provides one possible explanation for a non-causal associative link between hyperuricaemia and cardiovascular disease.

URIC ACID AS A MARKER OF INSULIN RESISTNCE:

Insulin resistance syndromes result in attenuation of insulin-mediated glucose utilization and confer a substantial increase in cardiovascular risk[117] through activation of several pathways including the sympathetic nervous system. Elevated serum uric acid is a consistent feature of the insulin resistance syndromes, which are also characterized by elevated plasma insulin level (fasting and post-carbohydrate),

blood glucose concentration and serum triglyceride concentration and raised body mass index and waist-hip ratio.[118]

Insulin has a physiological action on renal tubules, causing reduced sodium and uric acid clearance. Because plasma insulin concentration is characteristically elevated, hyperuricemia may arise as a consequence of enhanced renal insulin activity. Elevated serum uric acid concentrations predict subsequent development of diabetes mellitus and hypertension, even in the presence of normal creatinine clearance and plasma glucose concentrations and therefore may be a subtle, early marker of peripheral insulin resistance syndromes. Thus a link between elevated serum uric acid concentration and cardiovascular disease may arise through its non-causal relationship with insulin resistance syndromes, where cardiovascular risk is mediated by other factors.

DIRECT IMPACT OF URIC ACID ON VASCULAR FUNCTION:

The endothelium plays a central role in maintaining vascular tone through synthesis and release of nitric oxide, a potent vasodilator. Reduction of nitric oxide bioavailability is an important early step in the development of atherosclerosis. So-called endothelial dysfunction, associated with impaired endothelium-dependent vasodilatation may arise from excessive free radical activity, which disrupts synthesis and accelerates degradation of nitric oxide. Thus increased oxidative stress appears to have an important role in development and progression of atherosclerosis and is a characteristic finding associated with its major risk factors, such as diabetes mellitus, hypertension, hypercholesterolemia and smoking.[119,120]

Serum uric acid possesses antioxidant properties and contributes about 60% of free radical scavenging activity in human serum. Uric acid interacts with peroxynitrite

to form a stable nitric oxide donor, thus promoting vasodilatation and reducing the potential for peroxynitrite-induced oxidative damage. Thus, uric acid could be expected to protect against oxidative stresses.

However, uric acid has been found to promote low-density lipoprotein (LDL) oxidation *in vitro*, a key step in the progression of atherosclerosis[121] and these effects are inhibited by vitamin C indicating an important interaction between aqueous anti-oxidants. Uric acid can also stimulate granulocyte adherence to the endothelium and peroxide and superoxide free radical liberation. Therefore uric acid may have a deleterious effect on the endothelium through leukocyte activation and interestingly, a consistent relationship has been noted between elevated serum uric acid concentration and circulating inflammatory markers.[122]

Uric acid traverses dysfunctional endothelial cells and accumulates as crystal within atherosclerotic plaques[123].These crystals may contribute to local inflammation and plaque progression. Thus, while uric acid appears to make a significant contribution to serum anti-oxidant capacity, it could also lead directly or indirectly to vascular injury.

High uric acid is strong independent marker of impaired prognosis with moderate chronic heart failure[124]. Uric acid is also strong predictor of stroke with myocardial infarction and non insulin dependent diabetes mellitus.[125]

DIABETES MELLITUS AND CORONARY HEART DISEASE:

Cardiovascular disease (CVD) is the main cause of morbidity and mortality in patients with diabetes mellitus (DM). A combination of complex factors such as hyperglycemia, insulin resistance, dyslipidemia, hypertension, oxidative stress, endothelial dysfunction, inflammation and hypercoagulability, contribute not only to

the initiation and progression of atherosclerosis and thrombosis, but also to direct myocardial dysfunction in patients with DM.

The pathophysiologic mechanisms by which DM increases CVD are complex and multifactorial. DM is associated with an increased risk for atherosclerosis independent of the diabetes-induced increase of the known risk factors such as hypertension, dyslipidemia and insulin resistance. This increased atherosclerosis risk is likely related to the combination of hyperglycemia-induced, oxidative stress, altered lipoproteins, increased advanced glycation products, endothelial dysfunction and inflammation. Both insulin resistance syndrome (IRS) and hyperglycemia seem to affect the risk of CVD in patients with DM.[126]

Diabetes Mellitus is also associated with direct myocardial dysfunction, in addition to the increased risk of coronary atherosclerosis.[127]

Metabolic and Hemodynamic Abnormalities in DM Associated with Increased Risk of CVD (Atherosclerotic CVD):

- Metabolic and biochemical abnormalities

  Hyperglycemia, dyslipidemia (hypertriglyceridemia, reduced HDL-C, increased small dense LDL, hyperinsulinemia, insulin resistance, hyperhomocysteinemia, renin-angiotensin activation.
- Increased oxidative stress

  Increased generation of AGEs, activation of polyol pathway and hexosamine pathway, lipid peroxidation, protein kinase C activation, vascular smooth muscle hyperplasia and hypertrophy, intimal lipid accumulation.

- Endothelial dysfunction

  Decreased nitric oxide bioavailability, impaired endothelial vasorelaxation and impaired permeability of endothelial tight junction.

  Increased sympathetic nervous system activity and sodium retention.

- Low-grade chronic inflammation and fibrosis

  Increased CRP, cytokines, TNF-α, angiotensin -II, vascular endothelial growth factor (VEGF), increased P-selectin, vascular cell adhesion molecule (VCAM)-1, intracellular adhesion molecule (ICAM)-1.

- Prothrombotic state.

  Increased PAI-1, increased platelet activation, increased fibrinogen.

- Cardiac dysfunction.

  Impaired myocardial glycolytic oxidative metabolism; microcirculatory endothelial dysfunction, abnormal sympathetic function, impaired calcium cycling, reduced cardiac compliance and diastolic dysfunction.

- Associated risk factors

  Hypertension and obesity.

EFFECTS OF DIABETES ON THE PATHOGENESIS OF ATHEROSCLEROSIS[128]:

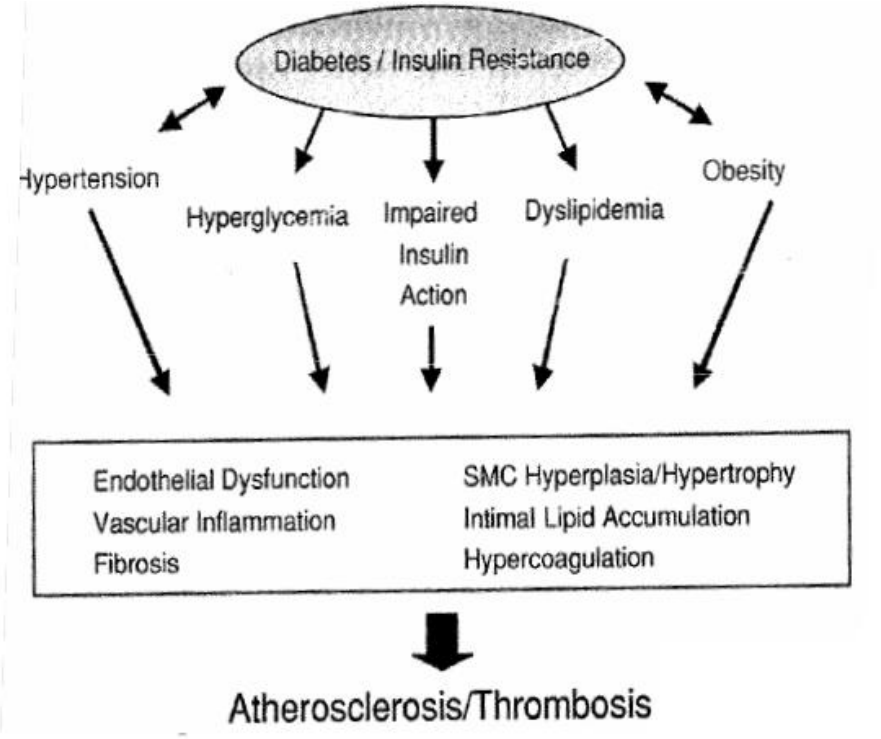


FIGURE 8: EFFECTS OF DIABETES ON ATHEROSCLEROSIS.

ENDOTHELIAL DYSFUNCTION IN DIABETES MELLITUS:

Studies have shown that chronic hyperglycemia and insulin resistance cause endothelial dysfunction. Endothelial dysfunction contributes to the development of atherosclerosis. The endothelial cell serves as an interface between circulating blood and vascular smooth muscle cells and therefore facilitates a complex array of functions in intimate interaction with the vascular smooth muscle cells, as well as cells within the blood compartment such as monocytes. The normal endothelium mediates vasodilatation, suppresses thrombosis and suppresses vascular inflammation and hypertrophy. Endothelial dysfunction, manifested as an impaired response to

vasodilators, and increased thrombosis, inflammation, and vascular smooth muscle cells growth and hypertrophy, is well documented in DM.[129,130]

Factors associated with endothelial dysfunction in DM include hyperglycemia, increased free fatty acids (FFAs), altered lipoproteins, oxidative stress, over expression of growth factors and cytokines, increased derivatives of glycation and activation of protein kinase C.

ALTERATIONS IN VASCULAR ENDOTHELIUM ASSOCIATED WITH DIABETS MELLITUS[131]:

Abnormality	Significance
↓ Release of and responsiveness to NO	Impaired endothelial function and reactivity
↑ Expression, synthesis, and plasma levels of endothelin-1	Vasoconstriction and hypertension
↑ Adhesion-molecule expression (VCAM-1; ICAM-1)	Increased monocyte adhesion to vessel wall
↑ Adhesion of platelets and monocytes	Foam cell formation, thrombosis, and inflammation
↑ Procoagulant activity (PAI-1; fibrinogen)	Thrombosis
↑ Advanced glycosylated end products	Increased stiffness of arterial wall
Impaired fibrinolytic activity	Decreased clot breakdown

↑, Increased; ↓, Decreased; NO, nitric oxide; PAI-1, plasmin activator inhibitor; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1

TABLE 2: ALTERATIONS IN VASCULAR ENDOTHELIUM IN DISBETES MELLITUS.

EFFECTS OF DIABETES ON VASCULAR COMPLIANCE AND ARTERIAL STIFFNESS:

Arterial stiffness is strongly associated with atherosclerosis. The Strong Heart Study[132] and the Atherosclerosis Risk in Communities Study[133] have reported increased arterial stiffness and reduced compliance in subjects with type 2 diabetes.

Several theories have been proposed to explain the increased arterial stiffening in diabetes. Increased glycation of ECM proteins, such as collagen and elastin, may increase covalent intermolecular cross-linking and thereby reduce elasticity.

Increased arterial stiffness may also result from arterial calcification, arterial-wall thickening or increased chronic vascular smooth muscle cells (VSMC) contraction due to an imbalance in vasoactive hormone activities. Impaired arterial elasticity might contribute to atherogenesis by increasing mechanical strain and shear forces, causing endothelial damage, activation of stretch-activated mechanoreceptors and increased release of trophic factors.[134]

METABOLIC DERANGEMENTS CONTRIBUTING TO CARDIOVASCULAR DISEASES IN DIABETES MELLITUS:

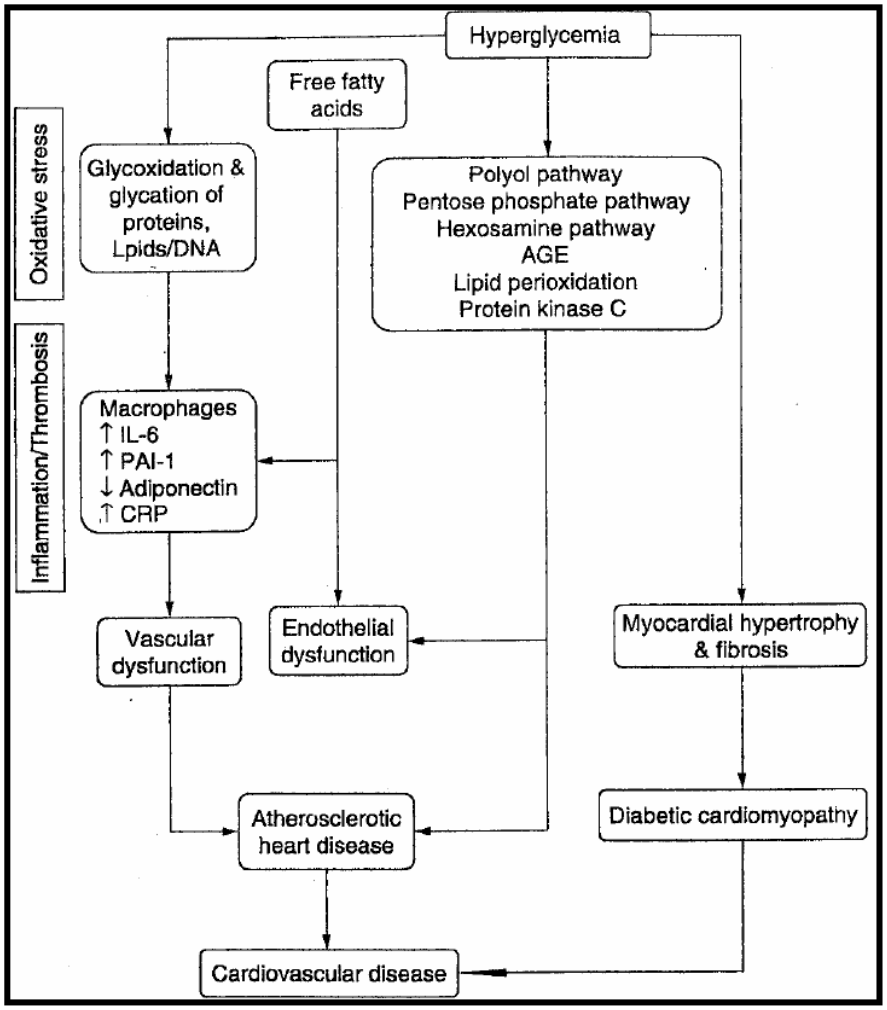


FIGURE 9: METABOLIC DERANGEMENTS IN DIABETES MELLITUS.

LOW-GRADE INFLAMMATION, COAGULATION AND THROMBOSIS IN DIABETES MELLITUS:

Diabetes mellitus is associated with excessive amount of adipose tissue. Visceral obesity contributes to the clustering of multiple risk factors for CVD.[135]

Recent advances in basic science have established a fundamental role for inflammation in mediating CVD through the initiation, progression and thrombotic

complications of atherosclerosis. Subclinical elevations of inflammatory markers including C-reactive protein (CRP), IL-6, fibrinogen and PAI-1 are observed in insulin resistance and type 2 DM.[136]

Increased visceral fat store is associated with macrophage infiltration that secretes proinflammatory molecules such as TNF-α, IL-6 and IL-1β. These cytokines augment inflammation and decrease insulin sensitivity. Cytokines from adipocytes may also contribute to insulin resistance by increasing TNF-α and IL-6 or decreasing adiponectin.[137]

Adiponectin is an insulin-sensitizing adipocytokine that is decreased with obesity, and DM. Indices of oxidative stress are significantly correlated with body mass index and inversely related to plasma adiponectin[138]. Moreover, inflammatory adipocytokines give rise to E-selectin and ICAM-1 in the endothelium, which participates in the migration of inflammatory cells to the subendothelial space, promoting the development of foam cells and unstable atherosclerotic plaque.[139]

HYPERTENSION AND CORONARY HEART DISEASE:

EFFECTS OF HTN ON HEART:

Hypertension places increased tension on the left ventricular myocardium that is manifested as stiffness and hypertrophy, which accelerates the development of atherosclerosis within the coronary vessels.

Abnormalities in Left Ventricular Function- the earliest functional changes in hypertension are in left ventricular diastolic dysfunction, with lower E/A ratio and longer isovolemic relaxation time.[140]

PATHOGENESIS[144]**:**

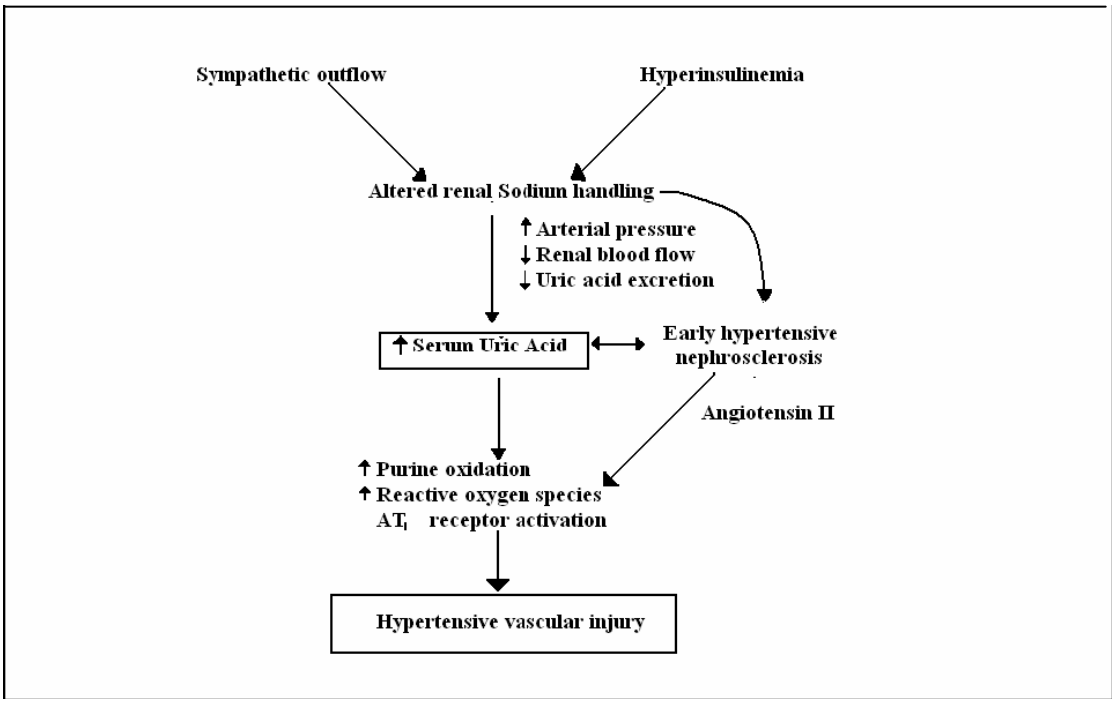


FIGURE 10: PATHOGENESIS OF HYPERTENSIVE VASCULAR INJURY.

LEFT VENTRICULAR HYPERTROPHY:

Hypertrophy as a response to the increased after load associated with elevated systemic vascular resistance can be viewed. Variety of dysfunctions accompany LVH, including lower coronary vasodilatory capacity, depressed left ventricular wall mechanics, and abnormal left ventricular diastolic filling pattern.[141]

CONGESTIVE HEART FAILURE:

The various alterations of systolic and diastolic function seen with LVH can progress into congestive heart failure. A 20 mmHg increment in systolic blood pressure conferred a 56% increased risk of CHF in the Framingham cohort.

When haemodynamically challenged by stress, persons with hypertension are unable to increase their end diastolic volume, because of decreased left ventricular relaxation and compliance. Consequently, a cascade begins, in which left ventricular end diastolic blood pressure rises, left atrial pressure increases and pulmonary edema develops.[142]

CORONARY HEART DISEASE:

Hypertension is a major risk factor for myocardial infarction and ischemia. Acute rise in blood pressure may follow the onset of ischemic pain; the blood pressure often falls immediately after the infarct if pump function is impaired. Once an MI occurs, the prognosis is affected by both the pre-existing and the subsequent blood pressure.[143]

The prevalence of silent MI is significantly increased in hypertensive subjects, and they have a greater risk for mortality after an initial MI.

METHODOLOGY

MATERIALS AND METHODS:

STUDY DESIGN: Cross sectional study

SOURCE OF DATA: RL Jalappa Hospital & Research centre, Kolar and RL Jalappa Narayana Hruduyalaya Hospital, Kolar.

With the calculation taking Odd's ratio as based on the mortality of CHD patients at 95% confidence interval and 80% power, sample size of 30 was determined.

STUDY GROUP: Consists of 60 individuals

Case group: 30 diagnosed cases of coronary heart disease

Control group: 30 healthy individuals.

CASE GROUP:

INCLUSION CRITERIA:

1. 30 diagnosed cases of coronary artery diseases which includes myocardial infarction, stable angina, and unstable angina of either sex admitted at Narayana Hruduyalaya and RL Jalappa Hospital and Research Centre, Kolar.
2. CHD cases with hypertension and diabetes mellitus.
3. Age above 18 years

EXCLUSION CRITERIA:

1. Acute infectious diseases.
2. Liver diseases.

3. Renal diseases.

4. Alcoholics and smokers

CONTROL GROUP:

1. Age and gender matched healthy population.

2. Control group will be screened for the complete blood tests and if they fall within normal reference range they were included as controls.

METHOD OF COLLECTION OF DATA:

- 5ml of blood was collected from the anti cubital vein under complete aseptic precautions after obtaining informed consent from the case and control groups and

- Ethical clearance from SDUAHER institutional ethical committee has been taken for the study.

PARAMETERS MEASURED:

In the present study following parameters were estimated by using the serum.

1. Blood glucose

2. CK-MB

3. Serum uric acid

4. High sensitive C-reactive protein.

Blood glucose, CK-MB, Serum uric acid were analyzed using semi-autoanalyzer.

Highly sensitive C-reactive protein was analyzed using immunoturbidimetric method.

ESTIMATION OF HIGH SENSITIVE – CRP[145]:

METHOD: Immunoturbidimetric method.

PRINCIPLE:

Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate is determined turbidimetrically.

REAGENTS:

R1: TBIS buffer with bovine serum albumin and mouse immunoglobulins.

R2: Latex particles coated with anti-CRP from mouse in glycine buffer.

Specimen: Serum or heparin / EDTA plasma

Reaction time: 12-70 min

Wavelength: 546nm

Units: mg/dl.

PROCEDURE:

	VOLUMES	***DILUENTS (H_2O)***
R1	82ul	42ul
R2	28ul	20ul
Sample	6ul	-

CALCULATION:

Cobas C system automatically calculates the analyte concentration in each sample.

MEASURING RANGE: 0.15-20.0 mg/L

REFERENCE RANGE:

The CDC/AHA recommended the following hsCRP cut-off points for CVD risk assessments.[44]

hsCRP LEVEL mg / L	***RELATIVE RISK***
<1.0	Low
1.0-3.0	Average
>3.0	High

TABLE 3: hs-CRP CUT –OFF LEVELS

CK-MB [146]:

METHOD: Antibody kinetic method.

PRINCIPLE:

Specific antibodies against CK M inhibit the complete activity of CK MM (main part of the total CK activity) and the CK M subunit of the CK MB. Only CK B activity is measured, which is half of the CK MB activity.

REAGENTS:

Reagent 1:

Imidazole - 60 mmol/l

Glucose - 25 mmol/l

N Acetyl cysteine - 25 mmol/l

Magnesium acetate - 12.5 mmol/l

EDTA - 2mmol/l

NADP - 2.5mmol/l

Hexokinase - 5 KU/l

Polyclonal antibodies (goat) against human CK MB ≥2500 u/l

Reagent 2:

Imidazole - 160 mmol/l

ADP - 10 mmol/l

AMP - 28 mmol/l

G6PDH - ≥15 k U/l

Di adenosine penta phosphate - 50 µol/l

Creatine phosphate - 150 mmol/l

Preservative - sodium azide 0.95 g /l

SAMPLE: Serum or Plasma

AUTOMATED PARAMETERS:

Wavelength : 340 nm

Temperature: 37^0 c

PROCEDURES:

	BLANK	***CALIBRATOR***	***SAMPLE***
CALIBRATOR	-	40 µl	-
SAMPLE	-	-	40 µl
DISTILLED WATER	40 µl	-	-
REAGENT	1000 µl	1000 µl	1000 µl

Mix read absorbance after 5 min start stop watch read absorbance again after 1, 2, 3, 4 and 5 min.

CALCULATIONS:

CK-MB [U/L] = {(Δ A / Min sample) / (ΔA/ min Calibrator)} X Conc. Calibrator [U/L]

REFERENCE INTERVALS:

CK (MEN):	>190 U/L
CK (WOMEN):	>167 U/L
CK MB:	>24 U/L

CK MB Activity is between 6 and 25 % of total CK activity.

URIC ACID[147]:

METHOD: Enzymatic colorimetric method.

PRINCIPLE:

Uric acid is converted by uricase to allantoin and hydrogen peroxide, which under the catalytic influence of peroxidase, oxidises 3, 5-dichloro-2-hydroxybenzenesulfonic acid and 4-aminophenazone to form a red-violet quinoneimine compound.

REAGENTS:

Reagent: Enzyme reagent

Uric acid standard: 5 mg/dl

SAMPLE: Serum, heparinised plasma or EDTA plasma, urine.

AUTOMATED PARAMETERS:

Wavelength: 520 nm

Incubation time: 10 min

Reaction temperature: 37^0 C

PROCEDURE:

	BLANK	STANDARD	TEST
SAMPLE	-	-	25µl
STANDARD	-	25µl	-
REAGENT	1000µl	1000µl	1000µl

Mix well, incubate at room temperature for 10 min at 37^0 C. Measure final absorbance of the sample (Ac) and standard (As) against the reagent blank. The colour is stable for 60 min at 20-25^0 C.

CALCULATION:

Ac/As x C = mg/dl Uric acid *Serum or plasma*

C= Concentration of standard

LINEARITY:

The method is linear up to a concentration of 25 mg/dl.

REFERENCE VALUES:

Serum:

Men: 3.4-7.0 mg/dl

Women: 2.4-5.7 mg/dl

Urine: 250-750 mg/dl

BLOOD GLUCOSE[148]:

METHOD: Glucose Oxidase-Peroxidase method.

PRINCIPLE:

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase (GOD). The hydrogen peroxide formed reacts, under catalysis of peroxidase (POD), with phenol and 4-aminophenazone (4AAP) to form a red violet quinonemine dye as indicator.

REAGENT:

- Enzyme reagent- Phosphate buffer 50 mmol/L, Phenol 15 mmmol/L, 4AAP 2.5mmol/L, GOD 18 KU/L, POD 2.5 KU/L
- Glucose standard- 100 mg/dL
- Preservative: Sodium azide (0.02%)

LINEARITY: The method is linear up to a concentration of 400mg/dL (200mmol/L).

AUTOMATED PARAMETERS:

Wavelength: 505nm (490-550nm)

Reaction temperature: 37^0 C

PROCEDURE:

	BLANK	STD	SAMPLE
SAMPLE	-	-	10µl
STANDARD(STD)	-	10µl	-
REAGENT	1000µl	1000µl	1000µl

Mix & incubate for 05min at 37^0 C. Measure absorbance of sample (AT) and standard (AS) against reagent blank at 505 nm.

CALCULATION:

TOTAL GLUCOSE(mg/dl) = AT/AS X Concentration of standard (100 mg/dl)

REFERENCE INTERVAL:

RANDOM BLOOD GLUCOSE: 75-140 mg/dl

Urine: <0.5 g/dl

STATISTICAL ANALYSIS

- The data collected was tabulated and analyzed using descriptive statistical tool.
- Mean and standard deviation was calculated for serum uric acid, hs-CRP, random blood sugar, CK-MB individually for cases and controls.
- This mean and standard deviation of cases and controls was compared using independent‘t’ test.
- Correlation between each parameter, i.e. correlation between CK-MB with hs-CRP and serum uric acid was done using Pearson’s correlation.
- p value of < 0.05 was taken statistically significant.

RESULTS

RESULTS

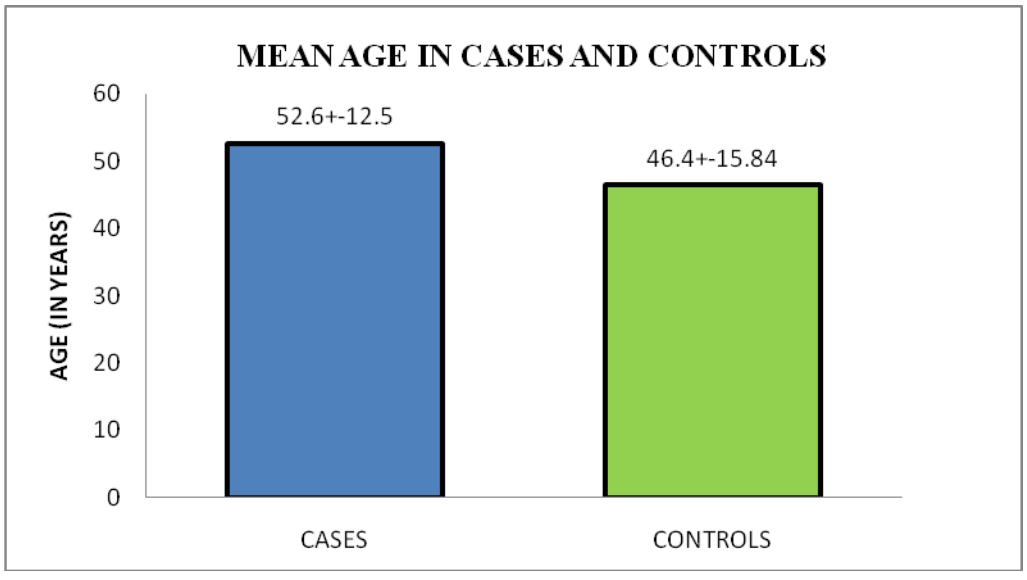
In the present study, 60 subjects were selected considering the inclusion and exclusion criteria stated in the methodology. Among them 30 were CHD cases and 30 were age and gender matched controls.

PRESENTATION OF DATA:

Master chart showing the blood glucose, serum uric acid, high sensitive C-reactive protein, CK-MB and blood pressure levels with hospital number, age and gender of the subjects obtained during the study in annexure 2.

AGE DISTRIBUTION OF CASES AND CONTROLS:

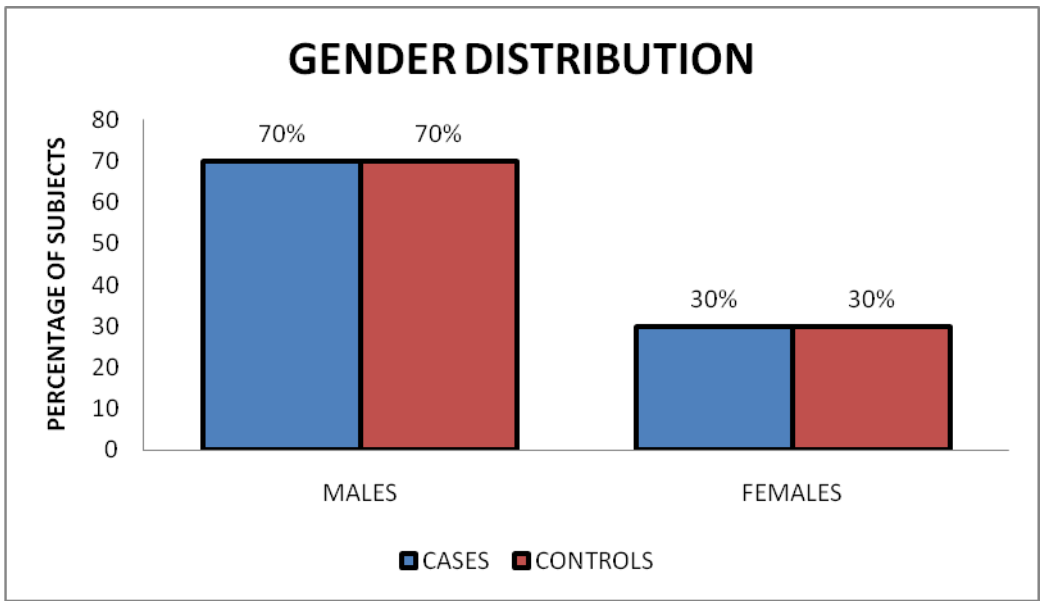
The mean age of CHD in cases and controls were 52.6 ± 12.5 and 46.4 ± 15.84 respectively as shown in graph 1.



GRAPH 1: MEAN AGE IN CASES AND CONTROLS.

GENDER DISTRIBUTION OF CASES AND CONTROLS:

The percentage of female in the cases and controls was 30% and the percentage of males in cases and controls was 70% as shown in the graph



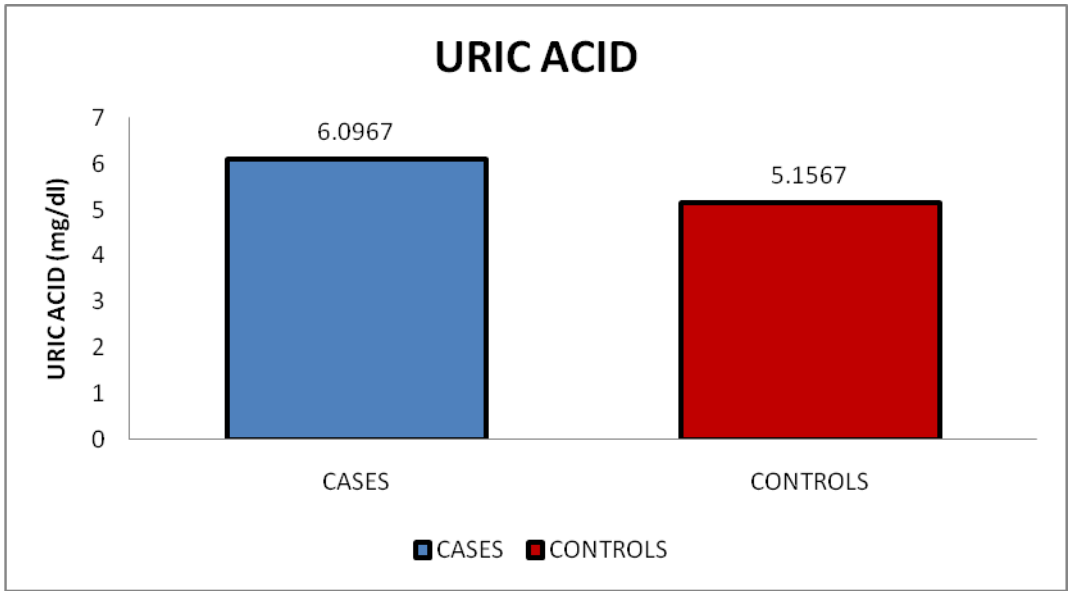
GRAPH 2: GENDER DISTRIBUTION

PARAMETER	*CASES*	*CONTROLS*	*t-VALUE*	*p-VALUE*
Random blood sugar (mg/dl)	222.63	87.36	7.6	<0.001**
Serum uric acid (mg/dl)	6.1	5.16	2.79	<0.008*
hs-CRP (mg/l)	7.1	0.185	4.66	<0.001**
CK-MB (u/l)	166.2667	16.0667	8.18	<0.001**

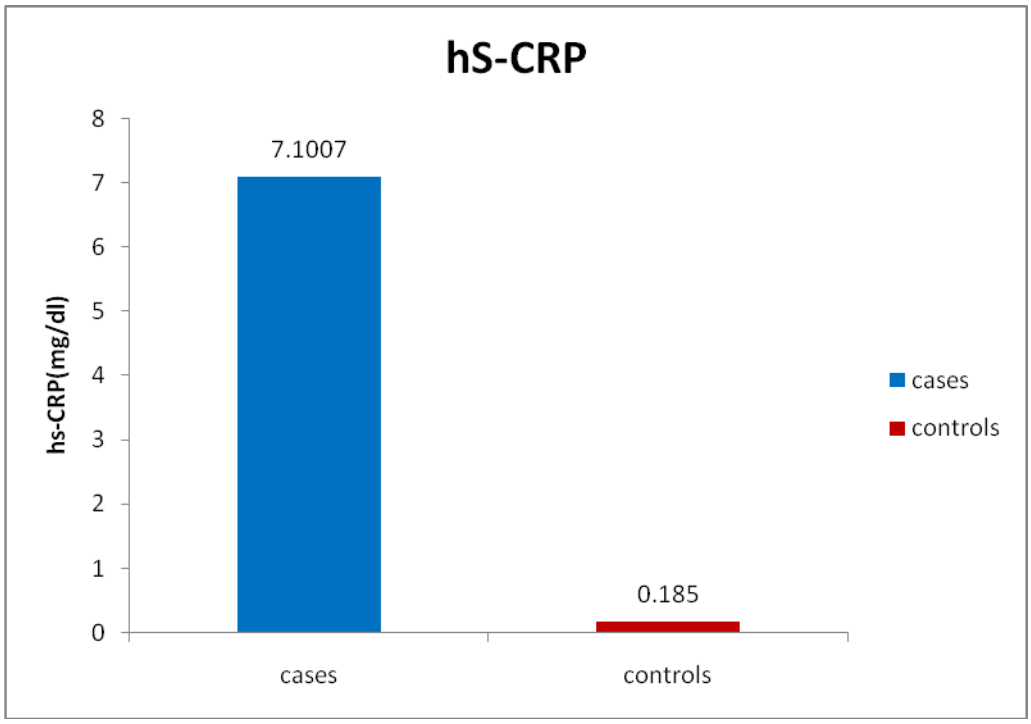
- SIGNIFICANT ** HIGHLY SIGNIFICANT

TABLE 4: INDEPENDENT t-TESTS COMPARING THE MEAN VALUES OF THE PARAMETERS BETWEEN THE CASES AND CONTROLS.

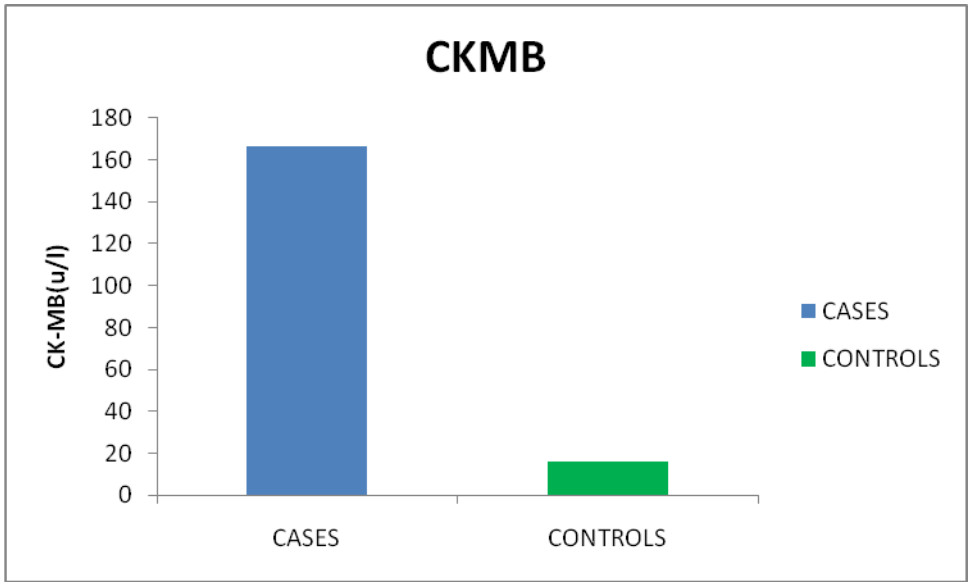
- The mean serum uric acid levels were raised in cases (6.1±1.54mg/dl) compared to the controls (5.16±1.007mg/dl) which was significant statistically ($p < 0.008$) as shown in the table 4 and graph 3.
- The mean hs-CRP levels were raised in cases (7.1±8.122mg/dl) compared to the controls (0.185±0.254mg/dl) which was highly significant statistically ($p < 0.001$) as shown in the table 4 and grapg 4.
- The mean CK-MB values were raised in cases (166.2667±100.37u/l) compared to the controls (16.0667±4.01u/l) which was highly significant statistically ($p < 0.001$) as shown in the table 4 and grapg 5.
- The mean blood glucose levels were raised in cases (222.63±96.19mg/dl) compared to the controls (87.36±8.70mg/dl) which was highly significant statistically ($p < 0.001$) as shown in the table 4 and graph 6.



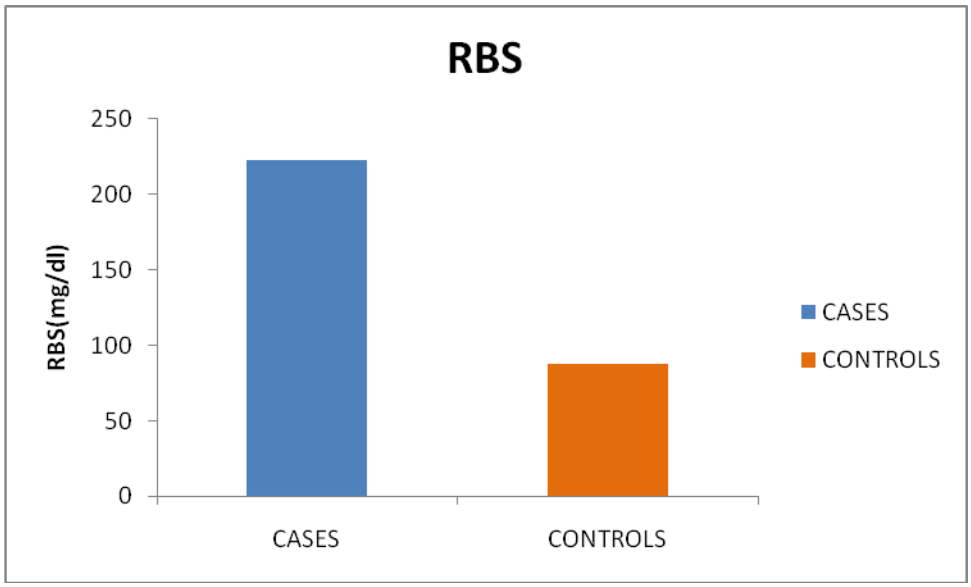
GRAPH 3: INDEPENDENT t TEST BETWEEN CASES AND CONTROLS



GRAPH 4: INDEPENDENT t TEST BETWEEN CASES AND CONTROLS



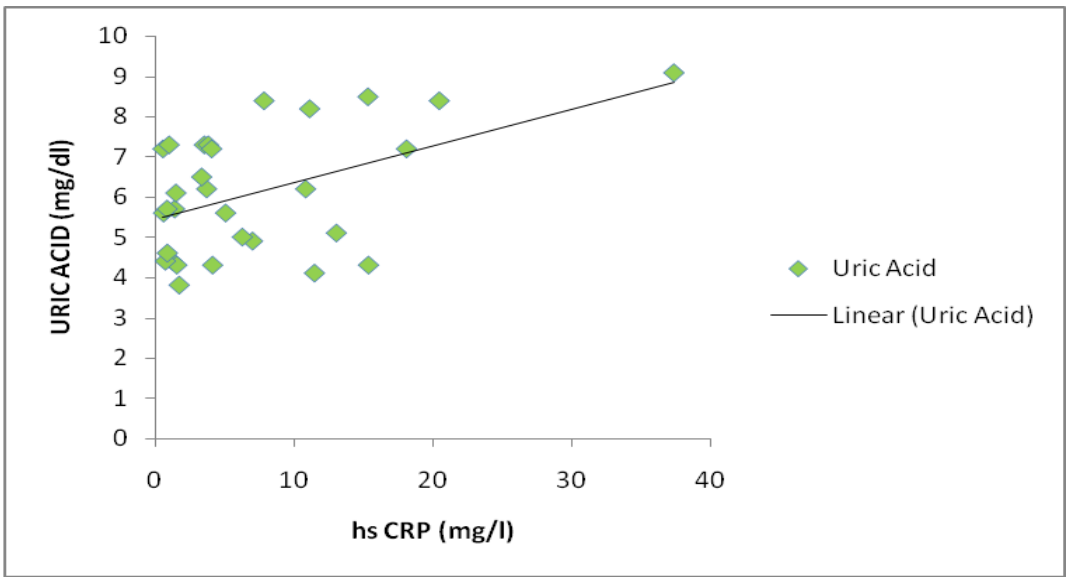
GRAPH 5: INDEPENDENT t TEST BETWEEN CASES AND CONTROLS



GRAPH 6: INDEPENDENT t TEST BETWEEN CASES AND CONTROLS

CORRELATION BETWEEN CK- MB AND HS-CRP IN CASES:

- Pearson's correlation, showed significant and positive correlation between serum uric acid and hs-CRP in cases ($r= 0.479$, $p < 0.007$) as shown in Graph 7.
- There was no significant correlation between CK-MB with hs-CRP and uric acid levels in cases.



GRAPH 7: CORRELATION BETWEEN SERUM URIC ACID AND hs-CRP IN CASES.

DISCUSSION

DISCUSSION

The present study was a cross sectional study done by selecting 60 subjects of which 30 were cases of coronary artery disease and 30 were age and gender matched normal healthy controls. The percentage of males in the cases and controls were 70% and females were 30%. Baseline parameters namely random blood glucose, CK-MB was estimated in cases and controls. Measurement of the serum levels of uric acid and high sensitive C-reactive protein in the cases was done and compared with the controls.

The levels of hs-CRP were found to be significantly increased in the cases when compared with the controls. This could be due to both atherogenic and thrombogenic vascular potential of hsCRP and there is substantial evidence that hsCRP might contribute directly to the pathogenesis of atherothrombosis[50]. Positive association of serum hsCRP with CHD found in the present study may be due to the indirect facilitatory role of hsCRP in atherosclerosis by endothelial injury or dysfunction.[89]

hsCRP is a ligand binding protein that binds to the phospholipids of plasma membranes of damaged cells with subsequent limited activation of the complement system. This complement system enhances the damaged cells to express cytokines which stimulates the liver to release CRP at the inflammatory site in an autocatalytic manner. hsCRP was found to amplify the pro-inflammatory effects of several mediators which lead to promotion of development of atherosclerosis.

hsCRP has been found to be a potent stimulator of tissue factor production by macrophages in vitro. Tissue factor is the main initiator of coagulation and atherosclerosis in vivo and so its local concentration in the arterial wall is clearly

related to coronary atherothrombotic events. Thus the capacity of hsCRP to enhance tissue factor production suggests a possible causative link between increased hsCRP values and coronary events.

Acute inflammation is a component of the pathophysiology of CAD. Therefore it suggests that inflammation is not only an important trigger mechanism of coronary syndrome related to plaque rupture, but also promoter of chronic atherosclerosis, as proposed that hs-CRP might play an atherogenic role through an interaction with low density lipoproteins.[149]

There are several potential mechanisms that may account for the observed relationship between blood pressure and CRP levels. Increased blood pressure may promote vascular inflammation by modulation of mechanical stimuli from pulsatile blood flow. Furthermore, elevated blood pressure is also known to promote generation of reactive oxygen species (ROS) as evident from a study where a significant correlation was observed between levels of CRP and mononuclear oxidative stress. Inflammation in hypertension decreases endothelium dependent relaxation, possibly by decreased capacity of endothelium to generate vasodilatory factors, particularly nitric oxide which in turn raises blood pressure. This is substantiated by several studies which have shown inflammatory markers such as CRP as an independent determinant of endothelium dependent vascular function among patients with CHD and this situation may also coexist with hypertensives. High levels of CRP may upregulate the levels of angiotensin receptors and enhance expression of plasmalogen activator inhibitor-1 by endothelial cells. Both these changes could raise blood pressure and promote atherogenesis.[87]

Takahashi Kinji and co-workers in 2006, have shown in their study that inflammation is closely related to insulin resistance and macro-angiopathy in type 2 DM and hs-CRP can be a useful marker for evaluation of pathophysiology in type 2 DM or vascular disease.[150]

A pilot study was done by Suman B in 2008 on hs-CRP and oxidative stress in young CAD patients in India, showed that elevated hs-CRP along with dyslipidemia and oxidative stress added to the predictive value of premature CAD.[151]

Soinio and colleagues reviewed data from 1045 patients with type 2 diabetes aged 45 to 65 years, over a 7 year follow up period and it revealed that the mean hs-CRP levels were significantly higher in 157 patients who died from CHD and 254 patients who had a fatal or non fatal CHD event.[152]

De Beer Fc and colleagues measured CRP and creatinine kinase MB levels in patients with definite MI, patients with spontaneous or exercise induced angina, subjects undergoing coronary arteriography and patients with non- cardiac chest pain. They found that all individuals with infarction developed raised CRP levels and there was significant correlation between the peak CRP and CK-MB levels as seen in our study.[153]

Haverkate et al measured CRP levels in 2121 outpatients with angina, enrolled in European Concerted Action on Thrombosis and Disabilities angina pectoris study and followed up to 2 years. They found that raised circulated concentrations of CRP predictors of coronary events in patients with stable or unstable angina. There was 2 fold increase in the risk of coronary events in patients whose CRP concentration was more than 3.6mg/dl.[154]

In our study we did not find statistically significant correlation between CK-MB levels with both hs-CRP and uric acid levels in cases.

In the present study, levels of serum uric acid were found to be significantly increased in cases when compared with controls. The increased uric acid levels in hypertensive cardiac diseases occurred mostly due to the decrease in renal blood flow, which may stimulate urate absorption.[109]

Hypertension also results in microvascular disease and this can lead to local tissue ischemia and this in turn leads to increased uric acid synthesis. In ischemia, ATP is degraded to adenine and xanthine and there is also increased generation of xanthine oxidase. This increased availability of xanthine and xanthine oxidase results in increased uric acid generation as well as oxidant formation.[155]

There is some evidence that serum uric acid could possibly promote, rather than preventing oxygenation of low-density lipoprotein cholesterol and lipid peroxidation. This can lead to carotid intima-media thickness and also an increase in platelet adhesiveness, resulting in thrombus formation that can contribute to the development of atherosclerosis, increasing the likelihood of the development of cardiovascular disease. The oxidative stress provoked by generation of oxygen free radicals also causes reduction in the availability of nitric oxide leading on to decreased endothelial regulated vascular relaxation.[121]

The increased oxidants and uric acid in ischemia is also associated with endothelial dysfunction and oxidative stress in heart failure and diabetes. Hyperuricemia has also been shown to play a role in endothelial dysfunction, produced either directly by increased serum uric acid levels or through elevated xanthine oxidase activity. Other potential mechanisms by which hyperuricemia and

elevated xanthine oxidase activity might produce vascular damage include increased platelet adhesiveness, smooth muscle proliferation and stimulation of inflammatory responses.[156]

The first National Health and Nutrition Examination Study(NHANES I) done by Freedman and his co-workers demonstrated that each 60 μmol/L increase in uric acid level was associated with a 48% increase in risk for incident ischemic heart disease among women.[157]

Fang and colleagues in the NHANES I epidemiologic follow up study done in United States in adults showed that increased levels of uric acid are related to increased cardiovascular mortality and morbidity.[158]

Madsen and colleagues suggested that in patients with significant CAD, high levels of uric acid could be a strong risk factor for adverse outcome and mortality.[159]

There is some evidence that serum uric acid could possibly promote, rather than preventing oxygenation of low-density lipoprotein cholesterol and lipid peroxidation. This can lead to an increase in platelet adhesiveness, resulting in thrombus formation that can contribute to the development of atherosclerosis, increasing the likelihood of the development of cardiovascular disease.[121]

High uric acid levels can also stimulate the release of free radicals, which have been shown to be involved in adhesion molecule expression by inflammatory cells as well as in inflammatory cell activation and adherence to the damaged endothelium.[160] This ultimately results in endothelial injury, again increasing the risk of cardiovascular disease development. This mechanism is supported by the study done by Levya et al in 39 male patients with chronic heart failure and 16 healthy controls,

where they measured circulating uric acid and markers of inflammation, which showed a positive correlation between elevated UA levels and chronic inflammation in chronic heart failure.[161]

A study by Kang et al[162] in 2005, showed an elevation in plasma UA concentration is associated with an increased level of C-reactive protein that has been identified as an important indicator of myocardial infarction, stroke and vascular death as seen in our study.

CONCLUSION

CONCLUSION

1. The levels of highly sensitive C-reactive protein were found to be higher in cases when compared to controls.
2. Hyperuricemia was seen in cases when compared to controls.
3. High levels of hsCRP may indicate coronary vascular inflammation state, which may be seen in patients with co-morbid conditions like hypertension, diabetes mellitus.
4. Hyperuricemia may indicate endothelial dysfunction and oxidative stress which is commonly seen in CHD.
5. Hence measurement of the levels of hs-CRP and serum uric acid in CAD might help in identifying the patient at increased risk of mortality.

SUMMARY

SUMMARY

This is a cross sectional study done in RLJH & RC, Tamaka, Kolar to estimate the levels of serum uric acid, high sensitive CRP in CHD patients. 60 subjects were chosen of which 30 were diagnosed cases of CHD and 30 were age and gender matched healthy controls. CHD patients showed hyperuricemia and elevated levels of highly sensitive CRP when compared to the controls.

These abnormalities may contribute as a risk factor for mortality and morbidity seen in CHD patients. Hence, early assessment of these parameters may help in decreasing the cardiovascular complications as their levels may serve as simple marker to identify patients at risk of mortality. They may also help in predicting the outcome and in effective management of these cases.

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ANNEXURES

ANNEXURES 1:

CASE HISTORY OF THE PATIENTS

Case No:

Name: Mr. /Mrs. OP No:

Age: IP No:

Gender: Ward:

Date: Occupation:

Weight:

Address:

PRESENTING COMPLAINTS:

1. Chest Pain
2. Dyspnoea
3. Palpitation
4. Giddiness
5. Syncopal attacks
6. Other symptoms

- Sweating, Vomiting, Nausea, Haemoptysis, Pedal oedema, Paralysis, Pain abdomen, Fever, etc.

PAST HISTORY:

- H/o Diabetes

(a) Duration of disease

(b) Treatment history

- H/o Hypertension (a) Duration

(b) Treatment

- H/o anginal attacks

- H/o suggestive of M.I

- H/o R.H.D.

FAMILY HISTORY:

Diabetes : yes/no if yes, duration:

Hypertension : yes/no if yes, duration:

Tuberculosis : yes/no if yes, duration:

OCCUPATIONAL HISTORY:

PERSONAL HISTORY:

Economic status:

Diet: vegetarian / mixed

Smoking: yes/no if yes, duration:

Alcohol: yes/no if yes, duration:

GENERAL PHYSICAL EXAMINATION:

Built: normal / below normal / well built / obese

Nourishment: well / poor nourished

Edema: Icterus:

Pallor: Clubbing:

Cyanosis: Lymphadenopathy:

Blood pressure: Pulse rate:

SYSTEMIC EXAMINATION:

CVS:

RS:

CNS:

PER ABDOMEN:

INVESTIGATION:

- Bl. Urea, S. Creatinine
- CK-MB
- Hs-CRP
- Troponin
- RBS
- Serum uric acid

- Complete Haemogram
- Fasting lipid profile
- Urine routine
- Chest X-ray PA
- ECG
- 2D-ECHO

OTHERS:

DIAGNOSIS:

TREATMENT:

CONSENT FORM

Informed consent:

The details of the study have been explained to me in my own language. I confirm that I have understood the above study and had the opportunities to ask questions. I understand that my participation in the study is voluntary and that am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose. I fully consent to participate in the above study.

Signature of the patient

CAD CASES

Sl.No.	HOSPITAL NO	Age	Gender	hs-CRP(mg/dl)	CK-MB(u/l)	Uric Acid(mg/dl)	RBS(mg/dl)	SBP(mmHg)	DBP(mmHg)
1	784711	68	M	37.38	72	9.1	234	140	90
2	637216	37	M	11.1	136	8.2	137	140	100
3	791883	62	M	20.45	300	8.4	612	150	100
4	793139	55	F	15.31	97	8.5	228	140	80
5	793405	50	M	3.5	237	7.3	189	160	90
6	793146	50	M	13.03	167	5.1	144	140	90
7	791030	54	M	6.98	92	4.9	135	140	90
8	793781	45	F	6.24	300	5	220	140	90
9	793786	38	M	3.79	300	7.3	175	130	90
10	805355	60	F	0.52	265	7.2	211	140	90
11	794182	49	M	1.18	280	4.4	140	140	90
12	794443	50	M	1.36	181	5.7	140	140	100
13	795092	31	M	18.09	154	7.2	130	140	90
14	795041	55	F	1.52	300	4.3	132	150	90
15	794791	45	M	1.69	33	3.8	158	160	110

Hs-CRP –highly sensitive C-reactive protein, CK-MB – Creatine kinase, RBS – Random blood sugar, SBP – Systolic blood pressure, DBP – Diastolic blood pressure.

Sl.No.	HOSPITAL NO	Age	Gender	hs-CRP(mg/dl)	CK-MB(u/l)	Uric Acid(mg/dl)	RBS(mg/dl)	SBP(mmHg)	DBP(mmHg)
16	794796	40	M	5.04	173	5.6	164	130	100
17	795480	54	M	10.81	250	6.2	355	180	110
18	796067	57	M	4.03	62	7.2	316	160	100
19	795473	28	M	11.45	224	4.1	253	140	90
20	795706	65	F	15.35	32	4.3	300	130	90
21	796761	70	F	0.96	92	7.3	201	170	110
22	787786	56	F	3.67	54	6.2	234	130	90
23	799602	65	F	1.45	55	6.1	186	140	100
24	811083	40	F	0.56	241	5.6	195	140	90
25	801185	40	M	4.1	144	4.3	211	150	100
26	804199	53	M	7.81	300	8.4	264	140	90
27	808724	69	M	0.69	21	4.4	312	160	110
28	805148	80	M	3.31	300	6.5	174	150	100
29	805454	42	M	0.82	81	5.7	298	140	90
30	805183	60	M	0.83	45	4.6	231	130	80

Hs-CRP –highly sensitive C-reactive protein, CK-MB – Creatine kinase, RBS – Random blood sugar, SBP – Systolic blood pressure, DBP – Diastolic blood pressure.

CONTROLS

Sl.No.	HOSPITAL NO	Age(years)	Gender	hs-CRP(mg/dl)	CK-MB(u/l)	Uric Acid(mg/dl)	RBS(mg/dl)	SBP(mmHg)	DBP(mmHg)
1	850358	38	M	0.35	16	4.5	82	120	80
2	851355	50	M	0.16	20	4.1	84	120	70
3	789532	45	F	0.12	18	3.8	91	110	70
4	804735	54	M	0.29	15	5.1	80	120	90
5	795465	50	M	0.29	16	4.8	96	110	80
6	762752	68	M	1.2	11	3.9	92	120	70
7	779817	37	M	0.21	12	4.8	87	110	80
8	856437	60	F	0.53	8	5.1	86	120	90
9	851610	50	M	0.42	18	5.4	76	120	80
10	851457	62	M	0.05	16	4.7	91	110	60
11	852497	49	M	0.15	18	3.8	75	110	70
12	804440	54	M	0.1	21	4.9	84	100	60
13	809011	57	M	0.05	21	4.7	106	120	90
14	804822	65	F	0.04	14	4.3	87	110	80
15	806250	70	F	0.11	15	4.8	75	100	70

Hs-CRP –highly sensitive C-reactive protein, CK-MB – Creatine kinase, RBS – Random blood sugar, SBP – Systolic blood pressure, DBP – Diastolic blood pressure.

Sl.No.	HOSPITAL NO	Age(years)	Gender	hs-CRP(mg/dl)	CK-MB(mg/dl)	Uric Acid(mg/dl)	RBS(mg/dl)	SBP(mmHg)	DBP(mmHg)
16	793820	28	M	0.2	18	5.3	108	130	80
17	851595	45	M	0.16	22	3.6	87	120	70
18	806280	55	F	0.11	15	4.1	89	110	70
19	851454	31	M	0.14	17	5.8	85	110	70
20	851260	40	M	0.32	11	4.9	81	120	70
21	801184	40	M	0.12	9	6.1	79	110	80
22	851344	69	M	0.41	8	4.7	92	130	80
23	851478	42	M	0.12	19	6.2	95	120	70
24	853642	60	M	0.28	24	5.3	79	110	80
25	854251	65	F	0.21	21	5.9	86	120	80
26	851119	40	F	0.06	18	6.7	104	120	70
27	852536	53	M	0.12	16	6.9	95	100	60
28	852497	70	M	0.05	17	6.4	75	120	90
29	851457	56	F	0.18	12	7.1	86	130	80
30	851610	70	M	0.06	16	7	88	110	80

Hs-CRP –highly sensitive C-reactive protein, CK-MB – Creatine kinase, RBS – Random blood sugar, SBP – Systolic blood pressure, DBP – Diastolic blood pressure.