CORRELATION OF HbA_{1c}, BLOOD GLUCOSE, CALCIUM, MAGNESIUM AND FASTING LIPID PROFILE IN MYOCARDIAL INFARCTION: A CASE-CONTROL STUDY IN RURAL KOLAR DISTRICT

BY

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Dissertation Submitted to
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M.D. (BIOCHEMISTRY)

Under the guidance of

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SRI DEVARAJ URS MEDICAL COLLEGE, KOLAR

MAY 2014

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled **"CORRELATION OF HbA_{1c}, BLOOD GLUCOSE, CALCIUM, MAGNESIUM AND FASTING LIPID PROFILE IN MYOCARDIAL INFARCTION: A CASE-CONTROL STUDY IN RURAL KOLAR DISTRICT"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. K.N. SHASHIDHAR, M.D, Professor and HOD, Dept of Biochemistry,** Sri Devaraj Urs Medical College, Kolar.

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This is to certify that the ethical committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has unanimously approved**, Dr. Harish. R** student in the Department of Biochemistry at Sri Devaraj Urs Medical College, Tamaka, Kolar to take up the dissertation work titled **"CORRELATION OF HbA_{1c}, BLOOD GLUCOSE, CALCIUM, MAGNESIUM AND FASTING LIPID PROFILE IN MYOCARDIAL INFARCTION: A CASE-CONTROL STUDY IN RURAL KOLAR DISTRICT"** to be submitted to Sri Devaraj Urs Academy of Higher Education and Research, Kolar.

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LIST OF ABBREVIATIONS

ACS – Acute coronary syndrome

AMI – Acute myocardial infarction

ACAT – Acyl-CoA cholesterol acyltransferase

Apo A – Apolipoprotein A

Apo B-48 – Apolipoprotein B-48

Apo C – Apolipoprotein C

Apo E – Apolipoprotein E

C – Unesterified cholesterol

CE – Cholesterol esters

Ca^{2+} - Calcium

CHD – Coronary heart disease

CETP – Cholesteryl ester transfer protein

DM – Diabetes mellitus

FBS – Fasting blood sugar

GHb – Glycated hemoglobin

HbA – Adult hemoglobin

HbA_{1c} – Glycated hemoglobin A_{1c}

HbF – Fetal hemoglobin

HDLc – High density lipoprotein cholesterol

HL – Hepatic lipase

HPLC – High performance liquid chromatography

IFG – Impaired fasting glucose

IGT – Impaired glucose tolerance

IDL – Intermediate density lipoproteins

LDLc – Low density lipoprotein cholesterol

LCAT – Lecithin:cholesterol acyltransferase

LPL – Lipoprotein lipase

LRP – LDL receptor-related protein

MACE – Major adverse cardiac event

MBG – Mean blood glucose

Mg^{2+} - Magnesium

Na^{+}-K^{+} ATPase – Sodium potassium ATPase

NSTEMI – Non-ST elevation myocardial infarction

Non-HDLc – Non high density lipoprotein cholesterol

OGTT – Oral glucose tolerance test

PPBS – Postprandial blood glucose

RMPs – Reference measurement procedures

STEMI – ST segment elevation myocardial infarction

TG - Triacylglycerols

VLDLc – Very low density lipoprotein

BACKGROUND:

Several investigations as well as prospective studies have shown a significant correlation between glucose metabolism and cardiovascular disease outcome in patients with or without diabetes mellitus (DM). Glycated hemoglobin (HbA_{1c}) has been shown to predict the development of myocardial infarction (MI). These studies prompted the hypothesis that HbA_{1c} could be considered to be an independent and crucial cardiovascular risk factor both in patients with or without diabetes.

OBJECTIVES:

1. To estimate glycated hemoglobin, blood sugar, calcium, magnesium and fasting lipid profile (Total cholesterol, HDL, LDL, Triglycerides & Non-HDL) in individuals with acute myocardial infarction (AMI) irrespective of diabetic status.
2. To compare glycated hemoglobin, blood sugar, calcium, magnesium and fasting lipid profile (Total cholesterol, HDL, LDL, Triglycerides & Non-HDL) in individuals with acute myocardial infarction irrespective of diabetic status.
3. To find correlation between glycated hemoglobin, blood sugar, calcium, magnesium and lipid profile in the study group.
4. To explore the possibility of using glycated hemoglobin, calcium and magnesium in conjunction with lipid profile as independent markers of coronary vascular events in non-diabetic individuals.

MATERIALS AND METHODS:

The present study is a case control study which includes 51 cases of acute myocardial infarction (AMI) irrespective of their diabetic status and 55 age and gender matched controls carried out in Sri Devaraj Urs Medical College during the period of February 2012 – July 2013. The cases were grouped into diabetic AMI and non-diabetic AMI patients.

RESULTS:

The results showed that males were more prone for myocardial infarction and increased risk is associated with smoking. There was no significant difference between the mean HbA_{1c} levels among myocardial infarction cases and controls irrespective of their diabetic status. The mean value of HbA_{1c} in AMI patients was 7.52±1.56% in comparison to 7.59±1.33% in controls. In the subgroup analyses, in the diabetic AMI group the mean was 8.63±0.997% and 8.79±0.9% in controls. In the non-diabetic AMI group the mean was 6.6±1.33% and 6.67±0.74% in controls. HbA_{1c} correlated strongly with FBS, PPBS but did not correlate significantly with macrometals and lipid profile. Fasting blood glucose was significantly elevated in non-diabetic AMI patients 95±17.54 mg/dl compared to non-diabetic controls 84.77±9.92 mg/dl ($p<0.05$). The serum calcium and magnesium were significantly increased in AMI compared to controls in both diabetic AMI and non-diabetic AMI patients within the reference range.

CONCLUSION:

HbA_{1c} helps to distinguish between stress induced hyperglycemia but fails to predict the risk for myocardial infarction. Fasting glucose in non-diabetic AMI patients not only reflects the acute glycometabolic state but also predicts risk and increased mortality. Increased serum calcium within the reference range is associated with increased cardiovascular risk.

Key words: acute myocardial infarction, glycated hemoglobin, calcium, magnesium, lipid profile

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Coronary heart disease (CHD) i.e. myocardial infarction is the leading cause of mortality globally with 7.2 million deaths and 12.2 percent of total deaths per year. The pooled estimates from studies conducted in India from 1990's till 2002 show that the prevalence rate of MI in urban areas is around 6.4 percent and 2.5 percent in rural areas. In urban areas the pooled estimate is around 6.1 percent for males and 6.7 percent for females. In rural areas, the estimate is around 2.1 percent for males and 2.7 percent for females. This shows a female preponderance of MI.[1]

Studies suggest that traditional cardiovascular risk factors such as diabetes mellitus, hypercholesterolemia and hypertension alone do not account for the excess of cardiovascular morbidity and mortality in the non–diabetic population. Several investigations as well as prospective studies have shown a significant correlation between glucose metabolism and cardiovascular disease outcome in patients with or without diabetes.[2,3]

The core of the issue is glycemic control by glycated hemoglobin has now been established as risk factor in predicting cardiovascular disease risk in individuals without diabetes. Elevated levels of glycated hemoglobin have been found to be associated with raised atherosclerotic lesions and fatty streaks in coronary arteries.[4]

Glycation is the non-enzymatic addition of a sugar residue to amino groups of proteins. Human adult hemoglobin (Hb) usually consists of HbA (97%), HbA_2 (2.5%) and HbF (0.5%). HbA is made up of 4 polypeptide chains- two alpha and two beta chains. Chromatographic analysis of HbA identifies several minor hemoglobins, namely $HbA_{1a,}$ HbA_{1b} and $HbA_{1c,}$ which are collectively referred to as HbA_1, fast hemoglobins, glycohemoglobins or glycated hemoglobins.[5]

Falsely elevated values are obtained when the charge on hemoglobin is altered by attachment of non-carbohydrate moieties, which may cochromatograph with glycated hemoglobin as in uremia (carbamylated hemoglobin), alcoholism, lead poisoning or chronic treatment with large doses of aspirin (acetylated hemoglobin). These substances interfere predominantly with HbA_{1a+1b}, and assays that measure HbA_{1c} have only slight alterations (< 1% glycated hemoglobin).[5]

HbA_{1c} concentration is dependent on the average blood glucose concentration. It is formed progressively and irreversibly over a period of time and is stable till half life of RBC. HbA_{1c} is unaffected by diet, insulin or exercise on the day of testing and thus reflects the average glucose levels over the last several weeks.[5]

Diabetes mellitus, a metabolic disorder characterized by hyperglycemia, results from decreased insulin secretion, defective insulin action or both. Glycated hemoglobin (HbA_{1c}) is a routinely used marker for long-term glycemic control particularly the mean blood glucose level (MBG) in association with dyslipidemia. Recently, it has been observed that HbA_{1c} is regarded as an independent risk factor for myocardial infarction (MI) in patients with or without diabetes. Positive relationship between HbA_{1c} and MI has been demonstrated in non-diabetic cases even within normal range of $HbA_{1c.}$[2] Reference range of HbA_{1c} estimated by cation exchange resin method: [6]

Non-diabetic: 4.5-8%

Good control: 8-9%

Fair control: 9-10%

Poor control: $\geq$10%

Even though many studies conducted at international and national level show a positive relation between the MBG and HbA_{1c} in the urban population, but this is not clear in rural population to find a correlation of these parameters.[7]

HbA_{1c} has been shown to predict the development of myocardial infarction (MI). It has been observed in a cohort study that there is a markedly increased risk of stroke in people with elevated HbA_{1c} of $\geq$ 7%. However studies conducted to find the correlation of this parameter with respect to MI are unclear.[8] These studies prompted the hypothesis that HbA_{1c} could be considered to be an independent and crucial cardiovascular risk factor both in patients with or without diabetes. This is an important finding as the comprehensive assessment of cardiovascular risk will improve the targeting of preventive treatment.

Ever since serum cholesterol was first linked up with cardiovascular pathophysiology, a number of additional markers like LDL, HDL, triglycerides and non-HDL have been identified, which help in defining the atherogenic potential of lipid profile better.[9] Studies correlating triglyceride levels and risk of coronary disease show that decrease in initial elevated levels are associated with decrease coronary heart disease compared with stable high triglyceride levels.[10]

A recent study correlated the relationship between the macro minerals such as calcium and magnesium in association with MI, and showed that magnesium plays a vital role in preventing heart attacks and also it is beneficial to reduce the cholesterol and keep the arteries healthy. Magnesium is a divalent and fourth most abundant intracellular cation. It is a major macro mineral. Magnesium has a unique role in energy metabolism pathways and is involved in more than 300 biochemical reactions in vivo.[11]

Calcium is an alkalizing nutrient which destroys the oxygen robbing acid in the body fluid, thus keeping the body alkaline and hence prevents diseases effortlessly. As in all muscles, heart muscle also requires these two cations because calcium causes cardiac muscle contraction and magnesium causes cardiac muscle relaxation. Studies conducted have shown that those who die from heart attacks have hypomagnesemia and hypercalcemia in their cardiac muscles. Hypomagnesemia is thought to be closely related to MI, where an inadequate supply or an increased demand (recession) may often lead to formation of clots in the heart and brain which may lead to the deposition of calcium in the blood vessels.[11]

Since we can trace every sickness, every disease and every ailment to a mineral deficiency, it is worth studying the association of these macro minerals particularly calcium and magnesium with glycated hemoglobin (HbA_{1c}) in myocardial infarction.

1. To estimate glycated hemoglobin, blood sugar, calcium, magnesium and fasting lipid profile (Total cholesterol, HDL, LDL, Triglycerides & Non-HDL) in individuals with acute myocardial infarction irrespective of diabetic status.
2. To compare glycated hemoglobin, blood sugar, calcium, magnesium and fasting lipid profile (Total cholesterol, HDL, LDL, Triglycerides & Non-HDL) in individuals with acute myocardial infarction irrespective of diabetic status.
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MYOCARDIAL INFARCTION (MI)

On July 21, 1768, William Heberden presented his paper "Some Account of a Disorder of the Breast" to the Royal College of Physicians, London, in which he quoted: "But there is a disorder of the breast marked with strong and peculiar symptoms, considerable for the kind of danger belonging to it, and not extremely rare. The seat of it, and sense of strangling and anxiety with which it is attended, may make it not improperly be called angina pectoris.[12, 13]

In 1901, Osler classified angina into several types, calling the anterior branch the "artery of sudden death." He agreed that coronary thrombosis was invariably fatal and later stated that "the tragedies of life are largely arterial."[14] However, in 1912, the concept that coronary thrombosis was always fatal was finally dispelled by James Herrick in his article titled "Clinical Features of Sudden Obstruction of the Coronary Arteries". He concluded "there is no inherent reason why the stoppage of a large branch of a coronary artery, or even of a main trunk, must of necessity cause sudden death."[15]

In the 1960's the "vulnerable plaque" hypothesis had gained enthusiastic support implicating plaque disruption leading to atherothrombosis, as a cause of acute coronary syndrome and sudden death. The role of inflammation as a primary or secondary event, supported by serum markers, led many to believe that atherosclerosis is at least a part of an inflammatory condition.[16]

Cardiac arrest was assumed to be irreversible. With the development of the external pacemaker by Paul Zoll (1952) and the defibrillator by William Kouwenhoven (1930s-1950s), used by Claude Beck (1947) and Zoll (1956), the rescue of victims of cardiac arrest became feasible. Beck dramatically stated that "The death factor in

coronary artery disease is often small and reversible .... The heart wants to beat and often it needs only a second chance." His concept that "the heart is too good to die" instilled optimism into the care of coronary patients.[17] The monitoring of patients in close proximity to skilled nursing personnel to treat ventricular arrhythmias and perform cardiopulmonary resuscitation was initiated in 1961 and then spread throughout the world. Since then, coronary care has gone through recognizable phases: resuscitation and the important role of the nurse, prevention of arrhythmias, hemodynamic catheter monitoring and treatment of pump failure, reduction of infarct size-first with beta blockers and now with thrombolytic therapy, and primary angioplasty.[18]

Myocardial infarction is the leading cause of mortality globally with 7.2 million deaths and 12.2 percent of total deaths per year. The pooled estimates from studies conducted in India from 1990's till 2002 show that the prevalence rate of MI in urban areas is around 6.4 percent and is 2.5 percent in rural areas.[1]

The increasing incidence of acute coronary syndromes (ACS) in Indians may be related to the changes in the lifestyle, the westernization of the food practices, the increasing prevalence of diabetes mellitus and probably genetic factors.[19]

Myocardial infarction is a fatal complication of atherosclerotic coronary heart disease. In most patients (80-95%) it results from thrombotic occlusion of the infarct related vessel. Following thrombosis, myocardial ischemia and necrosis sets in. This occurs as a wave front from the sub-endocardial region and progress to the sub epicardial region.[20]

CLASSIFICATION[20]

Patients with ischemic heart disease fall into two large groups:

1. Patients with chronic coronary heart disease (CHD) who most commonly present with stable angina and
2. Patients with acute coronary syndromes (ACS).

The latter group, in turn, is composed of patients with acute myocardial infarction (AMI) with ST-segment elevation on their presenting electrocardiogram (STEMI) and those with unstable angina and non ST segment elevation MI (Variant angina, NSTEMI).

CORONARY CIRCULATION

The epicardial coronary arteries take origin from the right and left coronary sinuses. The right and left coronary arteries are main arteries supplying blood to the heart as shown in Fig (1). In 85% of patients the right coronary artery (RCA) gives rise to the posterior descending artery (PDA) and this is referred to as right dominant circulation. In 8% cases the left circumflex gives rise to the PDA, posterior LV (PLV) branch and AV nodal artery, this being called the left dominant circulation. Regardless of the dominance the sinus node is supplied by RCA in 60% and left atrial branch of circumflex in 40% cases. The left main divides into left anterior descending (LAD) and left circumflex arteries (LCx). The LAD supplies the interventricular septum and anterolateral free wall of the LV. The RV branch and acute marginal supply the RA/RV. The RCA/PLV gives off AV nodal artery which supplies the AV node and bundle of HIS.[21]

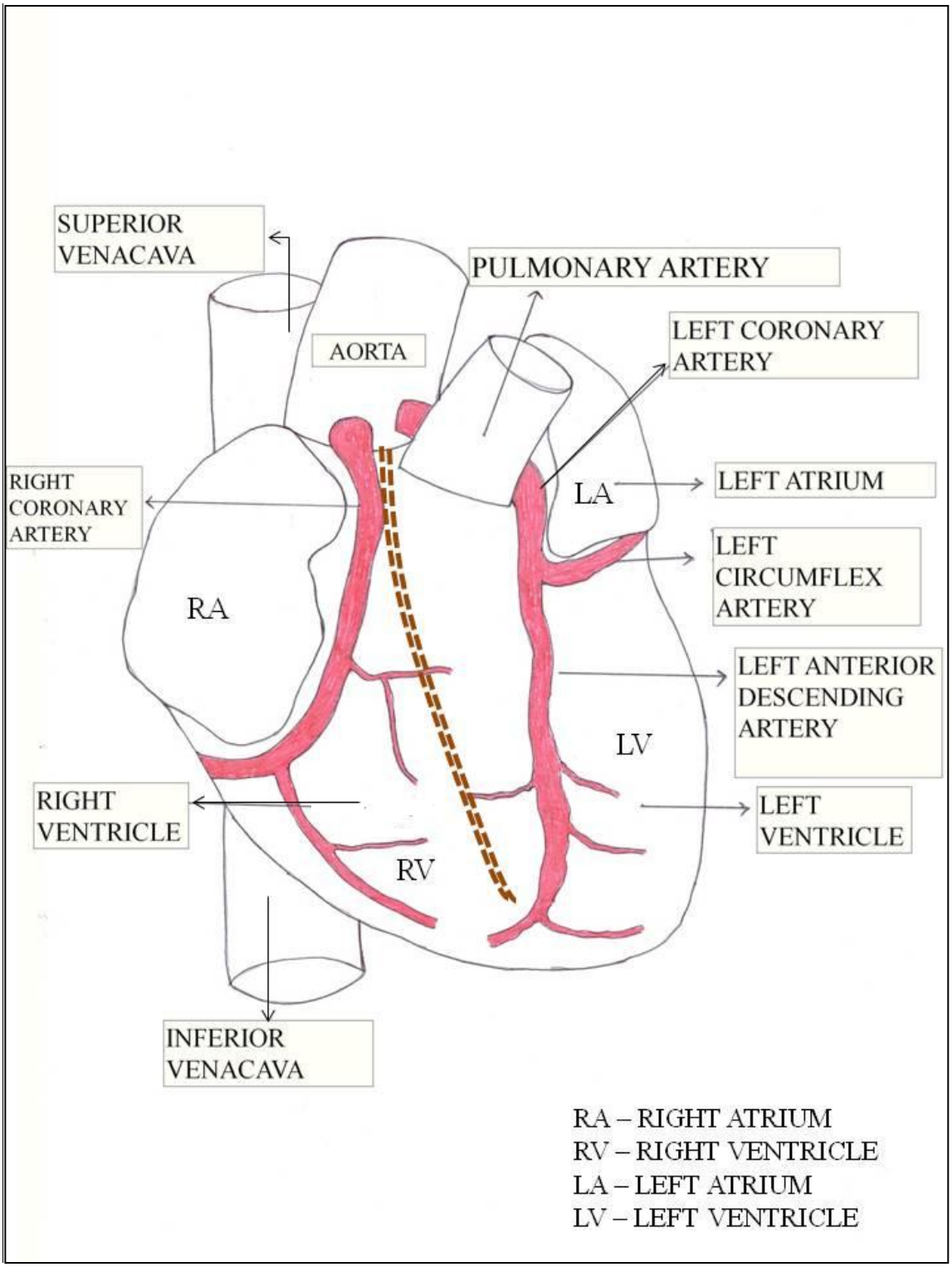


Fig (1) Anatomy of coronary arteries

Physiology of Coronary Circulation

The myocardium relies almost exclusively on oxidative metabolism for its energy needs. Even at rest the transmyocardial oxygen extraction is near maximal with a coronary venous O_2 saturation that is lowest in the body (25-35%). Any increase in myocardial O_2 can only be met by proportional increase in myocardial blood flow, chiefly mediated by reduction in coronary arteriolar resistance. The left coronary artery fills only in diastole while the RCA shows both systolic and diastolic coronary blood flow.[21]

Pathophysiology of AMI[20, 22]

The current knowledge of pathophysiology of acute myocardial infarction (AMI) started with the autopsy description by Dr. James Herrick from Chicago in1912 who concluded that the AMI results from the thrombotic occlusion of coronary artery and prophesized that the hope of salvaging the muscle lay with restoration of blood flow.[15] The theory of thrombosis was not accepted unequivocally until 1972 when coronary angiography performed during AMI revealed that in 85-90% of patients, the cause of AMI was indeed thrombotic occlusion of infarct related artery.[20]

Virtually all acute infarcts are caused by thrombosis developing on a culprit ruptured atherosclerotic plaque. Atherosclerosis involves the buildup of a plaque composed of variable amount of lipoprotein, extracellular matrix (collagen, proteoglycans, glycosaminoglycans), calcium, vascular smooth muscle cells, inflammatory cells (chiefly monocyte derived macrophages, and T lymphocytes, mast cells, dendritic cells) and new blood vessels angiogenesis (neovascularisation).[22]

A body of evidence at present suggests that atherosclerosis represent a chronic inflammatory response to vascular injury, caused by a variety of agents. These agents activate or injure endothelium and promote lipoprotein infiltration, retention and modification, along with inflammatory cell entry, retention and activation.[22]

Recent research suggests that one of the earliest steps in atherogenesis is endothelial activation or injury or dysfunction with infiltration, retention and modification of atherogenic lipoprotein (predominantly the apo-B containing lipoproteins) in the sub endothelial space of the vessel wall.[22]

Thrombus usually develops on a preexisting atheromatous lesion. Over a period of time, some of these lesions become unstable and are called as "vulnerable plaques". The cascade of events that lead to plaque rupture is thought to be inflammatory in character. Several factors precipitate plaque rupture. The most important factors are stress and catecholamine release which leads to increased shear forces by augmenting contraction and increasing blood pressure. The stress can be emotional, sepsis, trauma, anemia or a surgical operation. Infectious agents may also contribute to the inflammatory process and thus to destabilization of plaques.[20, 22]

LDLc plays a central role in atherogenesis. Higher levels of serum LDL leads to increased transport into the intima, where LDL becomes susceptible to a variety of modifications, including oxidation, enzymatic modification, nonenzymatic glycation, aggregation, and immune complex formation. All of these lead to enhanced macrophage uptake, foam cell formation, and initiation of the cascade of events resulting in progression of the atherosclerotic lesion. [23]

CLINICAL FEATURES OF ACUTE MYOCARDIAL INFARCTION[20]

Acute myocardial infarction presents itself as a sudden catastrophic incident and its definite clinical picture may vary from severe pain in the chest to minimal symptoms, with the disease remaining unrecognised.[24]

1. Pain in the Chest:

   In most of the cases this is the presenting complaint. It is a deep visceral pain, involving the central portion of the chest and epigastrium, described as tightness, heaviness or constriction in the chest. In most cases, it radiates to the arms and commonly it is often accompanied by weakness, sweating, nausea, giddiness and anxiety. It may occur during exertion and emotional outbursts, not relieved with rest and makes the patient to move about in an attempt to find a comfortable position.

2. Breathlessness

   Second most important symptom, breathlessness may be sudden in onset and intense or it may be exertional. It is common in those who had 'painless myocardial infarction' particularly diabetics and aged individuals, and those having complications like cardiogenic shock and pulmonary edema.[24]

3. Sudden loss of consciousness, a confusional state, and a sense of profound weakness or unexplained fall in blood pressure with giddiness, syncope

4. Auscultatory signs of ventricular dysfunction that may be present are, muffled heart sounds, atrial (S4) and ventricular (S3) gallop sounds and paradoxical splitting of the 2nd sound.

5. Some patients may present with a picture that of CCF.

DIABETES MELLITUS (DM)

Recognition of Diabetes Mellitus dates back to centuries before Christ as a medical condition producing excessive thirst, continuous urination, and severe weight loss has interested medical authors. Egyptian Papyrus (1500 BC) described it as a condition of "too great emptying of urine". The Hindu physicians, Charaka and Sushrutha, described about diabetes mellitus between 400 and 500 BC. They described the first clinical test for diabetes mellitus. They observed that urine from people with diabetes attracted ants and flies. Urine in diabetes was described by Charaka as 'madhumeha' or 'honey urine'. Apollonius of Memphis, around 230 B.C for the first tine used the term "diabetes", which in Greek means "to pass through". He and his contemporaries considered diabetes as a disease of the kidneys. Greek physician Aretaeus of Cappadocia in the 2nd century AD was the first to distinguish between diabetes mellitus and diabetes insipidus. The progress in the understanding of this disorder came slowly until middle of the 19th century. However during this period the course and complications of the disease were recognized.[25]

DIAGNOSIS OF DIABETES MELLITUS:

Diabetes mellitus is defined by the American Diabetes Association (ADA) as a 'group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia is associated with long term damage, dysfunction and failure of various organs especially the eyes, kidney, nerves, heart and blood vessels.[26]

The National Diabetes Data Group and World Health Organization have issued diagnostic criteria for Diabetes mellitus: [27]

Symptoms of diabetes plus random blood glucose concentration ≥ 200mg/dl or Fasting plasma glucose ≥ 126mg/dl or Two hour plasma glucose ≥ 200mg/dl during an oral glucose tolerance test.

In asymptomatic subjects, diabetes is diagnosed by a Fasting plasma glucose ≥ 126 mg/dl or a glucose ≥ 200 mg/dl 2 hours after a 75g Oral Glucose Tolerance test (Standard OGTT). This hyperglycemia should be confirmed on a second occasion. Impaired Fasting Glucose (IFG) is defined as fasting plasma glucose between 100mg/dl and 125mg/dl. Based on OGTT, Impaired Glucose Tolerance (IGT) is defined as plasma glucose between 140 mg/dl and 199 mg/dl. Individuals with IFG and/ or IGT, recently designated pre diabetes by ADA are at substantial risk for developing type 2 Diabetes mellitus and have an increased risk of cardiovascular disease but do not have associated micro vascular disease.[27] Because of high intra individual variation in plasma glucose in the fasting and 2 hour post prandial state, HbA_{1C} may provide useful confirmatory information for the screening and diagnosis of diabetes.[28, 29] Hence, at present ADA recommends $HbA_{1c} \geq$ 6.5% to be included as one of the diagnostic tool with the WHO criteria.[30]

GLYCATED HEMOGLOBIN (HbA_{1c})

Glycoproteins of special interest are glycated hemoglobin (GHb) and similar proteins, which are used to monitor long term glucose control in people with diabetes mellitus. It is a measure of the risk for the development of the complications of diabetes. In addition, GHb concentration has recently being shown to predict cardiovascular disease risk in individuals without diabetes.[2, 5]

Glycated hemoglobin (GHb) is formed by a post-translational, non-enzymatic, substrate concentration dependent, irreversible process of combination, of aldehyde group of glucose and other hexose with the amino terminal valine of the β chain of hemoglobin. Human adult hemoglobin (Hb) usually consists of HbA (97% of the total), HbA_2 (2.5%) and HbF (0.5%). Adult hemoglobin is made up of four polypeptide chains, two α and two β chains. Chromatographic analysis of HbA identifies several minor hemoglobins, namely HbA_{1a}, HbA_{1b}, HbA_{1c}, which collectively are referred to as HbA, or fast hemoglobins. HbA_{1c} is formed by the condensation of glucose with N-terminal valine residue of either β-chain of HbA to form an unstable Schiff base.[5]

The Schiff base may either be dissociated or undergo an amadori rearrangement to form a stable ketoamine as shown in figure (2).[5]

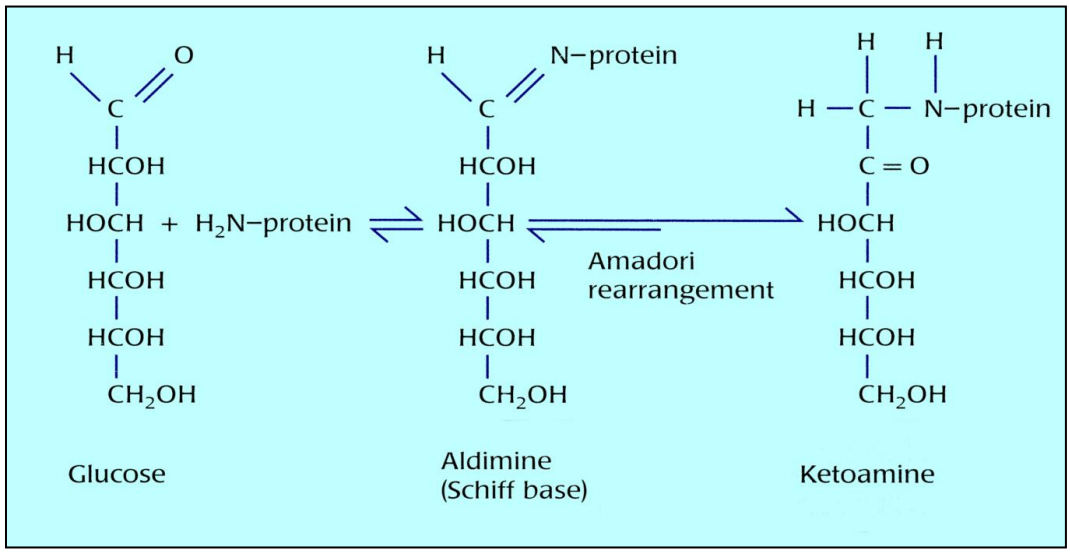


Fig (2) Formation of glycated hemoglobin

Formation of glycated hemoglobin is essentially irreversible and its concentration in blood depends on both the life span of the red blood cell (average120 days) and blood glucose concentration. Since the rate of formation of GHb is directly proportional to the concentration of glucose in the blood, the GHb concentration represents the integrated

values for glucose over the preceding 6 to 8weeks. This provides an additional criterion for assessing glucose control because GHb values are free of day-to-day glucose fluctuation and are unaffected by recent exercise or food ingestion. The contribution of the plasma glucose concentration to GHb depends on the time interval, with more recent values providing larger contribution than earlier values. The plasma glucose in the preceding 1 month determines 50% of the HbA_{1c}, whereas days 60 to 120 determine only 25%. The half-life of GHb is 35 days.[5]

The interpretation of GHb depends on the life span of the circulating red blood cells. Patients with hemolytic disease or other conditions with shortened red blood cell survival exhibit a substantial reduction in GHb. Similarly, individuals with recent significant blood loss have falsely low values owing to a higher fraction of young erythrocytes. The effects of hemoglobinopathies on GHb depend on assay method. Another source of error in selected methods is carbamylated hemoglobin. This is formed by attachment of urea, and is present in large amounts in renal failure. It co elutes with GHb and gives rise to falsely high values.[5]

METHODS FOR DETERMINATION OF GLYCATED HEMOGLOBIN

Significant analytical improvements have occurred since glycated hemoglobin (GHb), measured as total HbA_1, were first used in routine clinical laboratories around 1977.[31] There are more than 30 different methods for the determination of GHb. These methods separate hemoglobin from GHb using techniques based on charge differences (ion-exchange chromatography, HPLC, electrophoresis and isoelectirc focusing), structural differences (affinity chromatography and immunoassay) or chemical analysis

(photometry and spectrophotometry). Regardless of the method, the result is expressed as a percentage of total hemoglobin.

ION EXCHANGE MINI COLUMNS

Ion exchange chromatography separates hemoglobin variants on the basis of charge. The cation exchange by resin (negatively charged), packed in dispensable mini columns has an affinity for hemoglobin, which is positively charged. The patient's sample is hemolyzed and 1 aliquot of the hemolysate is applied to the column. A buffer is applied and the eluent is collected. The ionic strength and pH of the eluent buffer are selected so that GHbs are less positively charged than HbA and are eluted first. The GHbs are measured in a spectrophotometer. A second buffer of different ionic strength can be added to the column to elute the more positively charged main hemoglobin fraction. This is read in the spectrophotometer and GHb is expressed as a percentage of total hemoglobin. Most current commercial ion exchange method is HPLC.[5]

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

GHb and other hemoglobin fractions can be separated by HPLC, which employs cation-exchange chromatography. Several fully automated systems are commercially available. Assays require only 6µl of whole blood. Anticoagulated blood is diluted with a hemolysis agent containing borate. Samples are incubated at 37^0c for 30 minutes to remove Schiff base and inserted in the auto samples. Detection is performed at both 415 and 690nm and results are quantified by integrating the area under the peaks as shown in Fig (3).[5]

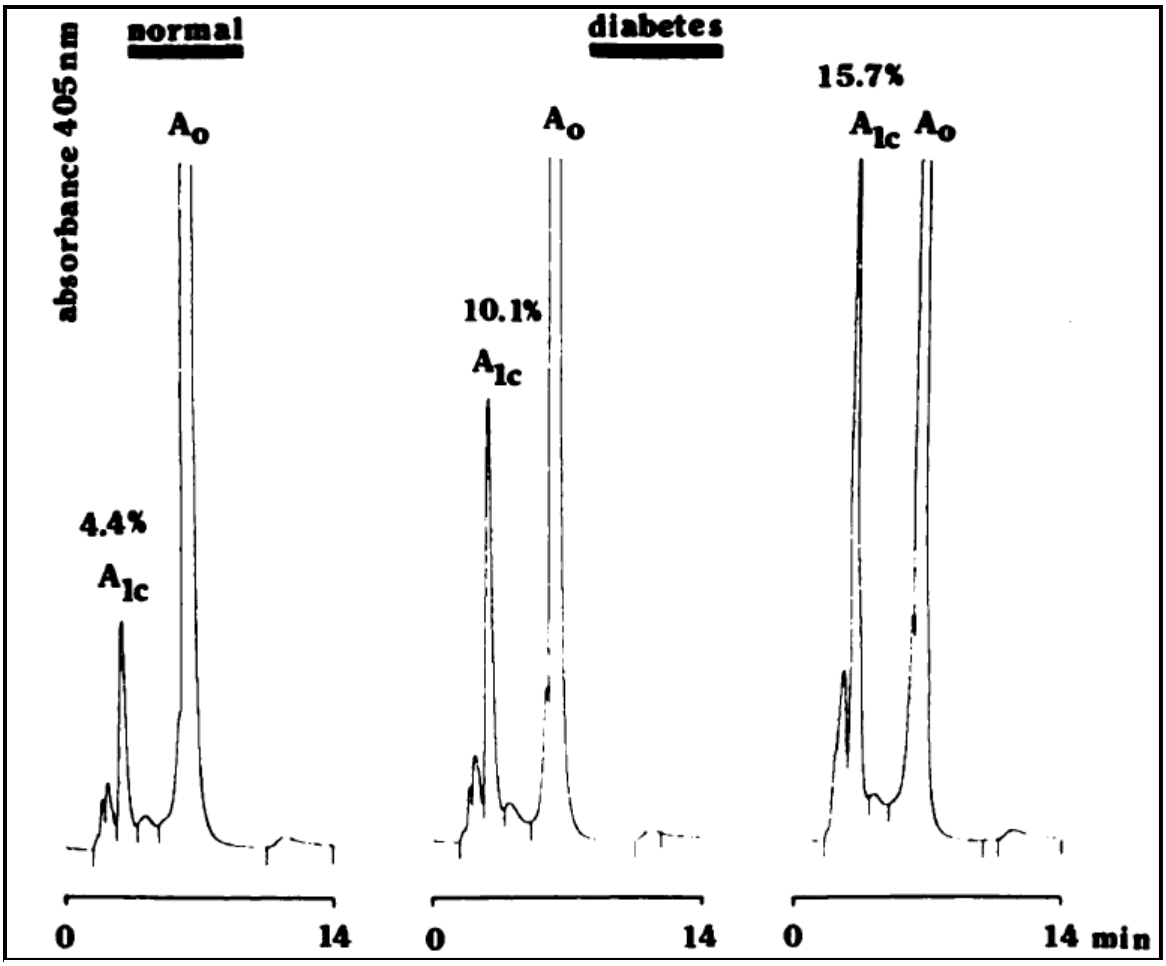


Fig (3) High Performance Liquid chromatography of GHb

Courtesy: Ellis G, Diamandis EP, Giesbrecht EE, et al. An Automated "High-Pressure" Liquid-ChromatographicAssay for HemoglobinA1C. Clin Chem. 1984;30: 1746-1752.

ELECTROPHORESIS

Agar-gel electrophoresis on whole blood hemolysates at pH 6.3, provides good resolution of HbA and GHb. The gel contains negatively charged moieties that interact with the hemoglobin. After 25 to 35 minutes, the GHb separates on the cathodic side of HbA. Quantification is performed by scanning densitometer at 415 nm.[5]

ISOELECTRIC FOCUSING

The hemoglobin variants separate on isoelectric focusing on the basis of their migration in gel containing a pH gradient. Ampholytes in the pH range of 6 to 8 establishes the gradient in 1 mm thick acrylamide gel slabs. On completion of isoelectric focusing, the gels are fixed and then scanned on a high resolution integrating microdensitometer.[5]

IMMUNO-ASSAY

Assays for GHb have been developed using antibodies raised against the Amadori product of glucose (ketamine linkage) plus the first few (4 to 8) amino acids at the N-terminal end of the β-chain of hemoglobin. GHb in whole blood is measured by inhibition of latex agglutination. The agglutinator, a synthetic polymer containing multiple copies of the immunoreactive portion of GHb, binds the anti-GHb monoclonal antibody that is attached to latex beads. This agglutination produces light scattering, which is measured as an increase in absorbance. GHb in the patients sample competes for the antibody on the latex, inhibiting agglutination, thereby decreasing light scattering.[5]

AFFINITY CHROMATOGRAPHY

Affinity gel columns are used to separate GHb, which binds to the column, from the nonglycated fraction. M-Aminophenyl boronic acid is immobilized by cross linking to beaded or another matrix (eg. Glass fiber). The boronic acid reacts with the enediol groups of glucose, bound to hemoglobin, to form a reversible five member ring complex, thus selectively holding the GHb on the column as shown in Fig (4). The non glycated hemogoblin does not bind. Sorbitol is then added to elute the GHb. Absorbance of the

bound and non bound fraction, measured at 415 nm, is used to calculate the percentage of GHb.[5]

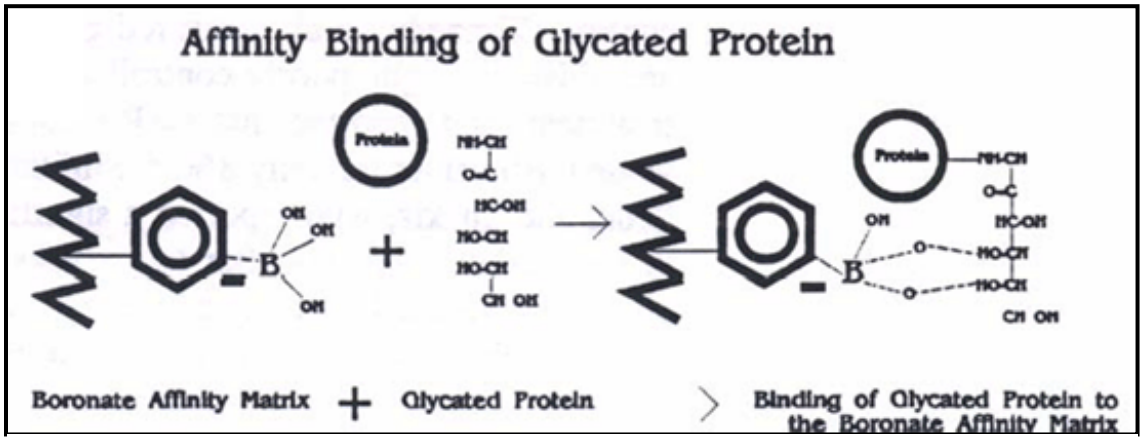


Fig (4) Affinity chromatography of GHb

Courtesy: National Health and Nutrition Examination Survey 1999 - 2000 Data Documentation, Codebook, and Frequencies. Glycohemoglobin (LAB10). Cited 2002 Jun. Last updated 2012 Mar. Last accessed 2013 Aug 13. Available from: http://www.cdc.gov/NCHS/images/NHANES/nhanes1999-2000/LAB10/GlycatedProtein.gif

BLOOD GLUCOSE

Claude Bernard observed and explained acute hyperglycemic response to stress more than a century ago.[32]

STRESS HYPERGLYCEMIA:

It is defined as hyperglycemia in previously euglycemic patients that gets corrected once the acute process resolves. This "diabetes of injury" exemplifies the obligatory metabolic rearrangements required to cope with critical stress. The concept evolved as glucose became identified as metabolic mirror of the severity and outcome of critical illness.[33]

Alajbegovic et al showed in 2003 that about 27.43% of patients with acute myocardial infarction had hyperglycemia and it had normalized in 90.27% of patients at

the time of discharge.[34] One third of all the patients admitted with hyperglycemia to general hospital do not have previously diagnosis of diabetes; in these patients hyperglycemia is a risk factor for adverse outcomes during acute illness.[35]

Admission hyperglycemia may not only be the cause of more severe myocardial damage but also its consequence. Large infarcts are more likely to cause catecholamine release, which effect fatty acid and glucose homeostasis. The catecholamine response is proportional to the severity of the infarct, as confirmed by the correlation between admission blood glucose and heart rate or Killip's class on admission.[36]

Various effects of acute hyperglycemia on AMI:

1. Electrophysiological changes:

Increased plasma glucose level has been demonstrated to be capable of inducing such electrophysiological alterations as to favor the occurrence of fatal arrhythmias.[37] This is consistent with the evidence that an acute increase of glycemia in normal subjects produces a significant QT elongation.[38]

2. Impaired left ventricular function:

Stress hyperglycemia is independently associated with impaired left ventricular function and also with a larger infarct size.[39, 40] Animal studies have shown that acute hyperglycemia abolishes ischemic pre-conditioning which would explain the worse myocardial performance in patients with acute MI and concomitant hyperglycemia.[41, 42]

3. Effect on coagulation:

The association of MI with increased thrombophilia is proved.[43] It has been reported that increased platelet activation after an MI is correlated with hyperglycemia in non-diabetic patients.[44] Acute hyperglycemia induces a shortening of the fibrinogen half-life and

increases in fibrinopeptide-A, fragments of pro-thrombin in factor VII, and in platelet aggregation, which are all phenomena suggesting increased activation of thrombosis.[45, 46]

4. Effect on inflammatory immune reaction:

Stress hyperglycemia was found to be associated with amplified inflammatory immune reactions and worse functional cardiac outcome.[47] There is evidence which suggest that MI is associated with local and systemic inflammation.[48]The inflammatory cells infiltrate the plaque; the infarcted heart appears to be enriched in activated T cells.[49]Although circulating immune markers are also chronically elevated in patients with chronic stable angina, a transient burst of T-cell activation can only be detected in patients with unstable angina and MI, suggesting that immune factors might precipitate plaque complications such as thrombus formation and vasoconstriction at the site of the culprit lesion. [50] Interestingly enough, acute hyperglycemia in healthy subjects and in patients with impaired glucose tolerance or overt diabetes produces a rise in inflammatory markers.[51,52,53] Following this line of thought, it might be speculated that the detrimental effect of stress hyperglycemia in acute MI might also stem from its ability to increase inflammation.

5. Effect on endothelial function:

Many studies have shown that hyperglycemia produces worsening of endothelial function.[54,55] The endothelial dysfunction plays a key role in cardiovascular disease.[56] Endothelial dysfunction is a common feature after an MI.[57] These studies suggest that hyperglycemia induced endothelial dysfunction can contribute to the adverse outcome of AMI.

6. Oxidative stress:

Oxidative stress is known pathogenic process for atherosclerosis and cardiovascular diseases.[58] Free radical production by hyperglycemia is the probable pathologic process.[59] Antioxidants can hinder some of the effects acutely induced by hyperglycemia, endothelial dysfunction, activation of coagulation, and inflammation, suggests that the action of acute hyperglycemia is mediated by the production of free radicals. [60, 61, 62]

It has been reported that during glucose oral challenge, a reduction in the antioxidant defenses and an increase in markers of oxidative stress is observed.[63] This gives direct evidence of effect of acute hyperglycemia on oxidative stress markers. 3-Nitrotyrosine is thought to be a relatively specific marker of oxidative damage mediated by peroxynitrite and it has recently been demonstrated to be an independent predictor of cardiovascular disease. [64, 65]

Nitrotyrosine formation is detected during acute hyperglycemia in the artery wall of monkeys, and in working hearts from rats during hyperglycemia, but also in the plasma of healthy and diabetic subjects.[66, 67, 68, 69] Hyperglycemia producing oxidative stress by itself can thus worsen outcome in AMI.

7. Therapeutic prospects

Diabetes and Insulin-Glucose Infusion in Acute Myocardial Infarction (DIGAMI) study showed that an insulin–glucose infusion followed by at least 3 months of multiple-dose insulin reduced long-term mortality in patients with diabetes who had an MI.[70, 71, 72] However, not all were convinced by the results. It is necessary to distinguish between a favorable metabolic effect of glucose–insulin infusion and the control of acute hyperglycemia. In terms of metabolic efficacy it has been suggested that insulin, by itself,

should have direct beneficial effect, particularly in reducing the level of free fatty acids (FFAs), which are known to be associated with a deterioration of clinical outcome and may have toxic effects of their own on the myocardium.[73, 74]

Glucose exerts several direct and powerful damaging effects, as described above, which are all able to worsen the prognosis of MI. Therefore, the true open question is whether hyperglycemia, when present during a MI, has to be treated with intensive insulin therapy even in non-diabetic patients. Insulin therapy has already shown a beneficial effect in critically ill patients.[75]

CALCIUM

Calcium (Ca^{2+}) is the king of all alkalizing nutrients. Calcium effectively douses acid the same way that water douses fire. It destroys oxygen robbing acid in the body fluid there by keeping the body alkaline so that it can prevent disease effortlessly and can reverse cancer.[11]

American journal of nutrition states that virtually no major organ system escapes calcium influence. Dietary supplements of calcium along with vitamins prevent further spread of cancer. [76, 77, 78]

Calcium exists in 3 major forms in plasma. Approximately 50% is in the free or ionized form, which is the physiologically important fraction, 40% is bound to albumin, and the remaining 10% is in soluble complexes with anions such as bicarbonate, phosphate, and lactate.[79]

The extracellular fluid (ECF) ionised calcium concentration is 10^4 times the concentration of the intracellular fluid (ICF) ionised calcium. Non-ionised calcium is predominantly found in bone providing an important structural function to the human

body, whereas the ionised calcium is responsible for a variety of physiological effects that are characteristic of the cell type (e.g. secretion, neuromuscular impulse formation, contractile functions, clotting). Normal plasma calcium, which consists of protein bound, ionised and complexed calcium, ranges from 2.10 - 2.55 mmol/L (Conversion factor: ÷ 0.25= 8.4-10.2 mg/dl). Normal plasma ionised calcium ranges from 1.15 - 1.30 mmol/L (Conversion factor: ÷0.25= 4.6-5.2 mg/dl) . Numerous hormones can influence calcium metabolism (e.g. 1,25 dihydroxycholecalciferol, parathyroid hormone, calcitonin, parathyroid hormone related protein, oestrogen, corticosteroids, thyroxin, growth hormone) although only 1,25 dihydroxycholecalciferol, parathyroid hormone, calcitonin are primarily concerned with the regulation of calcium metabolism.[80]

Calcium Deficiency[11]

Chronic calcium deficiency is associated with some forms of hypertension, prostate and colorectal cancer, some types of kidney stones, miscarriage, premenstrual problems, Joint and periodontal disease, sleep disturbances, mental depression, cardiovascular disease. Calcium levels are associated with arthritic or joint and vascular degeneration, calcification of soft tissues, hypertension, and stroke, mood and depressive disorders inhibits the cancer protective effects of vitamins.[78, 81] A weak heart rate means that calcium is deficient and the contraction phase is weak and short. This results in an increase in heart rate and also irregular heart rate because some contractions are missed entirely.

Calcium and atherosclerosis

Several mechanisms link arterial calcification to atherosclerosis. Cholesterol and its oxidation products accelerate coronary calcification. Smaller amounts of calcium have

been shown to be present in noncomplex; lipid-rich fibromuscular plaques.[82]

Computed tomography for measurement of calcium in the coronary arteries has been evaluated as a new non-invasive screening tool for predicting cardiovascular events. The coronary artery calcium score, which infers the presence of coronary atherosclerosis by measuring the amount of calcium in the coronary arteries, has been shown to predict the risk for cardiovascular events.[83]

MAGNESIUM

Magnesium (Mg^{2+}) is the fourth-most abundant cation in the body and the second most prevalent intracellular cation, next to potassium.[84] Magnesium is known to play an important role in carbohydrate metabolism and its imbalance has been implicated as a cause, consequence, or complications of diabetes mellitus. Magnesium serves as cofactor for about 300 cellular enzymes many of which are involved in energy and carbohydrate metabolism.[85]

The normal body content in the adult is approximately 2000 milli equivalent (mEq) or 24 grams. Magnesium is distributed unevenly, with greatest concentration in tissues having the higher metabolic activity such as brain, heart and kidney. Approximately 60% of the body magnesium is in the bone, one third of the skeletal magnesium has been shown to be exchangeable and this fraction may serve as a reservoir for maintaining a normal extracellular magnesium concentration.[86]

Extracellular magnesium accounts for only about 1% of total body magnesium content. The normal serum magnesium concentration is approximately 1.8 – 2.9 mg/dl (Conversion factor: x 0.411= 0.73-1.19 mmol/L). [87] About 70-75% of the plasma magnesium is ultra filterable of which the major portion is ionized. The non-filterable

portion is bound to plasma proteins, chiefly albumin. The remaining of the body magnesium is intracellular.

The concentration of total magnesium in cells varies with the tissues, but is of the order of 1-3 mmol/l (Conversion factor ÷0.411= 2.43-7.29 mg/dl). [88] In general, higher the metabolic activity of the cell, the higher the magnesium content. A low serum magnesium concentration usually implies magnesium deficiency. The serum magnesium however may not reflect intracellular magnesium. Intracellular magnesium depletion may exist despite a normal serum magnesium concentration.[89] Because tissue and cellular assays are difficult to perform and not widely available, the serum magnesium determination is the method by which magnesium deficiency is identified in clinical practice.

Myocardial Magnesium

The adult human body contains between 21 and 28 g of magnesium or about 3 mg/kg of fat free tissue. Over half of it is present in bone. Cardiac muscle has a significant concentration of magnesium (17.4-19.8 mEq/l) (Conversion factor: x 1.2= 20.88-23.76 mg/dl) higher concentration of magnesium is found in the ventricles then in the atria. There are no significant differences between magnesium concentration in the right and left ventricles or inter-ventricular septum.[90] Magnesium has been shown to be involved in ATP hydrolysis by myofibrils, and binding and release of calcium ions by sacrotubule reactions, which are essential to the contraction of heart muscle. Magnesium also stimulates oxidative phosphorylation in heart mitochondria, and affects Na^+-K^+ ATPase of heart membranes, and activates adenyl cyclase and probably phosphorylase kinase in the heart. Magnesium may have influence on muscle tone and conducting

system, though the myocardium may be less sensitive to magnesium than nervous tissue.[84, 91]

Epidemiological studies have suggested that the incidence of myocardial infarction and of sudden death is higher in areas of soft water intake.[92] Cardiac magnesium content has been reported to be low in patients whose death was attributed to myocardial infarction.[93] It is unknown however, if the low cardiac content proceeds the myocardial infarction or is result of it. Cardiac magnesium exchanges quite rapidly with plasma magnesium and a number of clinical studies have shown a fall in the serum magnesium concentration within the first 24 to 48 hours after myocardial infarction.[94] Infarcted myocardium has been repeatedly shown to have reduced magnesium content. But the results regarding the study of serum magnesium values in first 24 hours, following acute myocardial infarction has been variable. Some found no significant change of serum magnesium. It has, therefore been proposed that serum magnesium has an inverse relationship with coagulability of blood and serum cholesterol levels, following acute myocardial infarction.

Myocardial injury was established by histological examination of cardiac tissue. A significant rise in urinary magnesium excretion was observed during the first two hours after which the level declined but was still maintained above the control level. Magnesium content decreased significantly in infarcted myocardium. Magnesium depletion predisposes to vascular spasms, including coronary artery spasm and potentiates the contractile response to pressor agents such as angiotensin II and norepinephrine. Magnesium depletion potentially could worsen angina and precipitate acute myocardial infarction. The effectiveness of magnesium therapy in acute MI has

been reported to decrease infarction size, decrease the incidence of cardiac arrhythmias and lower mortality rate.[95, 96, 97]

LIPIDS IN CARDIOVASCULAR DISEASE

Much attention has been focused on lipids and lipoprotein mainly because of their strong association with coronary heart disease.[98] In the early 1980s, findings from the coronary primary prevention trial demonstrated for the first time that a decrease in plasma cholesterol concentration results in a reduction in the incidence of coronary heart disease. Since then, several secondary prevention trials using diet or drugs to lower blood cholesterol have also demonstrated a reduction in cardiovascular death and atherosclerotic clinical events.[99]

CHOLESTEROL

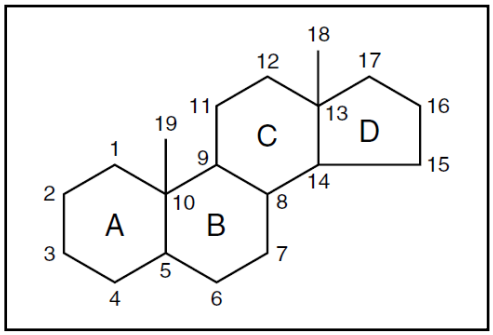


Fig (5) Steroid nucleus

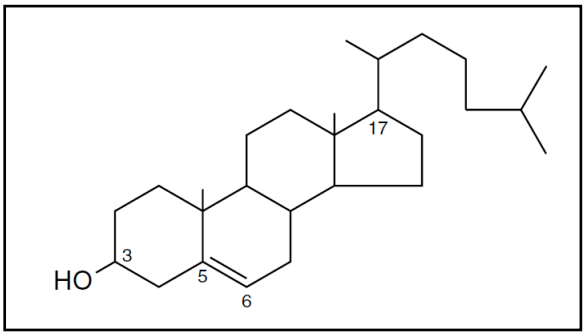


Fig (6) Structure of Cholesterol, 3-hydroxy-5, 6-cholestene

Cholesterol is the main sterol found exclusively in animals. Like other sterols, cholesterol is a solid alcohol of high molecular weight and possesses the cyclopentano perhydro phenanthrene skeleton. The molecule contains 27 carbon atoms.[100]

CHOLESTEROL ABSORPTION

Cholesterol is presented to the intestinal wall from 3 sources - the diet, bile and intestinal secretions and cells. Practically all cholesterol in the intestine is presented in the unesterified (free) form. To be absorbed, unesterified cholesterol is first solubilized through the formation of mixed micelles that contain unesterified cholesterol, fatty acids, monoglycerides (derived from triglycerides), phospholipids and conjugated bile acids. Most of the cholesterol absorption occurs in the small intestine (middle and terminal ileum). About 40-60 percent of the dietary and intestinal cholesterol is absorbed daily.

After the absorption in the intestinal mucosal cells, cholesterol together with triglycerides, phospholipids and a number of specific apoprotein is assembled into a large lipoprotein called chylomicron as shown in Fig (7). Chylomicrons enter the lymphatics, which empty into the thoracic duct and eventually enter the systemic venous circulations.[100]

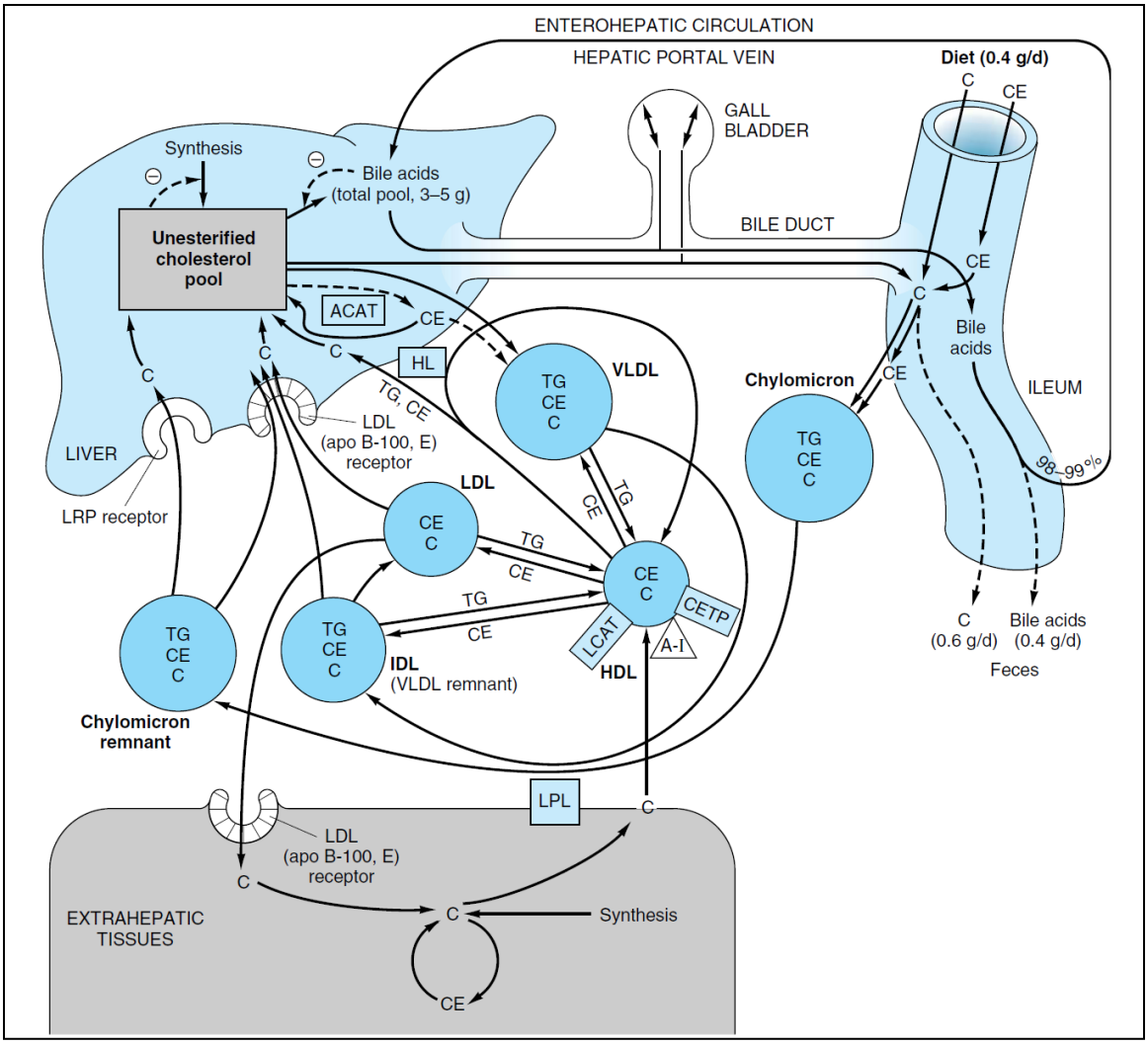


Fig (7) Transport of cholesterol

Transport of cholesterol between the tissues in humans. (C, unesterified cholesterol; CE, Cholesteryl ester; TG, triacylglycerol; VLDL, very low density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ACAT, acyl-CoA:cholesterol acyltransferase; LCAT, lecithin:cholesterol acyltransferase; A-I, apolipoprotein A-I; CETP, cholesteryl ester transfer protein; LPL, lipoprotein lipase; HL, hepatic lipase; LRP, LDL receptor-related protein.)

Courtesy: Murray RK, Bender DA, Botham KM, et al.Cholesterol Synthesis, Transport and Excretion. In: Harper's Illustrated Biochemistry. 28th ed. Mc-Graw Hill Companies. 2009. Available from: http:// www.accessmedicine.com

TRIGLYCERIDES

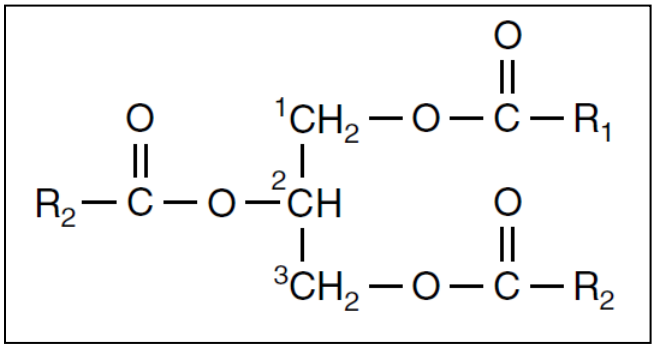


Fig (8) Structure of triacylglycerol

In human nutrition, triglycerides are the most prevalent glycerol esters. They constitute 95 percent of storage form of fat and is the predominant form found in plasma. Triglycerides are neutral fats present as fats and oils and are abundantly distributed in plant and animal kingdom. The vegetable seeds and nuts contain oil as a reserve food material. Lard, butter, fish liver oil are the common animal fat.[100]

Triglycerides are formed by esterification of three molecules of the same or different fatty acids with one molecule of glycerol. Thus, there are simple and mixed triglycerides. Usually, palmitic, oleic and stearic acids are the fatty acids present in natural fats, with small amounts of other fatty acids.[100]

Triglycerides are digested in the duodenum and proximal ileum. In the presence of bile salts and colipase, the pancreatic lipase hydrolyzes triglyceride to 2-monoacylglycerol and fatty acids. After the absorption of fatty acids and 2-monoacylglycerol, triglycerides are synthesized in the intestinal epithelial cells. Triglycerides along with the cholesterol and ApoB-48 give rise to chylomicrons as shown in Fig (9). Chylomicrons are then secreted into the lymphatic system.[100]

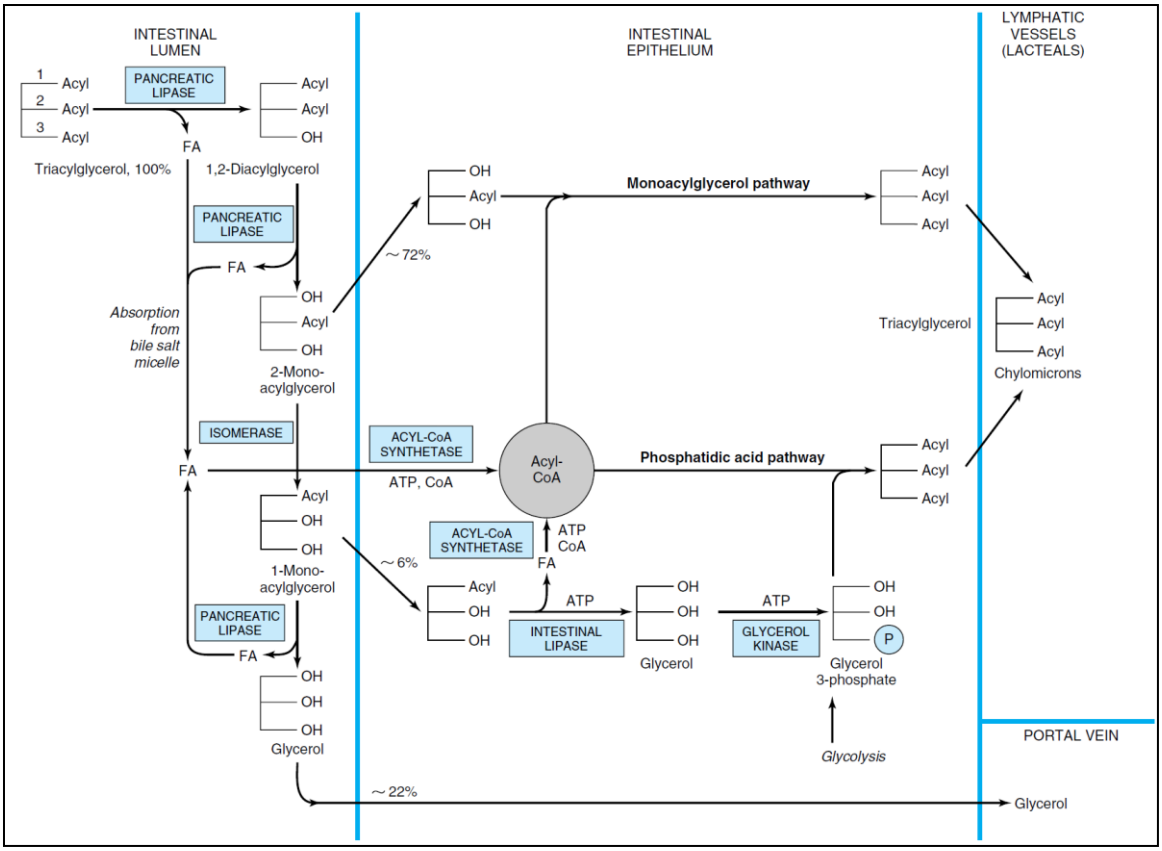


Fig (9) Digestion and absorption of triacylglycerols

Courtesy: Murray RK, Bender DA, Botham KM, et al. Nutrition, Digestion and Absorption. In: Harper's Illustrated Biochemistry. 28th ed. Mc-Graw Hill Companies. 2009. Available from: http:// www.accessmedicine.com

Lipids synthesized in the liver and the intestines have to be transported to various tissues to accomplish their metabolic function. Because of their insolubility, they are transported in plasma in macromolecular complexes called lipoprotein. Lipoproteins are spherical particles with non polar lipids (triglycerides and cholesterol esters) in their core and more polar lipids (phospholipids and free cholesterol) oriented near the surface. They also contain one or more specific proteins called apolipoproteins that are located on their surfaces as shown in Fig (10).[100]

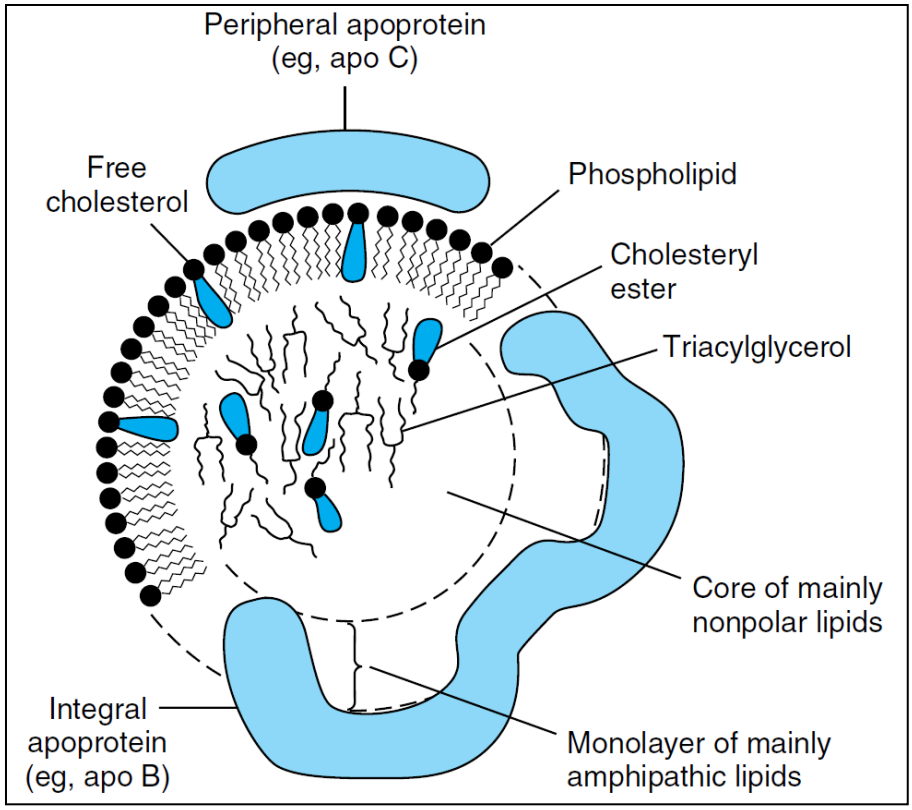


Fig (10) General structure of Lipoproteins

Courtesy: Murray RK, Bender DA, Botham KM, et al. Lipid Transport & Storage. In: Harper's Illustrated Biochemistry. 28th ed. Mc-Graw Hill Companies. 2009. Available from: http:// www.accessmedicine.com

Lipoproteins have different physical and chemical properties as they contain different preparation of lipids and proteins. Chylomicrons have the maximum lipid content whereas HDL contains the least. The lipoproteins have been categorized based on difference in their hydrated densities as determined by ultracentrifugation and by their mobility on agar gel electrophoresis as shown in Fig (11). In addition, they can be classified on the basis of the size and relative concentration of cholesterol or triglycerides and by their apoprotein content.[100]

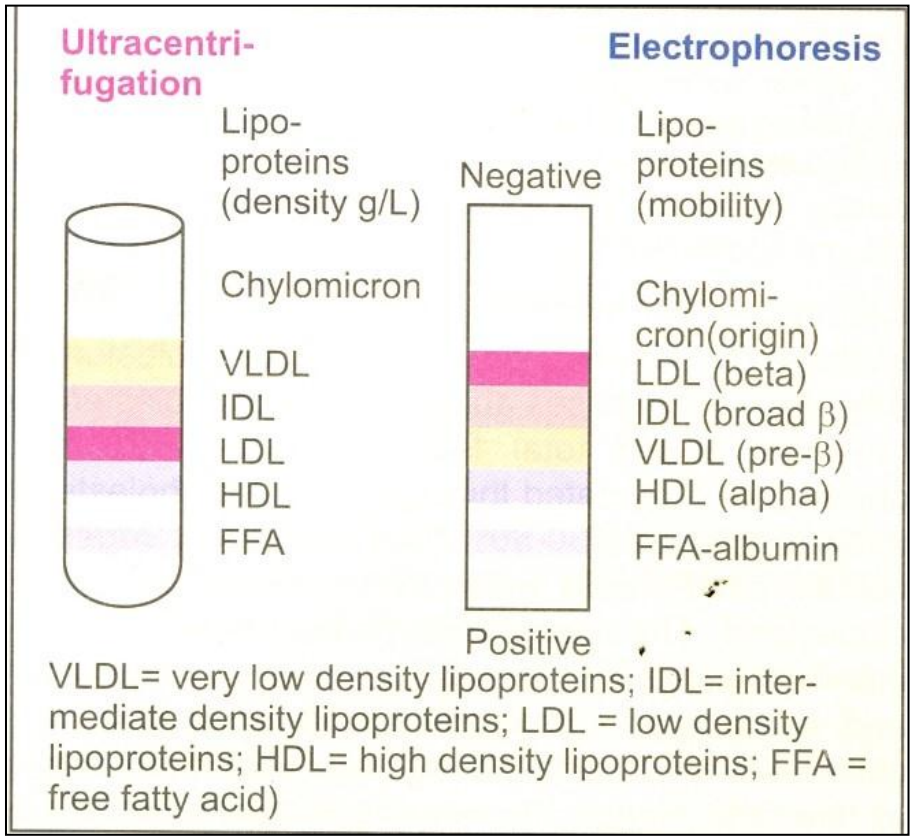


Fig (11) Plasma lipoproteins ultracentrifugation and electrophoresis patterns

Courtesy: Vasudevan DM, Sreekumari S, Vaidyanathan K. Cholesterol and lipoproteins. In: Textbook of Biochemistry for Medical students, 7th ed. Jaypee Brothers Medical Publishers, New Delhi.2013. p. 174.

The main lipoproteins are

1. Chylomicrons

2. Very low density lipoprotein (VLDL)

3. Intermediate density lipoprotein (IDL)

4. Low density lipoprotein (LDL)

5. High density lipoprotein (HDL)

TABLE (1): DIFFERENT TYPES OF LIPOPROTEINS

Lipoprotein	Source	Diameter (nm)	Density (g/mL)	Composition		Main Lipid Components	Apolipoproteins
				Protein (%)	Lipid (%)		
Chylomicrons	Intestine	90–1000	< 0.95	1–2	98–99	Triacylglycerol	A-I, A-II, A-IV,[1] B-48, C-I, C-II, C-III, E
Chylomicron remnants	Chylomicrons	45–150	< 1.006	6–8	92–94	Triacylglycerol, phospholipids, cholesterol	B-48, E
VLDL	Liver (intestine)	30–90	0.95–1.006	7–10	90–93	Triacylglycerol	B-100, C-I, C-II, C-III
IDL	VLDL	25–35	1.006–1.019	11	89	Triacylglycerol, cholesterol	B-100, E
LDL	VLDL	20–25	1.019–1.063	21	79	Cholesterol	B-100
HDL	Liver, intestine, VLDL, chylo-microns					Phospholipids, cholesterol	A-I, A-II, A-IV, C-I, C-II, C-III, D,[2] E
HDL_1		20–25	1.019–1.063	32	68		
HDL_2		10–20	1.063–1.125	33	67		
HDL_3		5–10	1.125–1.210	57	43		
Preβ-HDL[3]		< 5	> 1.210				A-I
Albumin/free fatty acids	Adipose tissue		> 1.281	99	1	Free fatty acids	

Courtesy: Murray RK, Bender DA, Botham KM, et al. Lipid Transport & storage. In: Harper's Illustrated Biochemistry. 28th ed.Mc-Graw Hill Companies, 2009. Available from: http:// www.accessmedicine.com

Chylomicrons

Chylomicrons are formed from the exogenous source of cholesterol. Chylomicrons are primarily triglyceride containing particles produced in the intestine, as a consequence of digestion of exogenous fat. Nascent chylomicron is assembled from dietary triglycerides and cholesterol in the enterocytes and packaged in secretory vesicles in the golgi apparatus. These particles are then introduced into circulation through the intestinal villi. The lipid content of nascent chylomicron consist mainly of triglycerides (90% by mass), whereas the protein components include apo B-48 and the apolipoprotein A (2% by mass).[100]

Shortly after entering the circulation, these particles acquire the apo C apolipoproteins and apo E from circulating HDL. Apo C-II now present on the surface of chylomicrons, activates the LPL attached to the luminal surface of the endothelial cells, which rapidly hydrolyze the triglycerides endothelial to free fatty acids. The fatty acids can be either taken up by muscle cells as an energy source or into adipose cells for storage. Simultaneously, some of the phospholipids and the apo-A lipoproteins are transferred from the chylomicron particles into HDL. The newly formed particle, the chylomicron remnant, contains 80-90% of the triglyceride content of the chylomicron. Because of the presence of apo-B-48 and apo E on its surface, the chylomicron remnant can by recognized by specific hepatic remnant receptors and internalized by endocytosis as shown in Fig (12).[100]

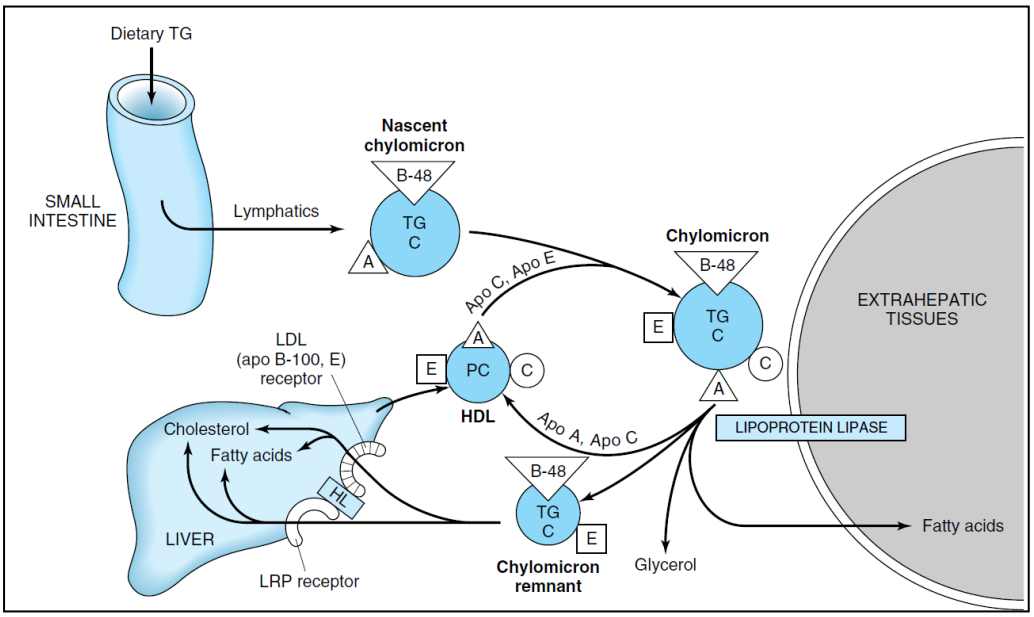


Fig (12) Metabolic fate of chylomicrons

(A, apolipoprotein A; B-48, apolipoprotein B-48; © , apolipoprotein C; E, apolipoprotein E; HDL, high-density lipoprotein; TG, triacylglycerol; C, cholesterol and cholesteryl ester; P, phospholipid; HL, hepatic lipase; LRP, LDL receptor-related protein.)

Courtesy: Murray RK, Bender DA, Botham KM, et al. Lipid Transport & Storage. Harper's Illustrated Biochemistry. 28th ed. Mc-Graw Hill Companies. 2009. Available from: http:// www.accessmedicine.com

VERY LOW DENSITY LIPOPROTEIN (VLDL) [95]

The endogenously made triglycerides and cholesterol are packed in secretory vesicles in the golgi apparatus, transported by exocytosis into the extracellular spaces, and introduced into circulation through the fenestrate of the hepatic sinusoidal endothelium in the form of nascent VLDL. This triglyceride rich particle (55% by mass) contains apo B-100, apo E and small amounts of apo C on its surface. Additional E apolipoproteins are transferred after secretion from circulating HDL.[100]

Apo C-II present on the surface of VLDL activates lipoprotein lipase present on the endothelial cells. This leads to hydrolysis of VLDL triglycerides and the release of free fatty acids. Normal VLDL is probably not atherogenic. The smaller and more cholesterol rich VLDL remnants appear to have atherogenic potential. Individuals with the genetic disorder of familial dysbetalipoproteinemia (Type III, or VLDL remnant lypolipidemia) have accelerated atherogenesis as shown in table (2). [100]

INTERMEDIATE DENSITY LIPOPROTEIN

During the hydrolysis of VLDL, triglycerides and the apo C apolipoproteins are transferred back to HDL. VLDL particles are thus converted to VLDL remnants. Some of which are taken up by the liver and the rest converted to smaller, denser particles called IDL. Surface materials from IDL including phospholipids, free cholesterol and apolipoprotein are transferred to HDL or form HDL de novo in the circulation. Cholesteryl esters are transferred from HDL to IDL. IDL undergoes further hydrolysis in which most of the remaining triglycerides are removed and all apolipoproteins except apo B -100 are transferred to other lipoproteins. This process ends with the formation of LDL.[100]

TABLE (2) Primary disorders of plasma lipoproteins (dyslipoproteinemias)

Name	Defect	Remarks
Hypolipoproteinemias Abetalipoproteinemia	No chylomicrons, VLDL, or LDL are formed because of defect in the loading of apo B with lipid.	Rare; blood acylglycerols low; intestine and liver accumulate acylglycerols. Intestinal malabsorption. Early death avoidable by administration of large doses of fat-soluble vitamins, particularly vitamin E.
Familial alpha-lipoprotein deficiency Tangier disease Fish-eye disease Apo-A-I deficiencies	All have low or near absence of HDL.	Tendency toward hypertriacylglycerolemia as a result of absence of apo C-II, causing inactive LPL. Low LDL levels. Atherosclerosis in the elderly.
Hyperlipoproteinemias Familial lipoprotein lipase deficiency (type I)	Hypertriacylglycerolemia due to deficiency of LPL, abnormal LPL, or apo C-II deficiency causing inactive LPL.	Slow clearance of chylomicrons and VLDL. Low levels of LDL and HDL. No increased risk of coronary disease.
Familial hypercholesterolemia (type IIa)	Defective LDL receptors or mutation in ligand region of apo B-100.	Elevated LDL levels and hypercholesterolemia, resulting in atherosclerosis and coronary disease.
Familial type III hyperlipoproteinemia (broad beta disease, remnant removal disease, familial dysbetalipoproteinemia)	Deficiency in remnant clearance by the liver is due to abnormality in apo E. Patients lack isoforms E3 and E4 and have only E2, which does not react with the E receptor.[1]	Increase in chylomicron and VLDL remnants of density < 1.019 (β-VLDL). Causes hypercholesterolemia, xanthomas, and atherosclerosis.
Familial hypertriacylglycerolemia (type IV)	Overproduction of VLDL often associated with glucose intolerance and hyperinsulinemia.	Cholesterol levels rise with the VLDL concentration. LDL and HDL tend to be subnormal. This type of pattern is commonly associated with coronary heart disease, type II diabetes mellitus, obesity, alcoholism, and administration of progestational hormones.
Familial hyperalphalipoproteinemia	Increased concentrations of HDL.	A rare condition apparently beneficial to health and longevity.
Hepatic lipase deficiency	Deficiency of the enzyme leads to accumulation of large triacylglycerol-rich HDL and VLDL remnants.	Patients have xanthomas and coronary heart disease.
Familial lecithin:cholesterol acyltransferase (LCAT) deficiency	Absence of LCAT leads to block in reverse cholesterol transport. HDL remains as nascent disks incapable of taking up and esterifying cholesterol.	Plasma concentrations of cholesteryl esters and lysolecithin are low. Present is an abnormal LDL fraction, lipoprotein X, found also in patients with cholestasis. VLDL is abnormal (β-VLDL).
Familial lipoprotein(a) excess	Lp(a) consists of 1 mol of LDL attached to 1 mol of apo(a). Apo(a) shows structural homologies to plasminogen.	Premature coronary heart disease due to atherosclerosis, plus thrombosis due to inhibition of fibrinolysis.

Courtesy: Murray RK, Bender DA, Botham KM, et al.Cholesterol Synthesis, Transport and Excretion. In: Harper's Illustrated Biochemistry. 28th ed. Mc-Graw Hill Companies. 2009. Available from: http:// www.accessmedicine.com

LOW DENSITY LIPOPROTEIN

LDL particles contain much less triacylglycerol than their VLDL predecessors and have a high concentration of cholesterol and cholesteryl esters. The primary function of LDL particles is to provide cholesterol to the peripheral tissues. They do so by binding to cell surface membrane LDL receptors that recognize apo B-100. The LDL particles are internalized in coated vesicles, which then fuse to form endosome. LDL dissociates from the receptor, which returns to the cell surface for reuse, whereas LDL migrates to the lysosome and are degraded by lysosomal enzymes, releasing free cholesterol, amino acids, fatty acids and phospholipids as shown in Fig (13).[100]

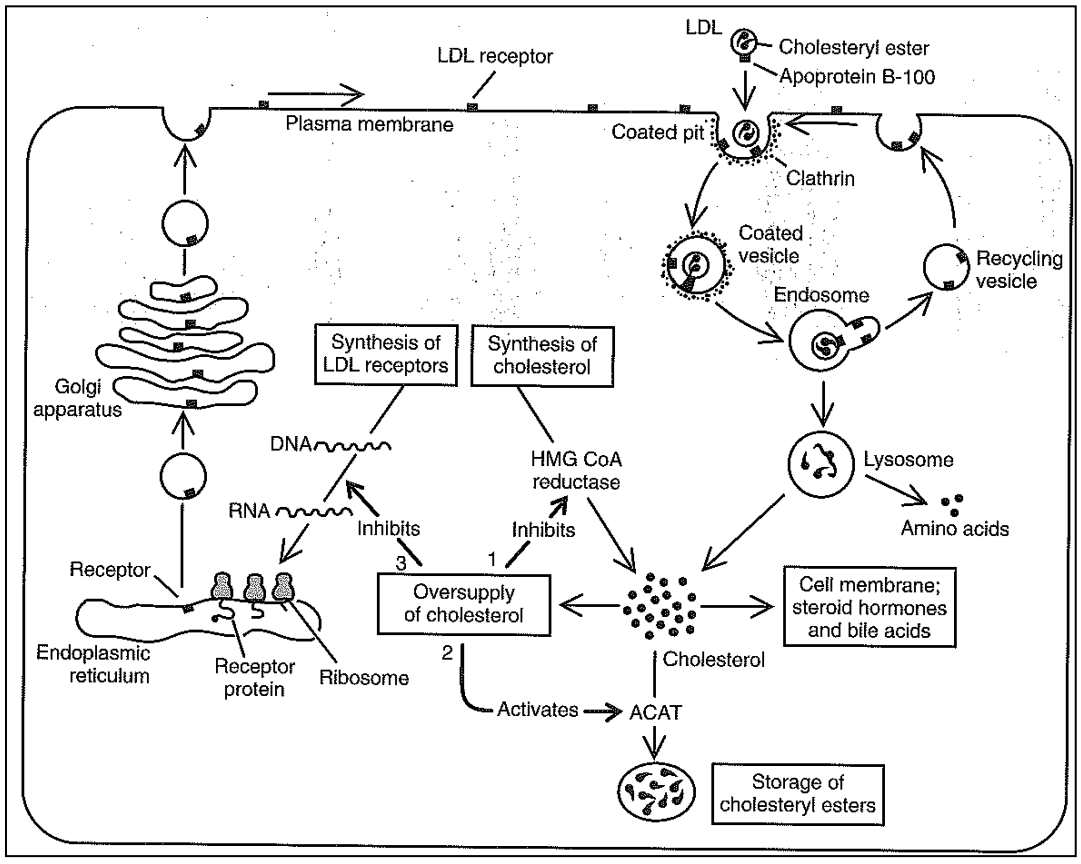


Fig (13) Low density lipoprotein receptor pathway

Courtesy: Rifai N, Warnick RG. Lipids, Lipoproteins, Apolipoproteins and other cardiovascular risk factors. In: Burtis CA, Ashwood ER, Bruns DE, editors, Tietz textbook of clinical chemistry, 4th ed, Philadelphia, W.B Saunders 2006; p.920.

UPTAKE OF CHEMICALLY MODIFIED LDL BY MACROPHAGE SCAVENGER

In addition to the highly specific and regulated receptor pathway for LDL uptake, LDL is also taken up by extrahepatic tissue through scavenger receptors or non receptor mediated pinocytosis. These scavenger receptors can bind to a broad range of ligands and mediate the endocytosis of chemically modified LDL, in which the lipid components of apo-B have been oxidized. Unlike the LDL receptors the scavenger receptor is not down regulated in response to increased intracellular cholesterol. Cholesteryl esters accumulate in macrophages and cause their transformation into "foam cells". These foam cells are considered to the earliest components of atherosclerotic lesion.[100]

HIGH DENSITY LIPOPROTEIN

High density lipoprotein are secreted from the liver or intestine as disk shaped nascent particles that consist primarily of phospholipid and apo A-1. Through the extracellular addition of surface components of triglyceride rich phospholipids, cholesterol and apolipoproteins, nascent HDL is converted to spherical particles.[100]

HDL performs the following functions:

1. HDL is a reservoir of apolipoproteins: HDL serves as a circulating reservoir of apo C- II and apo E.
2. HDL uptake of unesterified cholesterol : Nascent HDL by taking up cholesterol are converted to spherical particles
3. Esterification of cholesterol: When cholesterol is taken up by HDL it is immediately esterified by LCAT (Lecithin Cholesterol acyltransferase). This enzyme is synthesized by the liver. As nascent HDL accumulates cholesteryl

esters, it first becomes cholesteryl poor HDL_3 and eventually cholesteryl rich HDL_2 that carries these esters to liver.

4. Reverse cholesterol transport : HDL cholesteryl esters are delivered to the liver by the following mechanisms as shown in Fig (14)

   a) Cholesteryl esters are selectively taken up from HDL and HDL particles are returned to circulation for further transport.

   b) Cholesteryl esters are transferred from HDL to apo B-100 then taken up by liver through receptor mediated endocyctosis. [100]

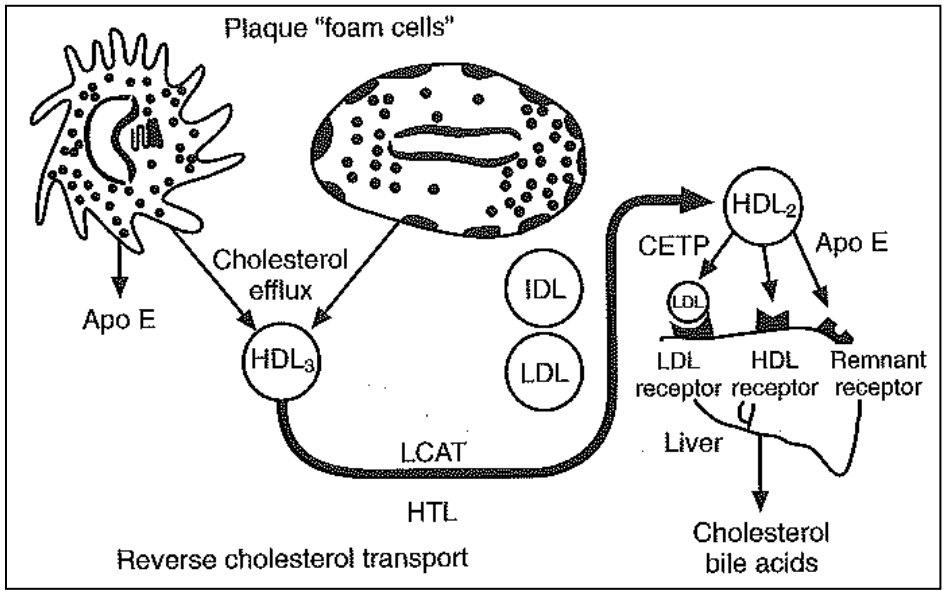


Fig (14) Reverse cholesterol transport pathway

Courtesy: Rifai N, Warnick RG. Lipids, Lipoproteins, Apolipoproteins and other cardiovascular risk factors. In: Burtis CA, Ashwood ER, Bruns DE, editors, Tietz textbook of clinical chemistry, 4th ed, Philadelphia, W.B Saunders 2006; p.921.

Different modifications of LDL convert it to a form recognized by macrophage scavenger receptors. Modifications that can favor foam cell formation in vitro include oxidation, aggregation, enzymatic modification, complexing with immunoglobulins and possibly others.[101] Furthermore oxidized LDL exhibits chemotactic and cytotoxic

activity.[102] Hypertriglyceridemia is a marker for increased risk for coronary artery diseaese.[103] Triglyceride related risk is partly the consequence of frequently associated changes in lipoprotein distribution, elevation of small dense readily oxidizable LDL and hemostatic disorders (increased factor VIIC and PAI-1). In addition, hypertriglycemidemia is associated with well-known cardiovascular risk factors like insulin resistance syndrome.[104] The correlation between triglyceride and coronary artery disease risk is the heterogenicity of triglyceride rich lipoproteins. The smaller and denser triglyceride rich particles are believed to have atherogenic potential.[105]

High HDL levels are protective against the development of atherosclerosis. In the Framingham study, risk for coronary artery disease increases sharply as HDL levels fall progressively below 40mg/dl.[106] In the Quebec cardiovascular study, for every 10% reduction in HDL, risk for MI increased by 13%.[107] The most important anti-atherogenic function of HDL is in its ability to drive reverse cholesterol transport. HDL reverses endothelial cell dysfunction, stimulating prostacyclin production, inhibits endothelial cell apoptosis, decreases platelet aggregability and inhibits LDL oxidation.[108]

Patients with coronary artery disease have low HDL phospholipids but elevated HDL triglycerides, plasma triglycerides, diglycerides and fibrinogen. Plasma lysolecithin is also diminished. Inhibition of coagulation is related to HDL phospholipids. The enhanced thrombus formation in coronary artery disease is related to altered HDL and plasma phospholipids, in particular to increased phosphotidyl ethanolamine. These adverse changes may result in increased deposition and reduced degradation and transport of lipids from arteriosclerotic lesion and thrombi and may therefore be a significant index of coronary artery disease.[109]

NON-HDL CHOLESTEROL

Recent studies conducted by Van Deventer HE et al on non-HDL cholesterol (non-HDLc) assays have showed overall the best concordance with reference measurement procedures (RMPs) for cardiovascular disease risk score classification of both normal and hypertriglyceridemic individuals. The non-HDL-c measure incorporates both LDL cholesterol and VLDL cholesterol, and thus reflects the cholesterol content of all apolipoprotein-B containing lipoproteins.[110]

Studies conducted by Shimamato T et al on Japanese population have shown that elevated triglyceride levels and low HDL-c is a strong predictor of MI. More over MI risk is high, precisely in those patients with mild to moderate hypertriglyceridemia and in whom measurement of non-HDL-c may be most useful.[111]

With respect to atherosclerosis two hypotheses are to be considered:

I. Lipid hypothesis of atherogenesis

II. Response to injury hypothesis

The lipid hypothesis of atherogenesis proposes that cholesterol in the blood is a major factor in cardiovascular disease and has been the focus of research seeking to prove or disprove its validity. Studies with lipid lowering agents such as statins and reduced cardiovascular risk have provided further associative evidence in support of lipid hypothesis.[112]

The response-to-injury hypothesis proposes that various, possibly different forms of insult may develop between the lining endothelium and the underlying cells of the artery wall. In hyperlipidemic individuals, they seem to be due principally to the lipids and lipoproteins associated with hyperlipidemia, whereas they may also result from

molecules yet to be identified with cigarette smoking, hypertension, diabetes, or possibly even some infectious agents. Diabetes is often complicated by varying forms of hyperlipidemia; thus, lipids may be critical, associative factors of atherogenesis in this disease.[113]

American Pathologist Earl Benditt has quoted "We cannot identify the unknown aspects of modern life that lead to atherosclerosis until we know the true nature of the characteristic atherosclerotic lesion". [114]

With all these perspectives it would useful to study the correlation of glycated hemoglobin, blood glucose, macrometals calcium, magnesium and fasting lipid profile in myocardial infarction.

Source of data: The present study is a case control study. The study group was selected from inpatients of RL Jalappa hospital and RL Jalappa Narayana Hrudayalaya Critical Care Unit. The control group was of same age and gender matched individuals irrespective of their diabetic status. This study included 106 subjects (cases-51 and controls-55) within a span of one year from February 2012 to July 2013. The study population comprised of men and women in the age group of 30-80yrs.

Sample size was calculated with $\alpha=0.05$ and $\beta=0.1$ (90% power)

$Z(1-\alpha/2) = 1.96$, $Z(1-\beta) = 1.282$ with the following formula[115]:

$$n= \frac{2\sigma^2 [Z (1-\alpha/2) + Z (1-\beta)]^2}{(\mu_1-\mu_2)^2}$$

Where

σ – Standard deviation

μ_1 and μ_2 – anticipated values of population means

STUDY GROUP:

Inclusion criteria:

1. Clinically proven cases of myocardial infarction in the age group of 30-80 yrs admitted in R L Jalappa Hospital and Research Centre and RL Jalappa Narayana Hrudayalaya -CCU, Kolar.
2. Patients with MI were included in this study irrespective of the history of diabetes mellitus.

Exclusion criteria

1. Patients with thyroid disorders, hemoglobinopathies, pregnancy and severely lipemic samples were excluded from this study because it may lead to altered HbA_{1c} levels.
2. Factors that are known to deplete magnesium such as those who are chronic alcoholics, thyroid dysfunction individuals and those who are on thiazide diuretics were excluded.
3. Patients with prostate and colorectal cancer or history of renal calculus, miscarriage, mental depression and those who are on calcium supplementation were excluded from the study because it may alter the serum calcium levels.

CONTROLS:

1. Included age and gender matched volunteers with no history of coronary heart disease.
2. After considering the inclusion and exclusion criteria the controls were selected accordingly.
3. Control group was screened for the same parameters which are done for cases.

Method of collection of data:

1. After obtaining informed consent from both cases and control groups, an overnight of minimum 8hrs fasting blood was collected for estimation of plasma glucose, serum lipid, calcium and magnesium. Fasting or post prandial whole blood for HbA_{1c} because values are not going to get altered. Post prandial plasma glucose was estimated in the 2 hr post prandial blood sample. All possible standard precautions were taken while collecting the blood samples. Sterile disposable needle and vacutainer were used for sample collection. The blood was drawn with the subject in sitting posture from the left antecubital vein.

Correct procedures were followed at every step such as site for venepuncture and pressure used to transfer into vacutainer on the whole the occurrence of hemolysis was prevented by this.

2. Biochemical parameters were done using suitable sample and specific methods-

   a. Estimation of HbA_{1c} was done by using whole blood mixed with lysing reagent to prepare a hemolysate and was analysed using weakly binding cation exchange resin and by using colorimeter at wavelength (λ)=415nm.[6]

   b. Plasma glucose was estimated by glucose oxidase and peroxidase method using dry chemistry analyzer –Vitros 250, from Johnson and Johnson.[116]

   c. Serum calcium estimation using colorimetric arsenazo III dye, λ=630nm.[117]

   d. Serum magnesium estimation by colorimetric xylidyl blue dye method, λ=505nm.[118]

   e. Since lipid profile is known to get altered due to non-fasting. Fasting lipid profile was done in Vitros 250 dry chemistry auto analyzer from Johnson and Johnson.

i. Total cholesterol[119]

ii. Triglycerides[120]

iii. HDL cholesterol[121]

Calculated parameters

iv. LDL cholesterol by Friedewald's formula [122] LDL = TC-[(TG/5) + HDLc]

v. Non-HDLc= [TC- HDLc] [110]

ESTIMATION OF HbA_{1C} BY ION EXCHANGE RESIN METHOD[6]

WEAKLY BINDING CATION EXCHANGE RESIN METHOD

PRINCIPLE:

Whole blood is mixed with lysing reagent to prepare a hemolysate. This is then mixed with a weakly binding cation exchange resin. The non-glycated hemoglobin binds to the resin leaving GHb free in supernatant. The GHb percentage is determined by measuring the absorbance of the GHb fraction and of the total Hb.

REAGENTS AND MATERIALS:

1. Resin reagent: 8mg/ml Cation-exchange resin buffered at pH 6.9
2. Lysing reagent: 10 mg Potassium cyanide surfactant added
3. Glycohemoglobin Standard: 10% Glycohemoglobin
4. Serum separators

PREPARATION OF REAGENTS

1. Glycohemoglobin lysing reagent: Bring the contents to room temperature.
2. Glycohemoglobin Cation-exchange resin: Bring contents to room temperature, swirl and gently invert a minimum of 10 times, swirl the bottle after addition of each tube.

REAGENTS STORAGE & STABILITY:

All reagents are stable at $2\text{-}8^0$ C till expiry date mentioned on the label. Do not freeze.

PHYSICAL OR CHEMICAL INDICATIONS OF INSTABILITY:

Alterations in the physical appearance of the reagents or values of control sera outside the manufacturer's acceptable range (4-20%) may be indications of reagents instability.

INSTRUMENT: Spectrophotometer or colorimeter set at 415 nm.

SPECIMEN COLLECTION AND PREPARATION:

Special preparation of the patient is unnecessary. Fasting specimens not required. No special additives or preservatives other than the anticoagulants are required. Collect venous blood with EDTA using aseptic technique.

INTERFERING SUBSTANCES:

Samples that are severely lipemic may cause elevated results. Fetal hemoglobin (HbF) has resin binding characteristic similar to Glycohemoglobin if present. Glycosylated HbS and HbC bind more tightly than HbA1 and produce lower values.

Other hemoglobinopathies (e.g beta thalassemia and hemolytic anemia) also produce lowered results.

MATERIALS REQUIRED:

1. 20 µl and 100µl micropipettes.
2. 500µl, 3ml and 5ml pipettes or dispensers.
3. 13 x 100 mm glass tubes.
4. Glass or plastic test tubes to hold 0.6 ml and 5 ml.
5. Rocker or rotator.
6. Glycohemoglobin controls: Normal level, Elevated level.

PROCEDURAL OUTLINE:

STEP A: **Hemolysate preparation:**

- Dispense 500 µl Lysing reagent into tubes Labeled: Standard, Control, Sample etc.

- Place 100 µl of the well mixed blood sample- Standard or control into the appropriately labeled tube. Mix well.
- Allow to stand for 5min.
- Plastic or glass tubes of appropriate size are acceptable.

STEP B: **Glycohemoglobin preparation**

1. Dispense 3.0 ml of Glycohemoglobin Cation exchange resin into 13 x 100 mm glass tube labeled Standard, Control, Sample etc.

   NOTE: Before use, mix the resin by inverting at least 10 times, swirl the bottle after addition to each tubes.
2. Add 100 µl of the prepared hemolysate (from Step A)
3. Position the filter separators in the tubes so that the rubber sleeve is approximately 1 cm above the liquid level.
4. Place the tubes on the rocker or rotator and mix continuously for 5min.
5. Remove the tubes from the rocker or rotator.
6. Push the filter separator into the tubes until the resin is firmly packed.
7. Supernatant may be poured into another tube or directly into a cuvette for absorbance measurement.
8. Adjust the instrument to zero absorbance at 415 nm with deionized water as the blank (Wavelength range: 390-420 nm)
9. Read and record the absorbance values for Standard, Control, Sample. These readings are glycohemoglobin.

STEP C: **Total Hemoglobin fraction:**

1. Dispense 5.0 ml deionized water into tubes labeled Standard, Control, Sample.
2. Place 20 µl of the hemolysate (from Step A) into appropriately labeled tube. Mix.
3. Adjust the instrument to Zero absorbance at 415 nm with deionized water as the blank.
4. Read and record the absorbance values for Standard, Control, and Sample. These readings are for total hemoglobin. Plastic or glass tubes of appropriate size are acceptable.

QUALITY CONTROL:

Glycohemoglobin Control: Normal, Elevated

CALCULATIONS:

Results for unknowns and controls are calculated as follows:

$$\text{Absorbance of Std} = \frac{\text{Absorbance of Std GHb}}{\text{Absorbance of Std THb}} = A1$$

$$\text{Absorbance of sample} = \frac{\text{Absorbance of Sample GHb}}{\text{Absorbance of Sample THb}} = A2$$

% GHb in sample= A2/A1x 10

Std Concentration=10

LIMITATION OF PROCEDURE:

Sample from patients with hemoglobinopathies or decreased erythrocytes survival time may show incorrect results.

EXPECTED VALUES:

Non-diabetics: 4.5-8.0 %

Good control: 8.0-9.0 %

Fair Control: 9.0-10.0 %

& Poor control: 10.0 % and above

LINEARITY:

The glycohemoglobin assay shows linearity for glycohemoglobin level in the range of 4.0-20.0 %. Blood samples with total hemoglobin greater than 18 g/dl should be diluted x 2 with deionized water before assay.

PRECISION:

Within run: The intra assay precision was established by assaying bloods with normal and elevated glycohemoglobin levels.

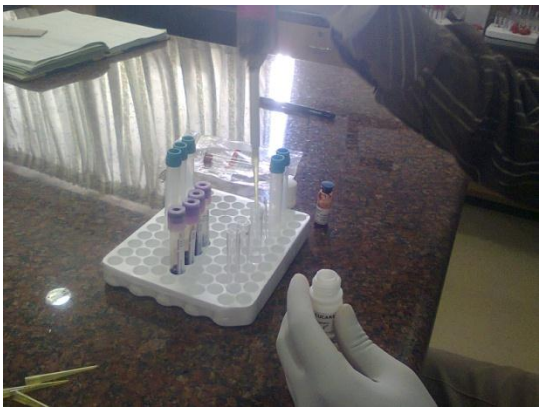
CORRELATION:

A comparative study of the glycated hemoglobin procedure and another widely used commercial method showed correlation (r) of 0.96.

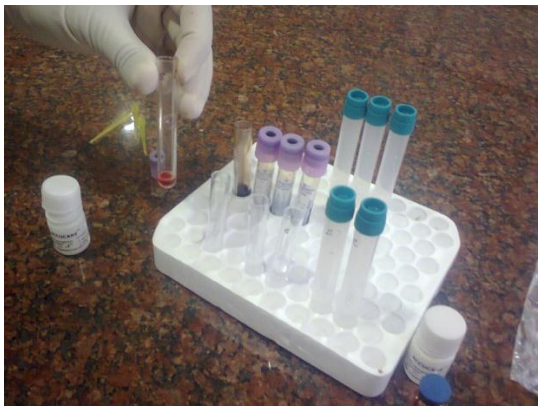
SENSITIVITY:

This glycohemoglobin procedure has a sensitivity of 0.02% glycohemoglobin per 0.001 units of absorbance.

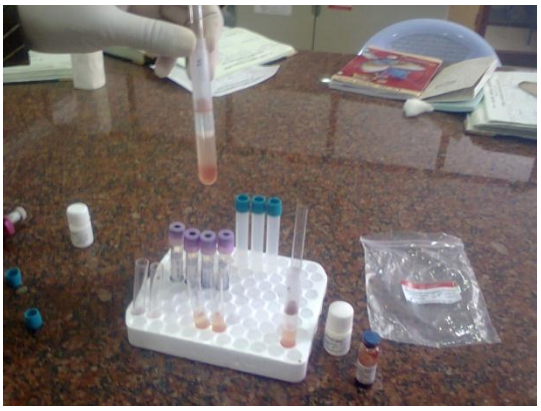
(a) HbA_{1c} estimation by cation exchange chromatography



(b) Preparation of hemolysate



(c) Hemolysate used for preparing total hemoglobin



(d) Hemolysate used for obtaining GHb in the cation resin tubes

(e) Eluent containing GHb

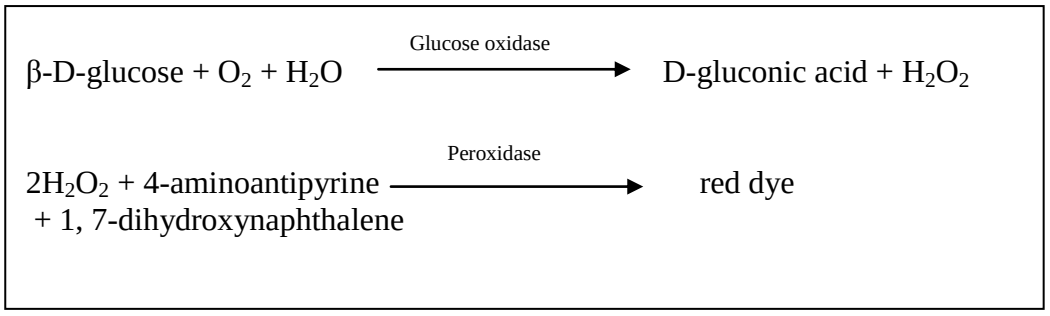
ESTIMATION OF GLUCOSE BY REFLECTANCE PHOTOMETRY[116]

INSTRUMENT: Vitros 250 dry chemistry auto analyzer based on reflectance photometry

PRINCIPLE:

The VITROS GLU Slide is a multilayered, analytical element coated on a polyester support.

A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. The oxidation of sample glucose is catalyzed by glucose oxidase to form hydrogen peroxide and gluconate. This reaction is followed by an oxidative coupling catalyzed by peroxidase in the presence of dye precursors to produce a dye. The intensity of the dye is measured by reflected light.



Incubation time: 5 min

Temperature: 37^0 C (98.6^0 F)

Wavelength: 540 nm

Reaction sample volume: 10µL

Sample: Plasma

Reagents

Reactive Ingredients per cm^2 of slide

Glucose oxidase (Aspergillus Niger, E.C.1.1.3.4) 0.77 U; peroxidase (horseradish root, E.C.1.11.1.7) 3.6 U; 1, 7- dihydroxynaphthalene (dye precursor) 67 μg and 4-aminoantipyrine hydrochloride (dye precursor) 0.11 mg.

Other Ingredients

Pigment, binders, buffer, surfactants, stabilizers and cross-linking agent.

Reagent Preparation

IMPORTANT: The slide cartridge must reach room temperature, 18–28 °C (64–82 °F), before it is unwrapped and loaded into the slide supply.

1. Remove the slide cartridges from storage.
2. Warm the wrapped cartridge at room temperature for 30 minutes when taken from the refrigerator or 60 minutes from the freezer.
3. Unwrap and load the cartridge into the slide supply.

Note: Load the cartridges within 24 hours after they reach room temperature, 18–28 °C.

Reagent Storage and Stability

VITROS GLU Slides are stable until the expiration date on the carton when they are stored and handled as specified. Do not use beyond the expiration date.

Interfering substances

- In fresh specimens, catalase released from the lysis of red blood cells causes a negative bias in glucose results. The degree of bias is proportional to the degree of hemolysis. In fresh samples, a negative bias of up to 10% may be observed with a level of hemolysis associated with a hemoglobin concentration of 250 mg/dL (2.5 g/L).

- Elevated lipids may limit diffusion of oxygen to the reactants. Dilute grossly lipemic samples twofold before analysis.

DETECTION LIMIT: 20-625 mg/dl

REFERENCE RANGE[27]:

The National Diabetes Data Group and World Health Organization have issued diagnostic criteria for Diabetes mellitus: Symptoms of diabetes plus random blood glucose concentration $\geq$ 200mg/dl (or) Fasting plasma glucose $\geq$ 126mg/dl (or) Two hour plasma glucose $\geq$ 200mg/dl during an oral glucose tolerance test.

QUALITY CONTROL: BIO-RAD internal and external quality assurance scheme followed strictly

ESTIMATION OF SERUM CALCIUM BY ARSENAZO III DYE METHOD[117]

At neutral pH, the Ca^{2+} with arsenazo III forms a complex, the color intensity of which is directly proportional to the concentration of calcium in the sample.

$$Ca^{2+} + \text{Arsenazo III} \xrightarrow{\text{pH 5.6}} \text{Colored complex}$$

REAGENTS:

Reagent 1: Arsenazo III reagent

Calcium standard: 10 mg/dl

SAMPLE:

Serum, heparinised plasma, 24 hr urine diluted 1:3 with distilled water (adjust the pH 3-4 with 0.4 N HCl)

REAGENT PREPARATION:

Reagent ready to use.

STABILITY: Upto expiry date when stored at room temperature.

AUTOMATED PARAMETERS

Type of reaction: End point

Wavelength: 650 nm

Incubation time: 5min

Cuvette: 1cm path length

Reaction temperature: Room temperature

Measurement: Against reagent blank

Sample/reagent ratio: 1:40

Blank absorbance limit: $\leq$ 0.8

Low normal: 8.8 mg/dl

High normal: 10.2 mg/dl

Linearity: 16.0 mg/dl

PROCEDURE:

Pipette into test tubes

	BLANK	STD	SAMPLE
SAMPLE	-	-	25µl
STANDARD(STD)	-	25µl	-
REAGENT	1000µl	1000µl	1000µl

Mix well; incubate at room temperature for 5 min. Measure final absorbance of the sample (T) and standard (S) against the reagent blank (B).

CALCULATION:

(T-B/S-B) x Concentration of standard = Ca^{2+} in *Serum* mg/dl

Concentration of standard=10 mg/dl

LINEARITY:

The method is linear upto a concentration of 16 mg/dl.

REFERENCE VALUES:

Serum: 8.8-10.2 mg/dl

DETECTION LIMIT: 4-16 mg/dl

Quality control: Accutestrol N- L- H

ESTIMATION OF SERUM MAGNESIUM BY XYLIDYL BLUE METHOD[118]

PRINCIPLE:

At alkaline pH magnesium reacts with xylidyl blue and produces a colored compound.

The intensity of color produced is proportional to magnesium concentration.

REAGENTS:

Reagent 1: Xylidyl blue reagent

Magnesium standard: 2.5 mg/dl

SAMPLE:

It is recommended to use serum.

When using plasma avoid EDTA which may increase results.

Urine should be previously taken to an acid pH value (pH 3-4) by adding some drops of HCl. Then dilute 1:5 with distilled water.

REAGENT PREPARATION:

Reagent ready to use.

STABILITY: Upto expiry date when stored at room temperature.

AUTOMATED PARAMETERS

Type of reaction: End point

Wavelength: 520 nm

Incubation time: 5min

Cuvette: 1cm path length

Reaction temperature: Room temperature

Measurement: Against reagent blank

Sample/reagent ratio: 1:100

Low normal: 1.9 mg/dl

High normal: 2.5 mg/dl

Linearity: 5.0 mg/dl

PROCEDURE:

Pipette into test tubes

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

Mix well; incubate at room temperature for 5 min. Measure final absorbance of the sample (T) and standard (S) against the reagent blank(B).

CALCULATION:

(T-B/S-B) x Concentration of Std = mg/dl in *Serum*

Concentration of standard=2.5 mg/dl

LINEARITY:

The method is linear upto a concentration of 5 mg/dl.

REFERENCE VALUES:

Serum: 1.9-2.5 mg/dl (1.6-2.0 mEq/L)

CSF: 2.4- 3.1 mg/dl (1.9-2.5 mEq/L)

Urine: 75-125 mg/ 24h (60-100 mEq/ 24h)

DETECTION LIMIT: 0.1-5mg/dl

Quality control: Accutestrol N- H

NOTE: Metallic ions and proteins do not interfere with assay. Use disposable plastic ware to run tests.

ESTIMATION OF SERUM TOTAL CHOLESTEROL BY REFLECTANCE PHOTOMETRY[119]

INSTRUMENT: Vitros 250 dry chemistry auto analyzer based on reflectance photometry

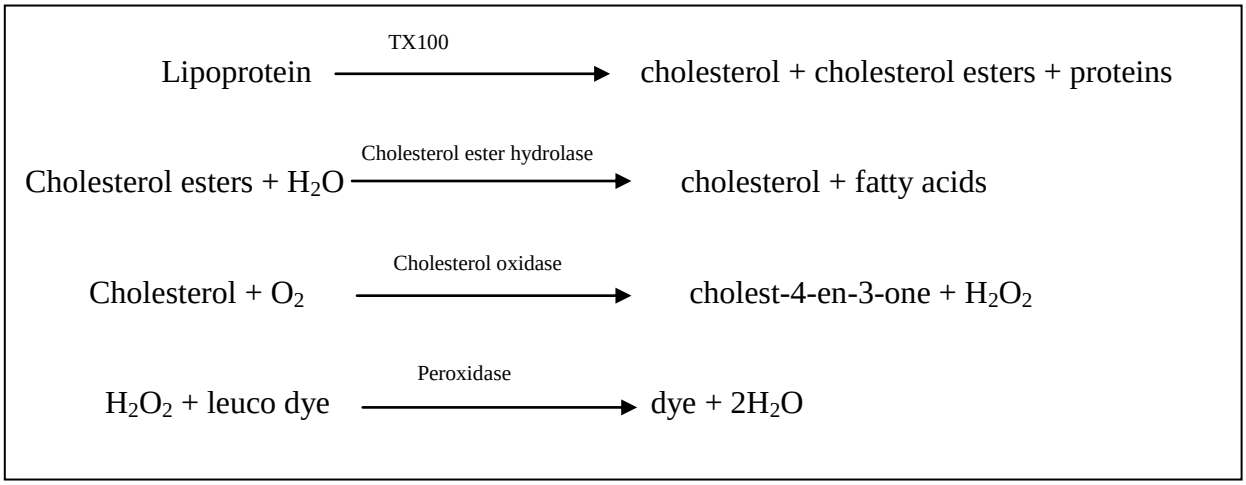
PRINCIPLE:

The VITROS CHOL Slide is a multilayered, analytical element coated on a polyester support. The method is based on an enzymatic method.

A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers.

The Triton X-100 (TX100) surfactant in the spreading layer aids in dissociating the cholesterol and cholesterol esters from lipoprotein complexes present in the sample. Hydrolysis of the cholesterol esters to cholesterol is catalyzed by cholesterol ester hydrolase. Free cholesterol is then oxidized in the presence of cholesterol oxidase to form cholestenone and hydrogen peroxide. Finally, hydrogen peroxide oxidizes a leuco dye in the presence of peroxidase to generate a colored dye.

The density of dye formed is proportional to the cholesterol concentration present in the sample and is measured by reflectance spectrophotometry.



Temperature: 37^0 C (98.6^0 F)

Wavelength: 540 nm

Reaction sample volume: 5.5µl

Sample: Serum

REAGENTS

Reactive Ingredients per cm^2 of slide

Triton X-100 0.81 mg; cholesterol oxidase (Nocardia or Cellulomonas, E.C.1.1.3.6) 0.4 U; cholesterol ester hydrolase (Pseudomonas, E.C.3.1.1.13) 2.0 U; peroxidase (horseradish root, E.C.1.11.1.7) 5.3 U; and 2-(3,5-dimethoxy- 4- hydroxyphenyl)-4,5-bis-(4-dimethylaminophenyl) imidazole (leuco dye) 0.17 mg.

Other Ingredients

Pigment, binders, buffer, surfactants, stabilizers and cross-linking agent.

Reagent Preparation

IMPORTANT: The slide cartridge must reach room temperature, 18–28 °C (64–82 °F), before it is unwrapped and loaded into the slide supply.

1. Remove the slide cartridges from storage.
2. Warm the wrapped cartridge at room temperature for 30 minutes when taken from the refrigerator or 60 minutes from the freezer.
3. Unwrap and load the cartridge into the slide supply.

Note: Load the cartridges within 24 hours after they reach room temperature, 18–28 °C.

Reagent Storage and Stability

VITROS CHOL Slides are stable until the expiration date on the carton when they are stored and handled as specified. Do not use beyond the expiration date.

INTERFERING SUBSTANCES

- Gentisic acid 5mg/dl
- N-acetylcysteine 10 mg/dl

DETECTION LIMIT: 50-325 mg/dl

REFERENCE RANGE[123]:

Desirable < 200 mg/dl

Borderline High 200–239 mg/dl

High ≥ 240 mg/dl

QUALITY CONTROL: BIO-RAD internal and external quality assurance scheme followed strictly

ESTIMATION OF SERUM TRIGLYCERIDES BY REFLECTANCE PHOTOMETRY[120]

INSTRUMENT: Vitros 250 dry chemistry auto analyzer based on reflectance photometry

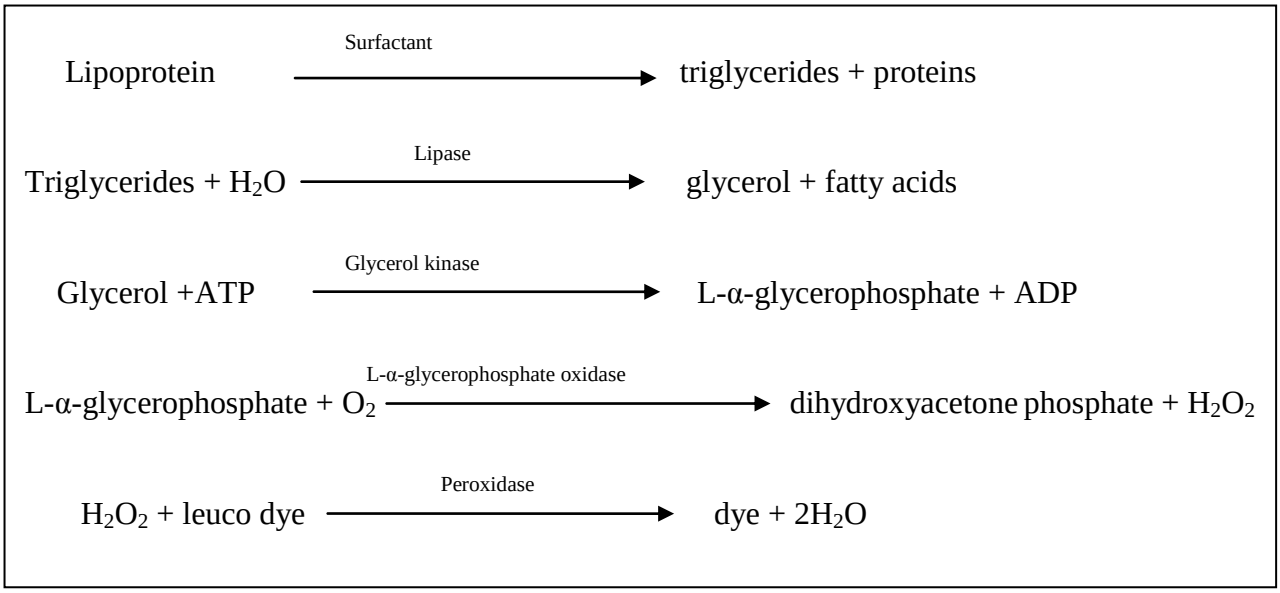
PRINCIPLE:

The VITROS TRIG Slide is a multilayered, analytical element coated on a polyester support. The analysis is based on an enzymatic method.

A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers.

The Triton X-100 surfactant in the spreading layer aids in dissociating the triglycerides from lipoprotein complexes present in the sample. The triglyceride molecules are then hydrolyzed by lipase to yield glycerol and fatty acids. Glycerol diffuses to the reagent layer, where it is phosphorylated by glycerol kinase in the presence of adenosine triphosphate (ATP). In the presence of L-α-glycerol-phosphate oxidase, L-α-glycerophosphate is then oxidized to dihydroxyacetone phosphate and hydrogen peroxide. The final reaction involves the oxidation of a leuco dye by hydrogen peroxide, catalyzed by peroxidase, to produce a dye.

The density of the dye formed is proportional to the triglyceride concentration present in the sample and is measured by reflectance spectrophotometry.



Temperature: 37^0 C (98.6^0 F)

Wavelength: 540 nm

Reaction sample volume: 5.5µl

Sample: Serum

REAGENTS

Reactive Ingredients per cm^2 of slide

Lipase (Candida rugosa, E.C.3.1.1.3) 0.15 U; Peroxidase (horseradish root, E.C.1.11.1.7) 0.52 U; glycerol kinase (Cellulomonas sp., E.C.2.7.1.30) 0.35 U; L-α-glycerophosphate oxidase (Pediococcus sp., E.C.1.1.3.21) 0.19 U; Triton X-100 0.62mg; 2-(3,5-dimethoxy-4-hydroxyphenyl)-4,5-bis(4-dimethylaminophenyl) imidazole (leuco dye) 0.04 mg; and adenosine triphosphate 0.14 mg.

Other Ingredients

Pigment, binders, buffer, surfactants, stabilizers, scavenger, enzyme co-factors, dye solubilizer and cross-linking agent.

Reagent Preparation

IMPORTANT: The slide cartridge must reach room temperature, 18–28 °C (64–82 °F), before it is unwrapped and loaded into the slide supply.

1. Remove the slide cartridges from storage.
2. Warm the wrapped cartridge at room temperature for 30 minutes when taken from the refrigerator or 60 minutes from the freezer.
3. Unwrap and load the cartridge into the slide supply.

Note: Load the cartridges within 24 hours after they reach room temperature, 18–28 °C.

Reagent Storage and Stability

VITROS TRIG Slides are stable until the expiration date on the carton when they are stored and handled as specified. Do not use beyond the expiration date.

INTERFERING SUBSTANCES

- Free (non-esterified) glycerol in serum is measured along with the glycerol from the hydrolysis of triglycerides and diglycerides. Certain clinical conditions (e.g., diabetes mellitus and cardiac ischemia) show high endogenous free glycerol levels. Some drugs used in the treatment of lipemia also produce elevated glycerol levels. Triglyceride results from samples of such patients will not reflect actual serum triglyceride content.
- Grossly lipemic samples show a slower rate of color development than do clear serums, which results in a negative bias.

DETECTION LIMIT: 10-525 mg/dl

REFERENCE RANGE[123]:

Normal < 150 mg/dl

Borderline High 150–199 mg/dl

High 200–499 mg/dl

Very High ≥ 500 mg/dl

QUALITY CONTROL: BIO-RAD internal and external quality assurance scheme followed strictly

ESTIMATION OF SERUM HDL CHOLESTEROL BY REFLECTANCE PHOTOMETRY[121]

INSTRUMENT: Vitros 250 dry chemistry auto analyzer based on reflectance photometry

PRINCIPLE:

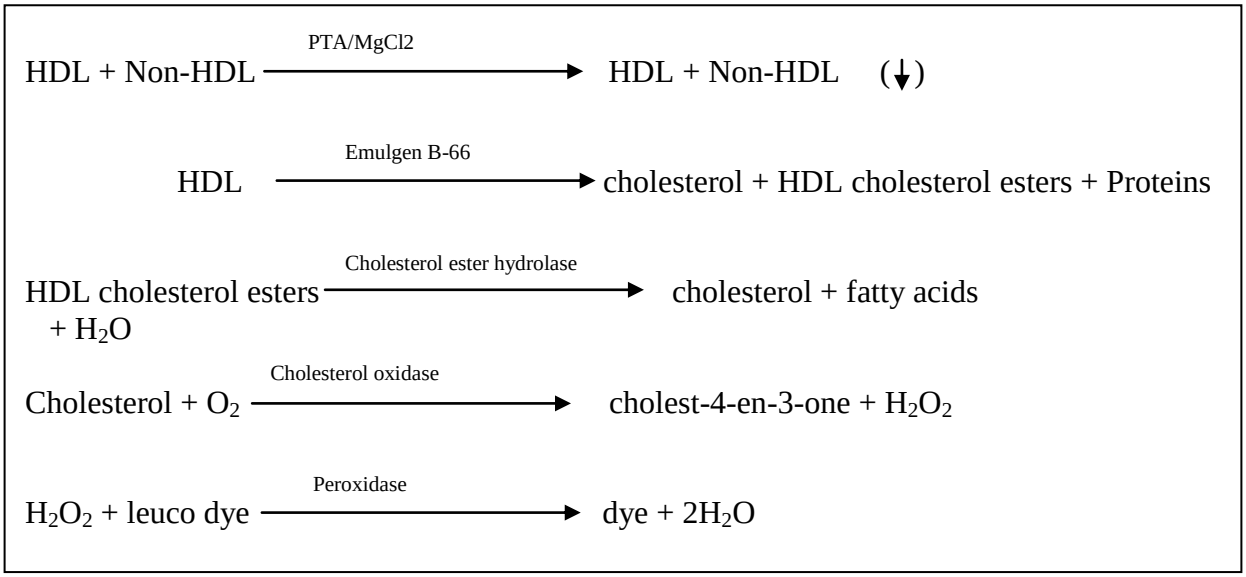
The VITROS dHDL Slide is a multilayered analytical element coated on a polyester support. The method is based on a non-HDL precipitation method.

A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers.

HDL is separated by the precipitation of non-High Density Lipoproteins (non-HDL) using phosphotungstic acid (PTA) and magnesium chloride ($MgCl_2$) in the spreading layer. The Emulgen B-66 surfactant in the spreading layer aids in the selective dissociation of the cholesterol and cholesterol esters from the HDL lipoprotein complexes present in the sample. Hydrolysis of the HDL-derived cholesterol ester to cholesterol is catalyzed by a selective cholesterol ester hydrolase. Free cholesterol is then oxidized in

the presence of cholesterol oxidase to form cholestenone and hydrogen peroxide. Finally, hydrogen peroxide oxidizes a leuco dye in the presence of peroxidase to generate a colored dye.

The density of dye formed is proportional to the HDL cholesterol concentration present in the sample and is measured by reflectance spectrophotometry.



Temperature: 37^0 C (98.6^0 F)

Wavelength: 670 nm

Reaction sample volume: 10µl

Sample: Serum

Reagents

Reactive Ingredients per cm^2 of slide

Emulgen B-66 0.7 mg; phosphotungstic acid 0.3 mg; magnesium chloride 0.2 mg, cholesterol oxidase (Cellulomonas, E.C.1.1.3.6) 0.8 U; cholesterol ester hydrolase (Candida rugosa, E.C.3.1.1.3) 1.2 U; peroxidase (horseradish root, E.C.1.11.1.7) 2.2 U;

and 2- (3,5-dimethoxy-4-hydroxyphenyl)-4,5-bis-(4-dimethylaminophenyl) imidazole (leuco dye) 0.02 mg.

Other Ingredients

Pigment, binders, buffer, surfactants, stabilizers, scavenger and cross-linking agent.

Reagent Preparation

IMPORTANT: The slide cartridge must reach room temperature, 18–28 °C (64–82 °F), before it is unwrapped and loaded into the slide supply.

1. Remove the slide cartridges from storage.
2. Warm the wrapped cartridge at room temperature for 30 minutes when taken from the refrigerator or 60 minutes from the freezer.
3. Unwrap and load the cartridge into the slide supply.

Note: Load the cartridges within 24 hours after they reach room temperature, 18–28 °C.

Reagent Storage and Stability

VITROS dHDL Slides are stable until the expiration date on the carton when they are stored and handled as specified. Do not use beyond the expiration date.

INTERFERING SUBSTANCES:

- Ascorbic Acid 3mg/dL (170 μmol/L)
- Bronidox 800 mg/dL (38 mmol/L)
- Dopamine 4 mg/dL (0.26 mmol/L)
- Gentisic Acid 5 mg/dL (0.32 mmol/L)
- N-acetylcysteine 10 mg/dL (0.61 mmol/L)

DETECTION LIMIT: 5-110 mg/dl

REFERENCE RANGE[123]:

Low < 40.0 mg/dl

High ≥ 60.0mg/dl

QUALITY CONTROL: BIO-RAD internal and external quality assurance scheme followed strictly

CALCULATION OF LDL CHOLESTEROL BY FRIEDEWALD'S FORMULA[122]

LDLc= TOTAL CHOLESTEROL – (TRIGLYCERIDES/5) – HDLc

Limitations of Friedewald's formula:

a. Division of the total plasma triglyceride concentration by the factor five yields a falsely low value for the "VLDL" and falsely high value for the "LDL" contribution to the total plasma cholesterol. Thus, when this formula is used, a Type III patient may be falsely classified as a Type II.

b. The anomalous lipoproteins in Type III are detectable with certainty only by ultracentrifugal isolation of VLDL and determination of either its electrophoretic mobility or cholesterol and triglyceride content.

c. Third, LDLc cannot always be accurately estimated when the plasma triglyceride concentration exceeds 400 mg/dl or when plasma triglyceride is less than 40mg/dl.

CALCULATION OF NON-HDL[110]

Non HDL-c = Total cholesterol – HDL-c

STATISTICAL ANALYSIS

The data collected was tabulated and analyzed using descriptive statistical tools- mean, standard deviation, and comparison between the groups was carried out by using independent student 't' test, Mann Whitney 'U' test and Pearson's correlation analysis. Complete analysis was carried out using SPSS package evaluatory version 14.

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented in Mean ± SD and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. The following assumptions on data are made, Assumptions:

1. Dependent variables should be normally distributed

2. Samples drawn from the population should be random, Cases of the samples should be independent

Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups on metric parameters.

Mann-Whitney 'U' test is a non-parametric test of the null hypothesis that two populations are the same against an alternative hypothesis and is as efficient as Independent't' test. It has been used for sub group analyses of data as the sample size in each subgroup is less than 30.

If the p-value associated with the t-test is small (< 0.05), there is evidence to reject the null hypothesis in favor of the alternative. In other words, there is evidence that the means are significantly different at the significance level reported by the p-value. If the p-value associated with the t-test is not small (> 0.05), there is not enough evidence to

reject the null hypothesis, and you conclude that there is evidence that the means are not different.

Pearson's correlation coefficient (r) is a measure of the linear correlation (dependence) between two variables X and Y, giving a value between +1 and −1 inclusive, where 1 is total positive correlation, 0 is no correlation, and −1 is negative correlation. It has been used in this as a measure of the degree of linear dependence between two variables. Pearson's correlation coefficient is obtained using the following formula:

$$r = \frac{\sum_{i=1}^{n}(X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\sum_{i=1}^{n}(X_i - \bar{X})^2}\sqrt{\sum_{i=1}^{n}(Y_i - \bar{Y})^2}}$$

Significant Figures

* Statistically significant (p value :< 0.05)

** Strongly significant (p value : <0.001)

The present study is a case control study which includes 51 cases of acute myocardial infarction irrespective of their diabetic status and 55 age and gender matched controls. This study was undertaken to estimate glycated hemoglobin, blood sugar, calcium, magnesium and fasting lipid profile (Total cholesterol, HDL, LDL, Triglycerides & Non-HDL) in individuals with acute myocardial infarction irrespective of diabetic status and also to correlate HbA_{1c} with other blood parameters. A series of statistical tables and graphs are used to present the results.

The cases comprised of 45 males and 06 females, showing a male preponderance with a male: female ratio of 15:2. The mean age was 57.19 $\pm$ 10.71 yrs (range 30-80yrs), suggesting an increased trend of AMI with advancing age and included 23 diabetics, 28 non-diabetics. Among the diabetics 19 were males and 04 females. The occurrence of STEMI (64.7%) was more than NSTEMI (35.3%) as shown in Fig (15). There was an increased risk of AMI in smokers (54.9%) than non-smokers (45.1%) (Odds Ratio 3.24, 95% Confidence Interval 1.4439 to 7.2988) ($p<0.05$) as shown in Fig (16).

The study group comprised of both diabetics and non-diabetics patients and respective controls. The subjects were grouped as follows:

Group I – All acute MI cases irrespective of their diabetic status

Group II – Controls

Group III – Diabetic AMI patients

Group IV – Non-diabetic AMI patients

FIG (15) DISTRIBUTION OF TYPES OF AMI

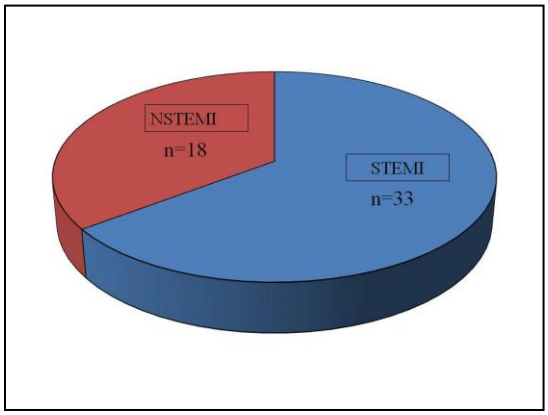


FIG (16) DISTRIBUTION OF SMOKING HABIT IN STUDY GROUP (Total Cases & Controls)

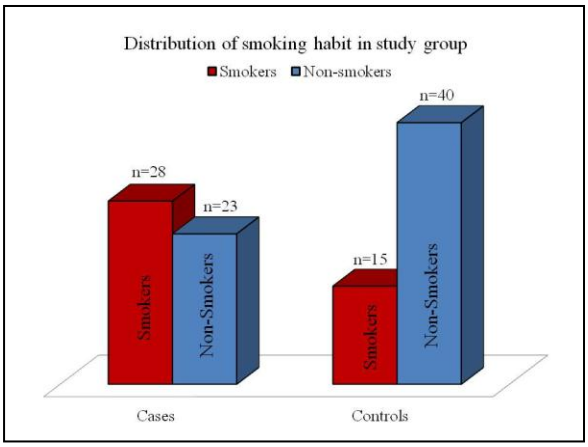
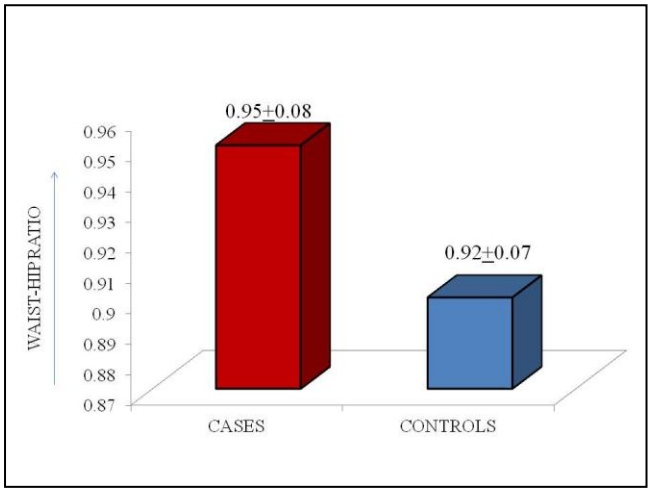


FIG (17) COMPARISON OF WAIST:HIP RATIO AMONG TOTAL CASES AND CONTROLS



In the cases waist:hip ratio was 0.95±0.08 and in controls 0.92±0.07 ($p<0.05$) however, BMI was not significantly elevated in cases in comparison to controls; suggesting that abdominal obesity was a better predictor of AMI regardless of the diabetic status as shown in Fig (17).

The biochemical parameters were compared between cases and controls using Independent't' test as shown in table (3). Subgroup analyses were done for diabetic AMI patients and non-diabetic AMI patients as shown in table (4) and table (5) using Mann-Whitney 'U' test as the sample size in each Group III and Group IV was less than 30. For the intra group association of HbA_{1c} with other biochemical parameters Pearson's correlation was done.

The mean value of HbA_{1c} in AMI patients was 7.52±1.56% in comparison to 7.59±1.33% in controls. In the subgroup analyses, in the diabetic AMI group the mean was 8.63±0.99% and 8.79±0.9% in controls. In the non-diabetic AMI group the mean was 6.6±1.33% and 6.67±0.74% in controls. The data has been represented in Fig (18), (19), (20).

TABLE (3) COMPARISON OF PARAMETERS BETWEEN ACUTE MYOCARDIAL INFARCTION CASES (GROUP I) AND CONTROLS (GROUP II) IRRESPECTIVE OF THEIR DIABETIC STATUS BY STUDENT 'T' TEST

PARAMETER	GROUP I (n=51) MEAN ± SD	GROUP II (n=55) MEAN ± SD	'p' VALUE
BMI (kg/m^2)	23.82±4.21	22.85±3.68	0.11
WAIST – HIP RATIO	0.95±0.08	0.92±0.07	<0.05*
FBS (mg/dl)	132.22±64.24	115.04±53.88	0.138
PPBS (mg/dl)	161.86±92.48	162.8±89.32	0.958
HbA_{1c} %	7.52±1.56	7.59±1.33	0.79
Serum calcium (mg/dl)	9.76±0.62	9.08±0.45	<0.001**
Serum Magnesium (mg/dl)	2.42±0.13	2.22±0.18	<0.001**
Total cholesterol (mg/dl)	163.9±40.08	172.5±36.81	0.253
Triglycerides (mg/dl)	145.59±83.64	148.87±58.61	0.814
HDL (mg/dl)	35.94±8.81	41.1±8.46	<0.05*
LDL (mg/dl)	100.64±32.92	101.6±31	0.878
Non – HDL (mg/dl)	127.04±39.94	131±35.8	0.592

* Statistically significant
** Strongly significant

TABLE (4) COMPARISON OF PARAMETERS BETWEEN ACUTE MYOCARDIAL INFARCTION WITH DIABETES MELLITUS (GROUP III) AND CONTROLS (GROUP II) BY MANN WHITNEY 'U' TEST

PARAMETER	GROUP III (n=23) MEAN ± SD	GROUP II (n=24) MEAN ± SD	'p' VALUE
BMI (kg/m^2)	25.61±4.99	24.15±3.55	0.496
WAIST – HIP RATIO	0.98±0.07	0.94±0.09	0.143
FBS (mg/dl)	177.52±71.37	154.12±62.1	0.263
PPBS (mg/dl)	230.39±100.62	240.5±85.88	0.573
HbA_{1c} %	8.63±0.997	8.79±0.9	0.22
Serum calcium (mg/dl)	9.85±0.737	9.23±0.42	<0.05*
Serum Magnesium (mg/dl)	2.43±0.11	2.17±0.139	<0.001**
Total cholesterol (mg/dl)	157.96±37.32	172.8±32.24	0.17
Triglycerides (mg/dl)	145.87±95.46	154.96±48.6	0.221
HDL (mg/dl)	34.39±8.43	38.91±6.56	<0.05*
LDL (mg/dl)	96.45±31.61	102.7±32.19	0.468
Non – HDL (mg/dl)	122.65±37.25	133.17±33.87	0.278

* Statistically significant
** Strongly significant

TABLE (5) COMPARISON OF PARAMETERS BETWEEN ACUTE MYOCARDIAL INFARCTION WITHOUT DIABETES MELLITUS (GROUP IV) AND CONTROLS (GROUP II) BY MANN WHITNEY 'U' TEST

PARAMETER	GROUP IV (n=28) MEAN ± SD	GROUP II (n=31) MEAN ± SD	'p' VALUE
BMI (kg/m^2)	22.34±2.75	21.36±3.55	0.242
WAIST – HIP RATIO	0.92±0.07	0.9±0.05	0.272
FBS (mg/dl)	95±17.54	84.77±9.92	<0.05*
PPBS (mg/dl)	105.5±17.45	102.65±11.71	0.704
HbA_{1c} %	6.6±1.33	6.67±0.74	0.308
Serum calcium (mg/dl)	9.68±0.52	8.96±0.44	<0.001**
Serum Magnesium (mg/dl)	2.42±0.15	2.27±0.21	<0.05*
Total cholesterol (mg/dl)	168.8±42.25	172.2±40.53	0.773
Triglycerides (mg/dl)	145.36±74.3	144.1±65.73	0.849
HDL (mg/dl)	37.21±9.06	42.8±9.44	<0.05*
LDL (mg/dl)	103.92±34.12	100.68±30.55	0.693
Non – HDL (mg/dl)	130.64±42.34	129.32±37.69	0.873

* Statistically significant
** Strongly significant

Fig (18) Comparison of HbA_{1c} between AMI total cases and controls

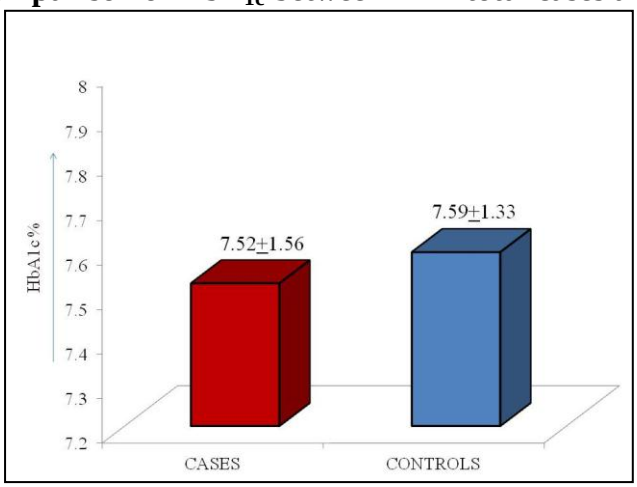


Fig (19) Comparison HbA_{1c} between Diabetic AMI patients and controls (Subgroup analyses)

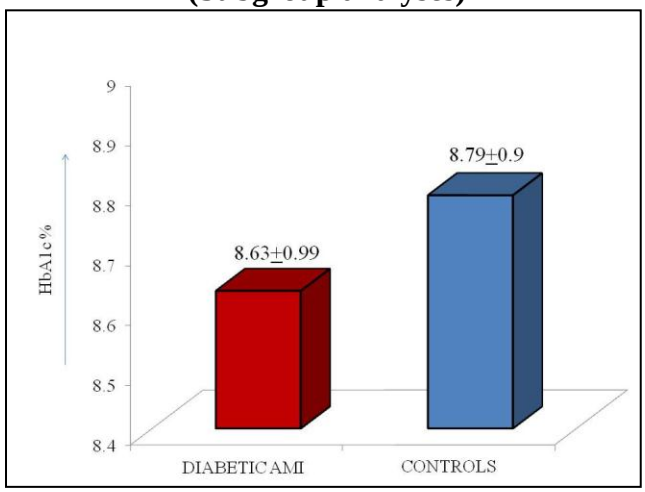
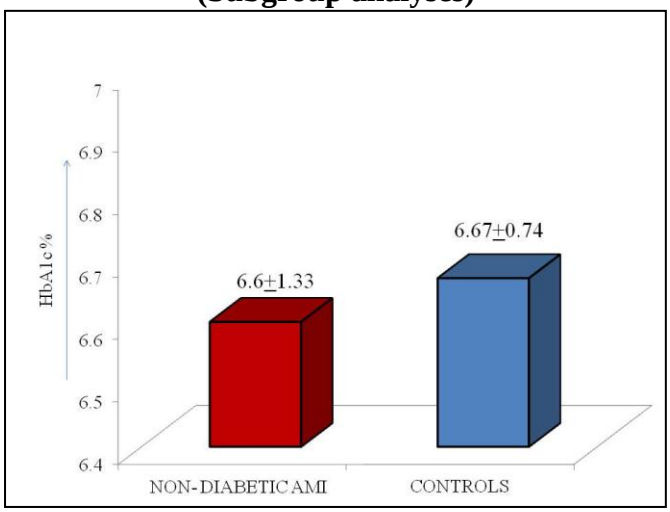


Fig (20) Comparison of HbA_{1c} between Non-diabetic AMI patients and controls (Subgroup analyses)



Pearson's correlation analysis was done for HbA_{1c} with other biochemical parameters as shown in table (6) and the scatter plots are represented as Fig (21) to (29).

TABLE (6) PEARSON'S CORRELATION OF HbA_{1c} WITH OTHER BIOCHEMICAL PARAMETERS IN ACUTE MYOCARDIAL INFARCTION CASES

PARAMETER	'r' VALUE	'p' VALUE
FBS (mg/dl)	0.522	<0.001**
PPBS (mg/dl)	0.53	<0.001**
Serum calcium (mg/dl)	0.146	0.307
Serum Magnesium (mg/dl)	0.194	0.173
Total cholesterol (mg/dl)	-0.123	0.391
Triglycerides (mg/dl)	0.055	0.7
HDL (mg/dl)	-0.013	0.928
LDL (mg/dl)	-0.144	0.319
Non – HDL (mg/dl)	-0.124	0.388

*Statistically significant
** Strongly significant

Fig (21) Pearson's correlation of HbA_{1c} with FBS in AMI cases

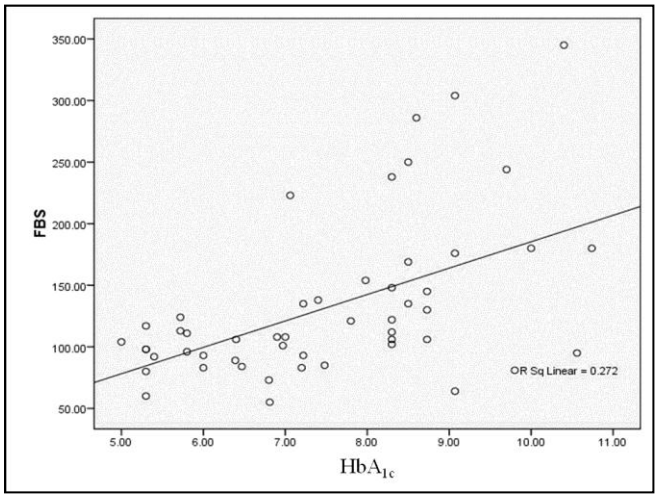


Fig (22) Pearson's correlation of HbA_{1c} with PPBS in AMI cases

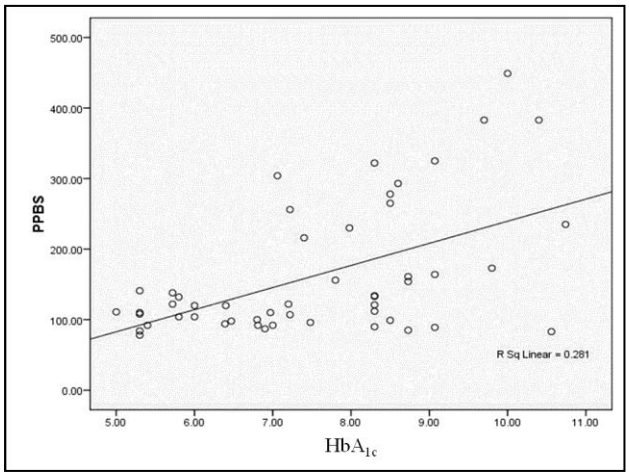


Fig (23) Pearson's correlation of HbA_{1c} with serum calcium in AMI cases

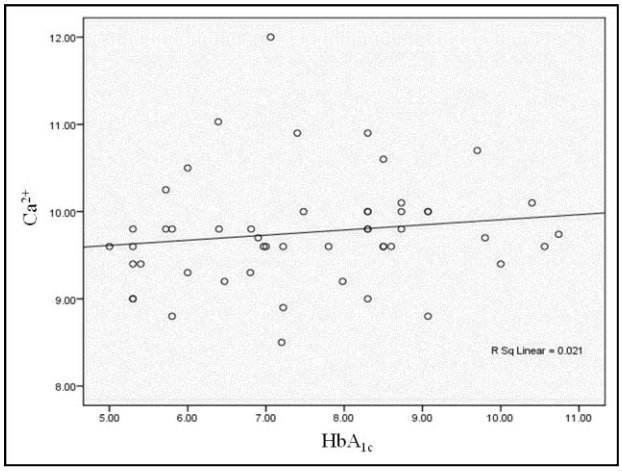


Fig (24) Pearson's correlation of HbA_{1c} with serum magnesium in AMI cases

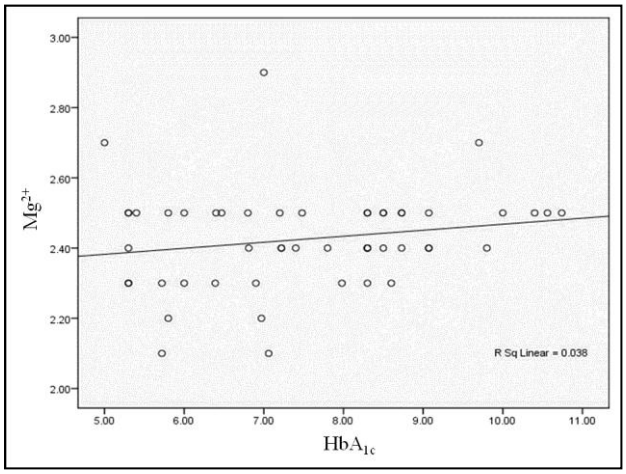


Fig (25) Pearson's correlation of HbA_{1c} with serum cholesterol in AMI cases

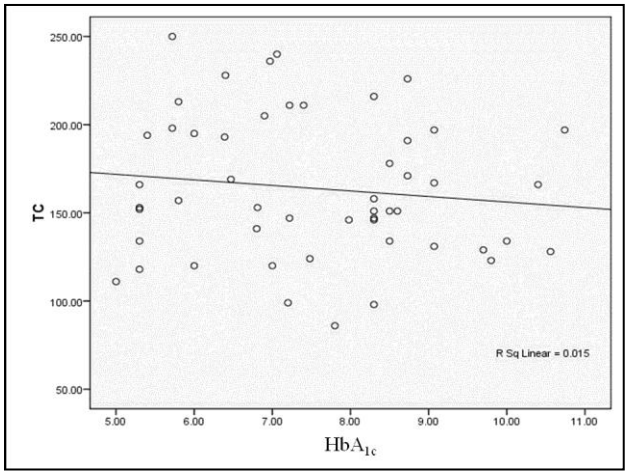


Fig (26) Pearson's correlation of HbA_{1c} with serum TG in AMI cases

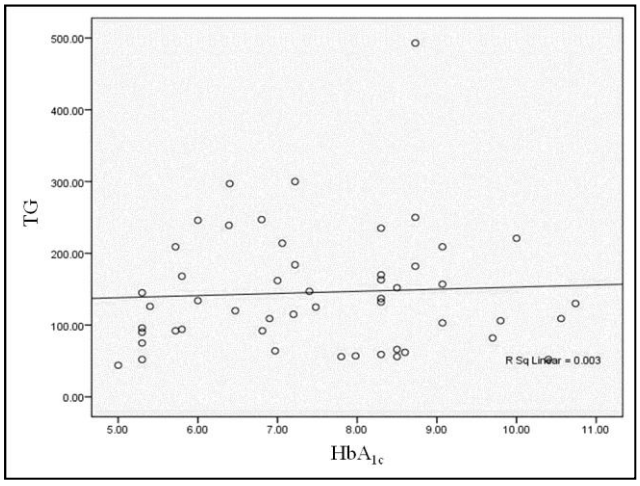


Fig (27) Pearson's correlation of HbA_{1c} with serum HDLc in AMI cases

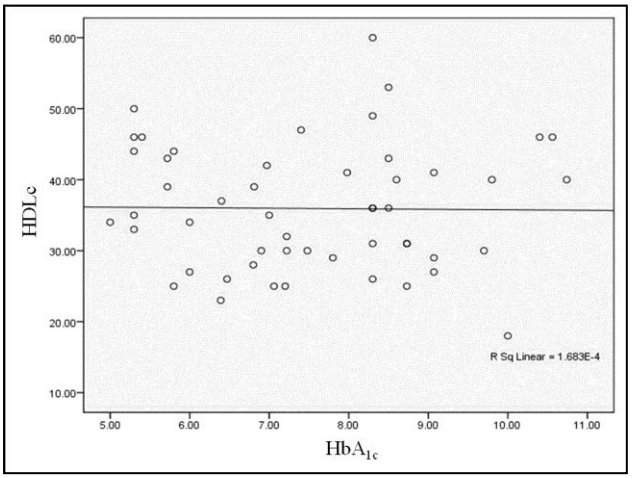


Fig (28) Pearson's correlation of HbA_{1c} with serum LDLc in AMI cases

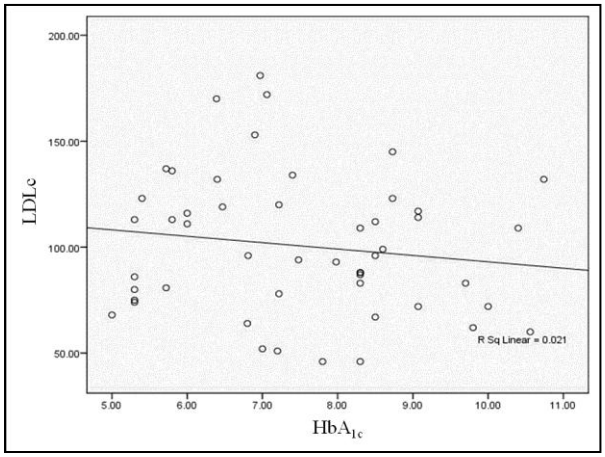
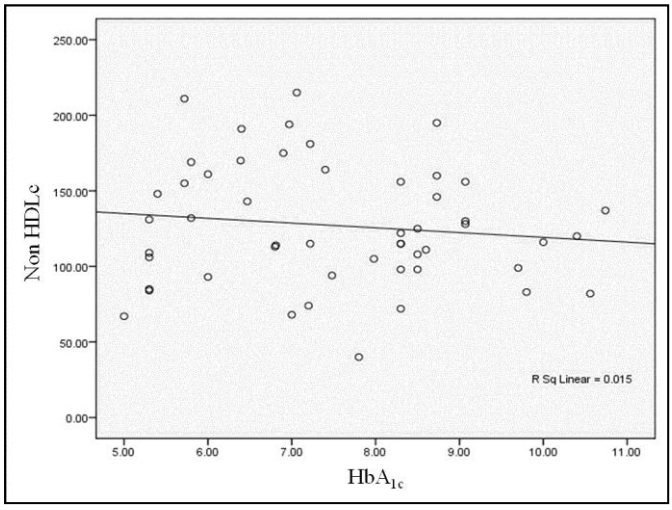


Fig (29) Pearson's correlation of HbA_{1c} with Non-HDLc in AMI cases



It was observed that HbA_{1c} was not significantly elevated in AMI irrespective of diabetic status and also in subgroup analyses of diabetics and non-diabetics. HbA_{1c} correlated strongly with FBS & PPBS ($p<0.001$ both groups). However, there was no strong correlation of HbA_{1c} with other parameters. This finding corroborates with that observed in other studies implying that HbA_{1c} is a predictor of chronic glycometabolic state rather than independent risk factor of AMI.[124, 125]

The fasting plasma glucose data of all groups total n=51 cases and controls n=55 has been represented in Fig (30) (31) (32). The fasting blood glucose levels were significantly increased in non-diabetic AMI patients 95 ± 17.54 mg/dl compared to non-diabetic controls 84.77 ± 9.92 mg/dl ($p<0.05$). This finding of elevated fasting glucose within the reference range is at par with the observations made by the DECODE study group.[126] However, this difference was not significant in diabetic AMI patients compared to diabetic controls.

The serum calcium was significantly elevated in AMI both groups when compared to respective controls but within the reference range and the same has been represented in Fig (33), (34), (35).The mean value of serum calcium in AMI group was 9.76 ± 0.62mg/dl and 9.08 ± 0.45mg/dl in controls ($p<0.001$) [Fig (33)].In the subgroup analyses, in the diabetic AMI group the mean was 9.85 ± 0.74mg/dl and 9.23 ± 0.42mg/dl in controls ($p<0.05$) [Fig (34)]. In the non-diabetic AMI group the mean was 9.68 ± 0.52mg/dl and 8.96 ± 0.44mg/dl in controls ($p<0.001$) [Fig (35)]. This finding is similar to the observation recorded in the Tromso study, a prospective cohort study.[79]

Fig (30) Comparison of FBS between total cases and controls

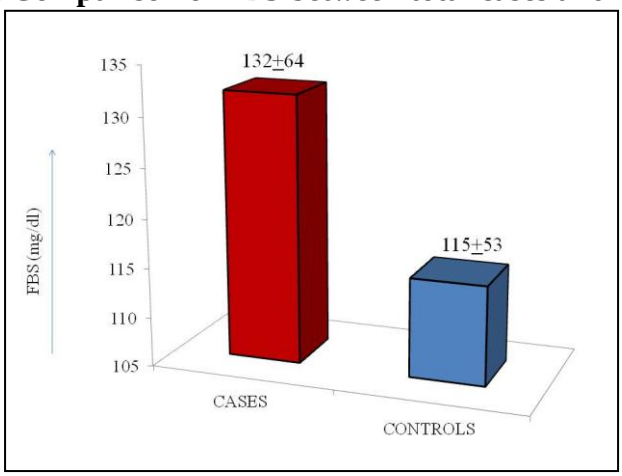


Fig (31) Comparison of FBS between Diabetic AMI and controls (Subgroup analyses)

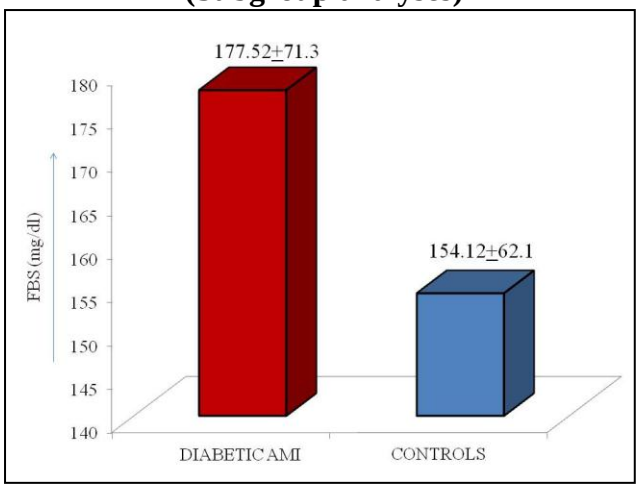


Fig (32) Comparison of FBS between Non-diabetic AMI and controls (Subgroup analyses)

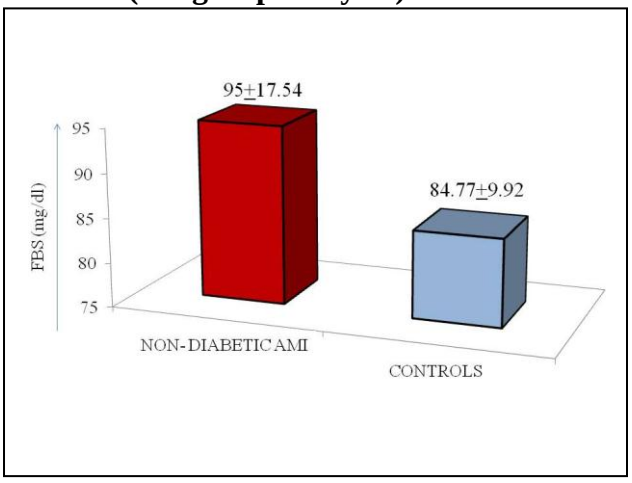


Fig (33) Comparison of serum calcium between AMI total cases and controls

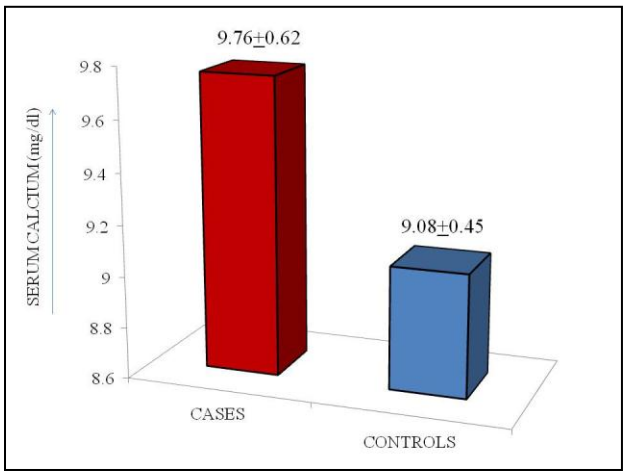


Fig (34) Comparison of serum calcium between Diabetic AMI and controls (Subgroup analyses)

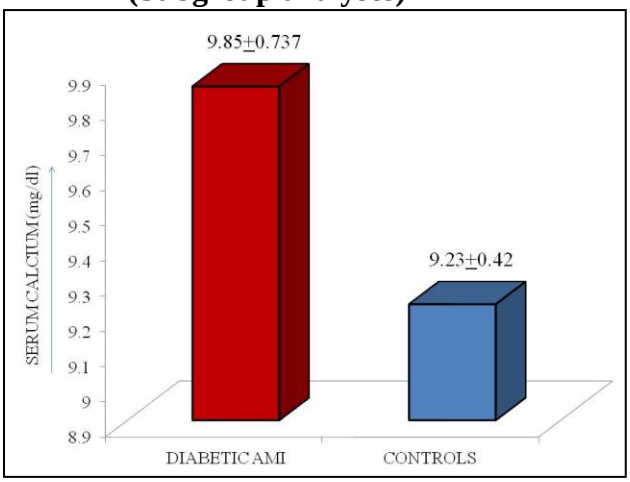
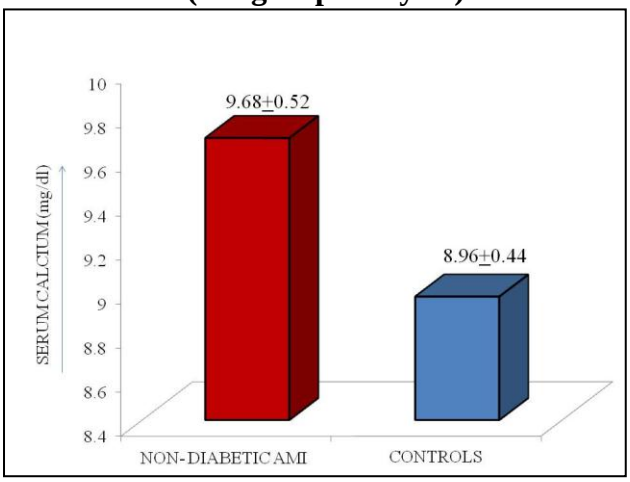


Fig (35) Comparison of serum calcium between Non-diabetic AMI and controls (Subgroup analyses)



The mean value of serum magnesium in AMI group was 2.42±0.13 mg/dl and 2.22±0.18 mg/dl in controls ($p<0.001$) [Fig (36)].In the subgroup analyses, in the diabetic AMI group the mean was 2.43±0.11mg/dl and 2.17±0.139mg/dl in controls ($p<0.001$) [Fig (37)]. In the non-diabetic AMI group the mean was 2.42±0.15mg/dl and 2.27±0.21mg/dl in controls ($p<0.05$) [Fig (38)].In this study serum magnesium levels was significantly increased in AMI patients irrespective of their diabetic status compared to controls, however the observed values were within the reference range. In view of hyperglycemia observed in this study elevation of serum magnesium is a probability of the acute glycometabolic state where magnesium is an essential cofactor for enzymes of carbohydrate metabolism. Another possibility could be due to impaired renal handling of magnesium in myocardial infarction.

In the lipid profile significance was found with respect to HDLc only which was significantly decreased when compared to controls. The mean value of serum HDLc in AMI group was 35.94±8.81mg/dl and 41.1±8.46mg/dl in controls ($p<0.05$) [Fig (39)].In the subgroup analyses, in the diabetic AMI group the mean was 34.39±8.43mg/dl and 38.91±6.56mg/dl in controls ($p<0.05$) [Fig (40)]. In the non-diabetic AMI group the mean was 37.21±9.06mg/dl and 42.8±9.44mg/dl in controls [Fig (41)]. Isolated low HDLc and increased risk of cardiovascular disease has been demonstrated by Huxley RR et al.[127] The present study supports this finding where significant difference was observed with respect to HDLc alone.

Fig (36) Comparison of serum magnesium between AMI total cases and controls

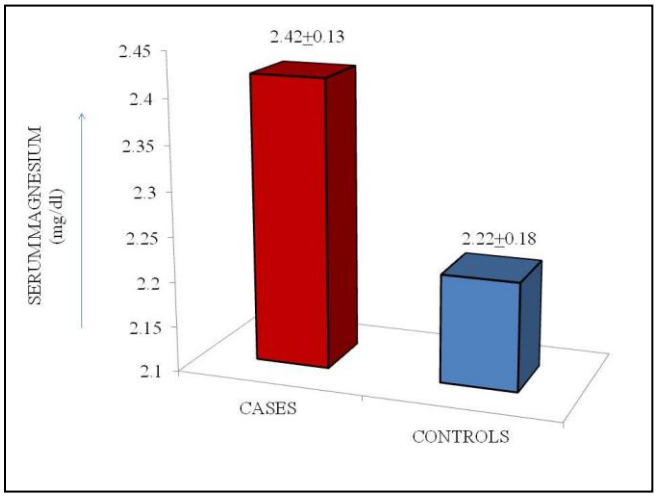


Fig (37) Comparison of serum magnesium between Diabetic AMI and controls (Subgroup analyses)

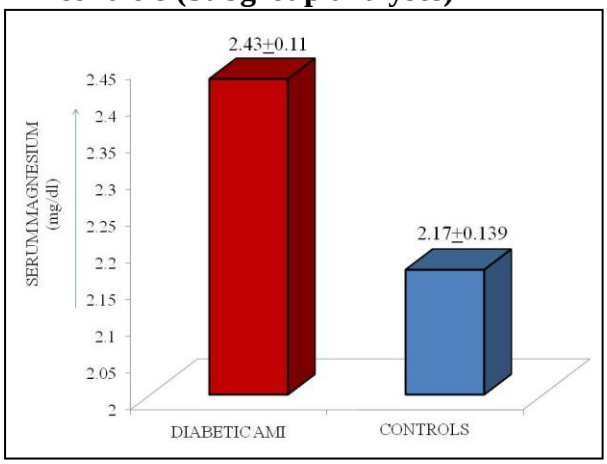


Fig (38) Comparison of serum magnesium between Non-diabetic AMI and controls (Subgroup analyses)

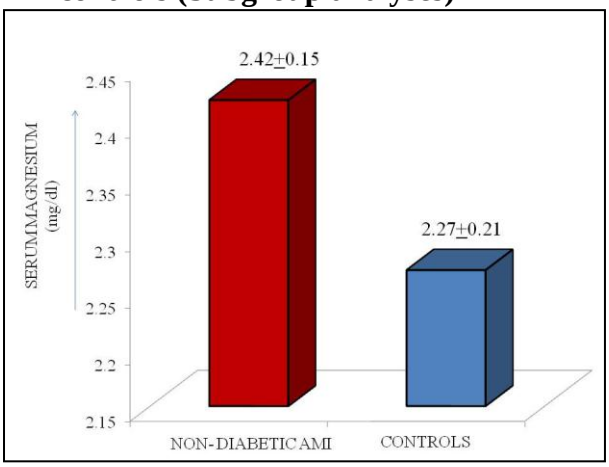


Fig (39) Comparison of serum HDLc between AMI total cases and controls

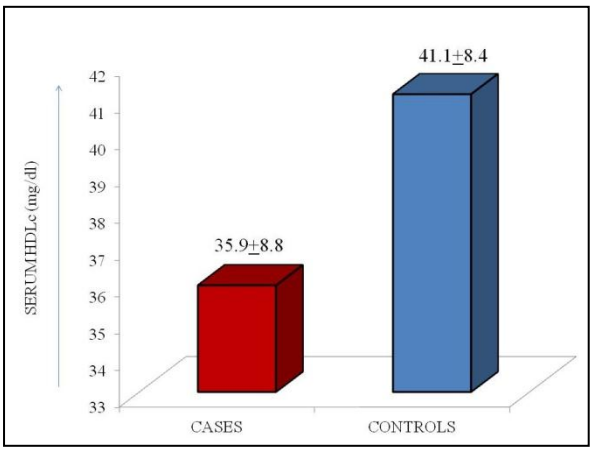


Fig (40) Comparison of serum HDLc between Diabetic AMI and controls (Subgroup analyses)

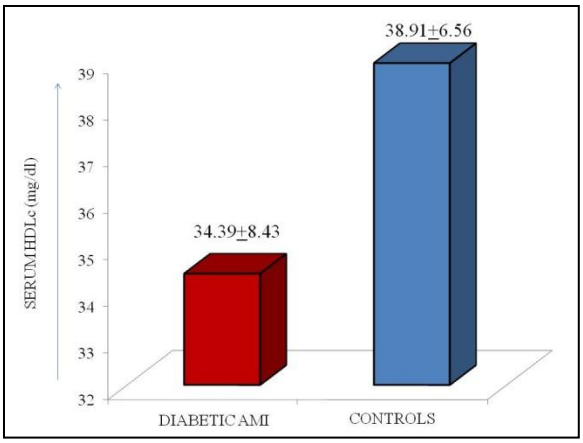
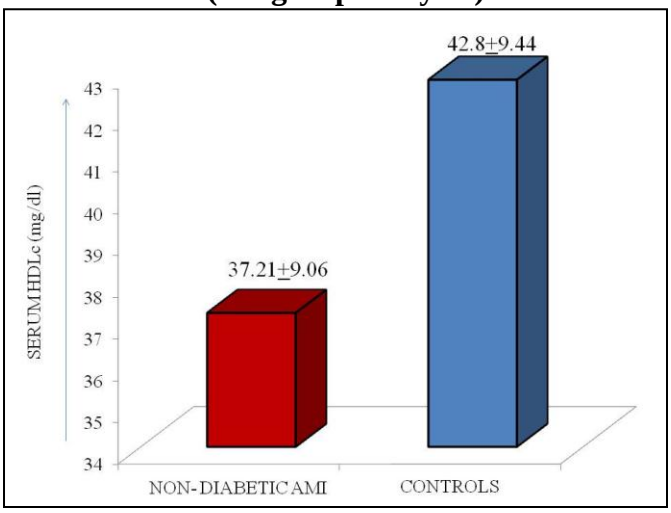


Fig (41) Comparison of serum HDLc between Non-diabetic AMI and controls (Subgroup analyses)



Acute myocardial infarction (AMI) is leading cause of death among non-communicable diseases in both developing and developed nations. Even in the rural population in India there is an increasing trend of the non-communicable diseases diabetes mellitus and MI due to sedentary life style and habits. Since myocardial infarction has multifactorial causation understanding its pathophysiology has shed light on the clinical utility of non-traditional risk markers of myocardial infarction.

In our study there was a male preponderance observed and the male: female ratio was 15:2. Males in this area are prone for AMI. The mean age was 57.19±10.1 yrs suggesting there was an increased risk of myocardial infarction with advancing age affecting them in their "golden years of life". The mean age in males with AMI was 57.3±10.63 yrs and in females with AMI the mean age was 56±9.38 yrs. With respect to anthropometric predictors of cardiovascular risk, it has been unclear which measure of obesity in particular body mass index (BMI), waist or hip circumference, or waist:hip ratio shows the strongest risk of cardiovascular disease. In our study, we observed that waist hip ratio showed statistically significant increase in acute MI patients compared to controls irrespective of their diabetic status implying that abdominal obesity is a better predictor of cardiovascular disease risk than BMI.[128]

Recently, glycosylated hemoglobin has been studied in depth as an independent risk marker for all the causes of mortality and death from any cause even in non-diabetics .[2,8] Elevated HbA_{1c} has now been regarded as an independent risk factor for MI in subjects with or without diabetes mellitus. This created interest in us with respect to this population to explore the possibility of using HbA_{1c} as a primetime tool in predicting AMI.

Studies conducted by Myint PK et al, it was observed that HbA_{1c} $\geq$7% was associated with increased risk of stroke even in the non-diabetics.[8] Even though the etiology and etiopathogenesis are considered same for both myocardial infarction and stroke it is not exactly true with respect to HbA_{1c} and myocardial infarction. In the EPIC-NORFOLK study, the possibility of using glycated hemoglobin as an independent predictor of death from cardiovascular disease was explored for the first time by Khaw KT et al.[129]

Studies conducted by Pasupathy et al to study the combinational effect of cardiac and biochemical markers in diabetic patients with cardiovascular disease, it was observed that the fasting plasma glucose level and glycosylated hemoglobin (HbA_{1c}) were elevated in cardiac patients with and without diabetes compared to healthy subjects.[7] However, in our study conflicting results were obtained with respect to HbA_{1c} which was not altered in AMI.

Even imaging studies have shown the association of elevated HbA_{1c} with cardiovascular disease. Studies conducted by Stakos et al, showed that higher GHb concentrations, even within 'normal' range, are independently associated with stiffer aorta and increased LV mass and thus may detect non-diabetic individuals at increased cardiovascular risk.[130] but we could not do the imaging studies and didn't concentrate on it as it has been already proved.

Studies conducted by Liu Y et al, it was shown that elevated HbA_{1c} level is an independent risk factor for mortality in CAD patients without diabetes, but not in patients with established diabetes mellitus. However, in our study subgroup analyses showed that

even in non-diabetics HbA_{1c} was not a predictor of myocardial infarction with a p=0.75 (Odds ratio 0.84, 95% Confidence Interval 0.27-2.55).[131]

In our study admission glycated hemoglobin was not elevated in comparison to controls. HbA_{1c} correlated strongly with fasting and postprandial plasma glucose but not with the macro metals and lipid parameters. This finding of lack of association of admission HbA_{1c} with cardiovascular risk is at par with the findings of Su G et al, who observed that admission glycemic variability was a strong predictor of major adverse cardiac events (MACE) but not admission HbA_{1c}.[124]

Studies conducted by Rasoul S et al, showed that among 30 day survivors neither admission glucose nor HbA_{1c} were predictors of long term mortality.[125] Studies done by Hadjadj et al, admission plasma glucose and HbA_{1c} were simultaneously measured in all patients consecutively hospitalized for acute myocardial infarction. It was observed that admission plasma glucose was higher in patients who had died by day 28, whereas HbA_{1c} was not altered.[132] This suggests that following acute myocardial infarction, acute glycometabolic state accounts for the prognosis rather than the chronic pre-existing glycometabolic condition.

In our study we observed that fasting blood glucose levels were significantly elevated in non-diabetic AMI patients than controls, 95 ± 17.54 mg/dl and 84.77 ± 9.92 mg/dl respectively ($p<0.05$). The DECODE Study group observed that slightly elevated glucose levels, even in the non-diabetic range, might be associated with increased cardiovascular disease risk. [126] In the Northern Sweden MONICA project which, in turn, is a part of the WHO MONICA Project (Monitoring of Trends and Determinants in Cardiovascular Disease) it was shown that in women with impaired glucose tolerance the

risk of silent MI was more than men.[133] However, in our study there was a male preponderance. The mean age in males with AMI was 57.3±10.63 yrs and in females with AMI the mean age was 56±9.38 yrs. There was not much difference in age. In AMI patients 54.9% were smokers and consumed tobacco all were males and the female patients did not have the habit of tobacco chewing. Hence, tobacco exposure could be the predisposing factor in males in our study.

Our study supports the findings of other studies described subsequently. Pradhan AD et al demonstrated HbA1c was a marker of long term glycemic control but was not significantly associated with the risk of cardiovascular disease.[134]

In the Atherosclerosis Risk in Communities (ARIC) Study, a prospective cohort study undertaken to assess the relation between HbA_{1c} level and incident MI during 8 to 10 years of follow-up of both diabetics and non-diabetics it was observed that in diabetic adults, the risk of MI increased throughout the range of HbA_{1c} levels whereas in non-diabetics HbA1c was not associated with MI risk.[135]

The findings of our study suggest that glycated hemoglobin is elevated in clinically advanced diabetes mellitus and its association with myocardial infarction may be due to coexistent traditional risk factors.

In the present study in non-diabetic AMI patients the fasting blood glucose was significantly elevated than non-diabetic controls ($p<0.05$). Studies conducted by Ishihara M et al, showed that fasting glucose and HbA_{1c}, but not admission glucose, were associated with 2-h postload glucose and were independent predictors of abnormal glucose tolerance. They also implied that fasting glucose and HbA_{1c} may be useful to

predict abnormal glucose tolerance of non-diabetic patients who survived AMI regardless of the presence or absence of admission hyperglycemia.[136]

Norhammar A et al demonstrated that in non-diabetic AMI patients there was a high prevalence of abnormal glucose metabolism; both HbA1c and fasting blood glucose were independent predictors of abnormal glucose metabolism.[137] In our study similar observations were made with respect to fasting glucose but was not true when it was HbA_{1c} was considered, even though clinically and hematologically they were not anemic and their hemoglobin was within normal range.

In our study serum calcium even though within the reference range was significantly elevated in both diabetics and non-diabetics with acute myocardial infarction compared to controls, $p<0.05$ and $p<0.001$ respectively supporting the findings of Lind L et al, who evaluated the serum calcium levels within the normal range as a prospective cardiovascular risk factor. It was found that serum calcium was an independent, prospective risk factor for MI in middle-aged males suggesting a role for extracellular calcium levels in the atherosclerotic process. [138] This is true even in our study.

Similar findings were observed in the Tromso study, where serum calcium was found to be independent cardiovascular risk factor and strongly correlated with hypertension and serum lipids.[79] Our findings with respect to the divalent cation calcium are at par with these studies establishing the role of increased serum calcium as a risk predictor of myocardial infarction.

In our study serum magnesium levels was significantly increased in AMI patients irrespective of their diabetic status compared to controls albeit in the reference range.

Studies have documented hypomagnesemia in myocardial infarction and magnesium therapy in acute myocardial infarction has also been advocated.[87, 139] In our study serum magnesium levels were estimated at the time of admission and contradicted the findings of hypomagnesemia where elevated serum magnesium levels with the reference range was observed.

Studies by Bunton RW have observed in patients who have undergone coronary bypass grafting that serum magnesium concentration was of no value for detecting perioperative myocardial infarction.[140] Since in our study serial monitoring of serum magnesium was not done this could not be commented on.

Studies conducted by Rector WG Jr et al, serum for magnesium and copper determinations was obtained daily for three days in patients suffering from myocardial infarction. The initial serum magnesium levels were normal in patients with ischemia but were low in some patients with myocardial infarction. Patients developing ventricular arrhythmias with myocardial infarction showed the lowest levels of serum magnesium. These data indicate that a decrease in serum magnesium as evaluated may be associated with ventricular arrhythmias in patients with myocardial infarction.[141]

Studies done by Woods JB et al to correlate the divalent cation concentration and ECG changes in canine myocardial infarction, it was observed that serum magnesium in canine decreased approximately 20% only after coronary artery occlusion and at 24 hours.[142] Studies conducted by Giesecke D et al, on serum magnesium levels where they followed their observation during coronary infarction and were correlated to the event with creatinine kinase activity. Those patients who were admitted early to the hospital showed initial increase in the serum magnesium levels which later fell during the event

and normalized subsequently.[143] Our study supports this findings and this could be one of the reasons for the elevated levels of serum magnesium in AMI patients as most patients were brought early to hospital due to improved access to tertiary health care in a rural place like Kolar.

In studies conducted by Shashidhar KN et al, it was observed even though hypomagnesemia is observed in diabetes mellitus, hypermagnesemia was demonstrated in chronic diabetics with end stage renal disease suggesting the primitive role of magnesium in glucose metabolism.[84] In view of hyperglycemia observed in our study elevation of serum magnesium is a probability of the acute glycometabolic state. Another possibility could be due to impaired renal handling of magnesium in myocardial infarction.

Lipid profile is a traditional marker of increased cardiovascular disease risk. However, in our study the total cholesterol, triglycerides, LDL and non-HDLc were not significantly increased in AMI patients irrespective of their diabetic status albeit HDL cholesterol which was significantly decreased in AMI patients both diabetics as well as non-diabetics when compared to controls ($p<0.05$). Serum lipids are known to decrease 24 hours following acute myocardial infarction and remain so for 2-3 months. The probable mechanisms are metabolic effect of stress, hormones, increased LDL receptor activity and increased cholesterol catabolism.[144]. With respect to triglycerides the factors could be the individual variability being high and the non-normal distribution of triglycerides.

The present study supports the findings of Zareen S et al; serum total cholesterol level was not significantly different in MI patients compared to controls.[145] In the present

study the serum HDL cholesterol levels were significantly reduced in AMI patients irrespective of their diabetic status ($p<0.05$, in both diabetic and non-diabetic group). There is increasing evidence that HDLc can predict cardiovascular risk independently of LDLc.[146] Studies have shown that low HDLc in conjunction with normal LDLc and triglycerides has a cardiovascular risk equivalent to elevated LDLc.[147] Similar observations were made in our study that of isolated low HDLc levels with normal triglycerides and LDLc levels. The present study supports the findings of Huxley RR et al, who observed in an Asian population that individuals exhibiting isolated low HDLc are at increased risk of MI.[127]

- Even though glycosylated hemoglobin (HbA_{1c}) is useful in assessing average glucose control, and has clear prognostic implications in the outpatient setting, its value in predicting outcomes in the setting of hospitalisation for AMI is limited.
- Admission HbA_{1c} levels in acute myocardial infarction is a useful inexpensive test for differentiating between stress induced hyperglycemia and diabetes mellitus.
- HbA_{1c} failed to predict risk of myocardial infarction. More longitudinal and prospective studies in rural areas of India are needed to establish causal relationship so that HbA_{1c} can be used as a prime time tool in clinical evaluation of myocardial infarction.
- Fasting blood glucose in non-diabetics is not only a marker of glucose dysregulation but also increased cardiovascular disease risk probably due to the elevated free fatty acids and increased thrombotic properties of platelets, enhanced oxidative stress, the activation of blood coagulation and platelets, stimulation of inflammation, and endothelial cell dysfunction.
- Increased serum calcium level but within the normal range is associated with increased risk for myocardial infarction. This causal relationship needs further validation as there is a possibility of impaired renal handling of divalent cations in myocardial infarction.
- The physiologically important form is the free or ionized form of calcium and serum calcium was not corrected for serum albumin in our study. Hence, the results must be interpreted with caution.

- Increased serum magnesium in our study was observed in cases at the time of admission compared to controls.
- Usually magnesium depletion occurs in myocardial infarction within 24 hours to 48 hours and later normalizes by 72 hours. Initial elevation could be due to release of magnesium from the necrosed myocardium and also magnesium exerts a cardioprotective effect against calcium.
- The possibility of impaired renal excretion also cannot be ruled out. There exist mechanisms in magnesium-calcium metabolism that are not well defined yet.
- Isolated low HDL cholesterol is a novel lipid phenotype with increased myocardial infarction.
- Non-HDL cholesterol calculation improves cardiovascular risk prediction by overcoming the limitations of calculating LDL by using Friedewald's formula.
- However, in our study non-HDL cholesterol failed to predict cardiovascular risk but decreased HDL cholesterol levels was still able to.
- Lipoproteins and lipids are useful for monitoring the effect of lipid lowering therapies and cardiovascular risk reduction but in these individuals with isolated low HDLc rather than lipid lowering therapies, smoking cessation and life style modification would prove beneficial.
- Pertaining to the relation between measures of obesity and myocardial infarction abdominal obesity is a better risk indicator of cardiovascular disease risk than body mass index. It can be implied to the clinicians that along with biochemical parameters anthropometric measurements such as waist-to-hip ratio also has to be considered rather than BMI to help determine the risk of MI in their patients.

- Though in the rural areas where there is a lack of good laboratory and healthcare services, these biochemical markers can be evaluated to be used as simple and cost effective tools of myocardial infarction risk along with the clinical findings.
- Any conclusion to postulate a hypothesis with respect to HbA_{1c}, serum calcium, serum magnesium, blood glucose and alterations of lipoproteins and lipids in myocardial infarction has to be considered with caution.
- Thus, a large population group and other risk factors including the genetic factors has to be considered before coming to any conclusion to find the association of glycated hemoglobin, diabetic status, calcium, magnesium and lipids with respect to myocardial infarction.

The present study was carried out in a rural tertiary health care center. The objective of this study was to find the correlation of HbA_{1c}, blood glucose, macro metals serum calcium and magnesium, and fasting lipid profile in acute myocardial infarction. The study comprised of 51 cases of acute myocardial infarction and 55 age and gender matched controls.

The following facts were derived from our study:

1. There is a male preponderance for myocardial infarction associated with the history of risk factor smoking.
2. Even though HbA_{1c} helps to distinguish between stress induced hyperglycemia and diabetes mellitus but it fails to predict the risk of myocardial infarction.
3. Fasting glucose in non-diabetic AMI patients not only reflects the acute glycometabolic state but also predicts risk and increased mortality.
4. Increased serum calcium but within the reference range is associated with increased cardiovascular risk.
5. Serum magnesium levels seem to be elevated in AMI reflecting the glucose dysregulation, where magnesium is a cofactor for enzymes of carbohydrate metabolism.
6. Possibility of impaired renal handling of the divalent cations i.e. calcium and magnesium could not be ruled out.
7. Isolated low HDLc seems to be a new lipid phenotype, than lipid lowering therapies, life style modifications and smoking cessation which may be beneficial in cardiovascular disease risk reduction.

8. However, myocardial infarction has a multifactorial causation and in view of this even simple biochemical markers with anthropometric measurements can prove helpful in predicting the risk of MI.
9. In continuation with this, genetic factors and geography should not be forgotten while doing any study on non-communicable diseases.

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ANNEXURE (1)

TITLE OF THE STUDY: CORRELATION OF HbA_{1c}, BLOOD GLUCOSE, CALCIUM, MAGNESIUM AND FASTING LIPID PROFILE IN MYOCARDIAL INFARCTION: A CASE-CONTROL STUDY IN RURAL KOLAR DISTRICT

CASE HISTORY OF THE PATIENTS

Case No:

Name: Mr/Mrs OP No:

Age: IP No:

Gender: Ward:

Date: Occupation:

Weight:

Address:

CHIEF COMPLAINTS:

HISTORY OF PRESENTING ILLNESS:

PAST HISTORY:

Hypertension : yes/no if yes , duration:

Diabetes : yes/no if yes , duration:

Tuberculosis : yes/no if yes , duration:

Heart diseases : yes/no if yes , duration:

Liver diseases : yes/no if yes , duration:

Drug ingestion : yes/no if yes , duration & details:

Others :

Thyroid disorders:

Prostate/ colorectal cancer:

Mental depression:

Miscarriage:

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 : Below poverty level/Above poverty level

Diet: Vegetarian / mixed/non-vegetarian

Vegan/Non-vegan

Eggitarian/Non eggitarian

Smoking : yes/no if yes, duration:

Alcohol : yes/no if yes , duration:

MENSTRUAL HISTORY : Regular/irregular/not applicable/other abnormalities

GENERAL PHYSICAL EXAMINATION:

Ht: Wt: BMI:

Waist hip ratio: Abdominal girth:

Built : normal / below normal / well built / obese/athletic

Nourishment : well / poor nourished

Oedema : Icterus :

Pallor : Clubbing :

Cyanosis : Lymphadenopathy :

Blood pressure : Pulse rate : Brachial dance:

SYSTEMIC EXAMINATION :

CVS :

RS :

CNS :

PER ABDOMEN :

CLINICAL DIAGNOSIS :

INVESTIGATIONS :

BLOOD :

PLASMA FBS: mg/dl

PLASMA PPBS : mg/dl

WHOLE HbA_{1c}: %

SERUM MAGNESIUM: mEq/dl

SERUM CALCIUM: mg/dl

LIPID PROFILE

→ SERUM TOTAL CHOLESTEROL: mg/dl

→ SERUM TRIGLYCERIDES: mg/dl

→ SERUM HDLc: mg/dl

→ SERUM LDL (calculated): mg/dl

→ NON HDLc (calculated): mg/dl

HEMOGLOBIN : g/dl

PERIPHERAL BLOOD SMEAR:

URINE(Spot) :

Albumin - Sugar –

OTHERS:

ANNEXURE (2)

Patient details:

Name: **Age:** **Gender:** **Hospital No.**

Title of the Study: Correlation Of Hba1c, Blood Glucose, Calcium, Magnesium And Fasting Lipid Profile In Myocardial Infarction: A Case-Control Study In Rural Kolar District

INFORMED CONSENT

I understand that I remain free to withdraw from this study at any time.

I have read or had read to me and understand the purpose of this study and the confidential nature of the information that will be collected and disclosed during the study.

I have had the opportunity to ask my questions regarding the various aspects of this study and my questions have been answered to my satisfaction.

I, the undersigned agree to participate in this study and authorize the collection and disclosure of my personal information as outlined in this consent form.

---------- ----------

Participant's Name & signature Date

---------- ----------

Signature of the witness Date

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Signature of the principal investigator Date

Master chart - Cases

ANTHROPOMETRIC DATA OF CASES

SI. No.	Age (yrs)	DM	DURATION (yrs)	HTN	DURATION (yrs)	MI	Sex	Smoking	Ht (cms)	Wt (kgs)	BMI	Waist - Hip ratio
1	60					STEMI	M	N	165	65	23.8	0.9
2	65					NSTEMI	M	Y	163	65	24.5	1
3	63					NSTEMI	M	N	170	65	22.4	1
4	70					STEMI	M	Y	180	100	30.8	1.04
5	60	Y	1	Y	1	STEMI	M	Y	178	65	20.5	1
6	40					STEMI	M	Y	178	64	20.2	1
7	52					STEMI	M	Y	178	65	20.5	1
8	50	NEW				STEMI	M	N	163	66	24.9	1
9	58	Y	4			NSTEMI	M	Y	176	102	33	1.08
10	45					NSTEMI	M	N	167	63	22.6	0.9
11	49	NEW				NSTEMI	M	Y	171	72	24.6	1.02
12	45	NEW				STEMI	M	N	170	89	30.7	1.05
13	61	Y	2	Y	2	STEMI	M	Y	174	114	37.7	1.1
14	52	Y	1			STEMI	M	Y	173	108	36.1	1.09
15	65	Y	2	Y	2	NSTEMI	M	Y	167	67	24.1	1
16	62	Y	2	Y	2	STEMI	M	N	159	56	22.2	0.98
17	38					STEMI	M	Y	175	76	24.8	1
18	64					STEMI	M	Y	163	67	25.2	1
19	60					STEMI	M	Y	159	60	23.8	0.9
20	56	Y	5	Y	5	STEMI	M	Y	156	60	24.6	1
21	78	Y	12			STEMI	M	Y	163	65	23.8	1
22	61	Y	5	Y	5	STEMI	M	Y	160	68	26.5	1.05
23	45	NEW				STEMI	M	Y	178	78	24.68	1
24	80	NEW				STEMI	M	N	165	61	22.42	1
25	45	Y	2	Y	2	STEMI	M	Y	173	78	26	1.02
26	70					STEMI	M	Y	160	67	23.8	1
27	58			Y	5	STEMI	M	N	170	68	23.5	1
28	65					STEMI	M	N	162	50	19	0.8
29	44	Y	2			NSTEMI	F	N	163	78	29.4	0.95
30	62	NEW				STEMI	M	N	176	65	21	0.9

Master chart - Cases

SI No.	Age (yrs)	DM	DURATION (yrs)	HTN	DURATION (yrs)	MI	Sex	Smoking	Ht (cms)	Wt (kgs)	BMI	Waist - Hip ratio
31	53	Y	5	Y		NSTEMI	F	N	165	60	22.05	0.85
32	60					NSTEMI	M	Y	172	60	20.33	0.9
33	69	Y	30	Y	1	STEMI	F	N	158	75	30.1	1.05
34	65					NSTEMI	F	N	174	60	19.8	0.8
35	51	Y	15			STEMI	M	N	159	54	21.4	0.9
36	52					STEMI	M	N	168	65	23.04	1
37	47					NSTEMI	M	Y	171	54	18.4	0.8
38	50					NSTEMI	F	N	158	50	20.08	0.85
39	48			Y		NSTEMI	M	N	169	74	25.9	1.02
40	86					STEMI	M	Y	184	80	23.66	1
41	73			Y	5	STEMI	M	N	165	65	23.8	0.9
42	55					NSTEMI	M	Y	170	65	22.49	0.85
43	70	Y	12	Y		STEMI	M	N	165	60	22.05	0.9
44	60					STEMI	M	N	171	57	19.5	0.85
45	55	NEW				NSTEMI	F	N	156	55	22.6	0.9
46	63	NEW		Y	5	STEMI	M	N	165	51	18.75	0.8
47	51					NSTEMI	M	Y	171	60	20.5	0.9
48	37					NSTEMI	M	Y	168	60	21.2	0.9
49	48					STEMI	M	Y	174	71	23.5	1
50	55					STEMI	M	Y	178	55	17.4	0.8
51	46					NSTEMI	M	Y	168	60	21.2	0.9

Master chart - Cases

BIOCHEMICAL DATA OF CASES

SI No.	FBS (mg/dl)	PPBS (mg/dl)	HbA1c %	Ca (mg/dl)	Mg (mg/dl)	TC (mg/dl)	TAG (mg/dl)	HDLc (mg/dl)	LDL (mg/dl)	Non HDLc (mg/dl)
1	92	92	5.4	9.4	2.5	194	126	46	123	148
2	104	111	5	9.6	2.7	111	44	34	68	67
3	93	104	6	9.3	2.3	195	246	34	111	161
4	106	120	6.4	9.8	2.5	228	297	37	132	191
5	106	85	8.73	9.8	2.5	191	182	31	123	160
6	117	141	5.3	9.8	2.5	152	96	46	86	106
7	98	108	5.3	9.4	2.4	118	52	33	75	85
8	135	99	8.5	9.6	2.5	134	152	36	67	98
9	244	383	9.7	10.7	2.7	129	82	30	83	99
10	108	87	6.9	9.7	2.3	205	109	30	153	175
11	180	449	10	9.4	2.5	134	221	18	72	116
12	130	154	8.73	10.1	2.4	226	250	31	145	195
13	138	216	7.4	10.9	2.4	211	147	47	134	164
14	148	134	8.3	10	2.4	146	137	31	88	115
15	83	122	7.2	8.5	2.5	99	115	25	51	74
16	286	293	8.6	9.6	2.3	151	62	40	99	111
17	73	100	6.8	9.3	2.5	141	247	28	64	113
18	111	132	5.8	9.8	2.5	157	94	25	113	132
19	83	120	6	10.5	2.5	120	134	27	116	93
20	169	265	8.5	10.6	2.4	178	66	53	112	125
21	250	278	8.5	9.6	2.5	151	56	43	96	108
22	345	383	10.4	10.1	2.5	166	52	46	109	120
23	145	161	8.73	10	2.5	171	493	25		146
24	121	156	7.8	9.6	2.4	86	56	29	46	40
25	238	322	8.3	9.8	2.5	151	163	36	83	115
26	122	121	8.3	9.8	2.5	98	132	26	46	72
27	106	133	8.3	10.9	2.4	147	59	49	87	98
28	108	92	7	9.6	2.9	120	162	35	52	68
29	112	112	8.3	10	2.4	158	170	36	88	122

Master chart - Cases

SI No.	FBS (mg/dl)	PPBS (mg/dl)	HbA1c %	Ca (mg/dl)	Mg (mg/dl)	TC (mg/dl)	TAG (mg/dl)	HDLc (mg/dl)	LDL (mg/dl)	Non HDLc (mg/dl)
30	176	164	9.07	8.8	2.4	167	103	29	117	128
31	223	304	7.06	12	2.1	240	214	25	172	215
32	89	94	6.39	11.03	2.3	193	239	23	170	170
33	180	235	10.74	9.74	2.5	197	130	40	132	137
34	95	83	10.56	9.6	2.5	128	109	46	60	82
35	304	325	9.07	10	2.4	131	157	27	72	130
36	113	122	5.72	10.25	2.3	250	209	39	80.8	211
37	64	89	9.07	10	2.5	197	209	41	114	156
38	85	96	7.48	10	2.5	124	125	30	94	94
39	93	107	7.22	9.6	2.4	211	300	30	120	181
40	84	98	6.47	9.2	2.5	169	120	26	119	143
41	60	84	5.3	9	2.3	134	75	50	74	84
42	80	78	5.3	9	2.3	166	90	35	113	131
43	81	173	9.8	9.7	2.4	123	106	40	62	83
44	98	110	5.3	9.6	2.5	153	145	44	80	109
45	135	256	7.22	8.9	2.4	147	184	32	78	115
46	154	230	7.98	9.2	2.3	146	57	41	93	105
47	96	104	5.8	8.8	2.2	213	168	44	136	169
48	124	138	5.72	9.8	2.1	198	92	43	137	155
49	102	90	8.3	9	2.3	216	235	60	109	156
50	101	110	6.97	9.6	2.2	236	64	42	181	194
51	55	92	6.81	9.8	2.4	153	92	39	96	114

Master Chart - Controls

ANTHROPOMETRIC DATA OF CONTROLS

SI No	Age (yrs)	DM	DURATION (yrs)	HTN	DURATION (yrs)	Smoking	Sex	Ht (cms)	Wt (kgs)	BMI	Waist - Hip ratio
1	47					N	M	178	54	17.08	0.8
2	70	Y	5			N	F	165	50	18.38	0.85
3	44					N	M	164	50	18.65	0.9
4	70					N	F	158	48	19.2	0.8
5	60					N	M	165	65	23.89	1
6	75					Y	M	180	54	16.66	0.9
7	42					N	F	152	35	15.15	0.8
8	75					N	M	158	45	18.07	0.9
9	75					N	M	174	54	17.8	0.95
10	50			Y	10	N	F	165	80	29.41	1.05
11	60	Y	10	Y	10	N	F	164	78	29.1	0.95
12	65	Y	10			Y	M	180	60	18.5	0.9
13	45	Y	5			Y	M	178	80	25.31	0.9
14	45					N	M	165	65	23.8	0.85
15	40					N	M	178	72	22.7	0.9
16	52					N	M	169	55	19.2	0.9
17	60					Y	M	165	55	20.2	0.9
18	51					Y	M	174	63	20.8	0.9
19	40					N	M	156	56	23.04	1
20	47	Y	10			N	M	178	75	23.7	0.95
21	42	Y	5			N	M	182	78	23.56	1
22	50	Y	3			Y	M	176	70	22.65	0.9
23	56					N	M	170	65	22.49	0.9
24	53					Y	M	164	65	24.2	0.95
25	56					N	M	172	61	20.6	0.9
26	55					N	M	178	56	17.7	0.9
27	58					N	M	172	59	20	0.9
28	60					N	M	170	73	25.2	1
29	39					N	M	165	67	24.63	0.95
30	56	Y	5			N	M	158	71	28.5	1.05
31	52					N	M	159	53	21.03	0.9
32	61					N	M	180	62	19.1	0.9
33	60					Y	M	178	75	23.7	0.95
34	65					N	F	160	60	23.43	0.8
35	51	Y	5			Y	M	178	65	20.5	0.9
36	60	Y	5			N	M	165	75	27.5	0.9
37	62					Y	M	150	55	24.4	0.95

Master Chart - Controls

SI No	Age (yrs)	DM	DURATION (yrs)	HTN	DURATION (yrs)	Smoking	Sex	Ht (cms)	Wt (kgs)	BMI	Waist - Hip ratio
38	55					N	M	176	57	18.56	0.9
39	60					N	M	178	78	24.68	0.9
40	63					N	M	176	60	19.4	0.9
41	58	Y	10			N	M	174	80	26.4	1
42	46	Y	5			N	M	175	87	28.43	1.05
43	63					N	M	180	89	27.46	0.9
44	54	Y	5			N	M	170	55	19.03	0.95
45	62	Y	10			N	F	158	68	27.3	1.09
46	57	Y	6			N	F	148	66	30.1	0.9
47	60	Y	10			Y	M	172	73	24.1	0.93
48	65	Y	5			N	M	157	52	21.1	0.81
49	65	Y	8			Y	M	169	61	21.4	0.71
50	70	Y	10			Y	M	163	57	21.5	1.03
51	61	Y	5			Y	M	166	72	26.6	1.06
52	62	Y	5			Y	M	178	65	20.5	1.04
53	75	Y	2			N	M	159	69	27.3	0.9
54	80	Y	5			N	M	160	58	22.6	1.02
55	50	Y	5			N	F	160	66	25.7	0.8

Master Chart - Controls

BIOCHEMICAL DATA OF CONTROLS

SI No.	FBS (mg/dl)	PPBS (mg/dl)	HbA1c %	Ca (mg/dl)	Mg (mg/dl)	TC (mg/dl)	TAG (mg/dl)	HDLc (mg/dl)	LDL (mg/dl)	Non HDLc (mg/dl)
1	91	86	6.64	8.8	2.2	186	170	40	112	146
2	83	130	9.8	9.3	2.2	187	170	37	116	150
3	90	87	5.3	8	1.6	111	61	29	70	82
4	98	110	6.47	8	1.7	177	136	40	110	137
5	60	122	6.55	8.1	2.4	216	226	54	117	162
6	71	91	5.97	9.2	2.5	127	128	39	63	88
7	90	117	6.55	9.3	2.5	124	80	31	77	90
8	90	97	5.3	9.4	2.5	178	61	47	119	131
9	74	88	7.81	9.8	2.4	103	82	28	59	75
10	92	78	5.8	9.3	2.3	202	153	44	127	158
11	117	113	6.81	9.6	2.3	175	136	42	106	133
12	131	187	6.47	9.4	2.4	158	177	32	91	126
13	194	236	9.2	10.2	2.2	126	85	19	90	107
14	85	100	6.47	8.6	2.4	214	161	50	131	164
15	85	109	6.81	8.8	2	164	285	44	63	120
16	84	119	6.3	9.2	2	237	293	44	135	193
17	94	112	5.3	8.6	2.1	157	116	72	62	85
18	73	94	7.06	8.8	2.2	151	75	44	92	107
19	91	105	6.47	9.2	2.3	195	126	25	145	170
20	98	156	8.73	8.8	2.1	213	151	32	151	181
21	251	397	9.15	8.8	2	148	234	41	60	107
22	173	256	9.91	8.8	2.5	164	102	38	105	126
23	102	120	6.97	9.3	2.4	142	186	31	74	111
24	97	120	7.81	8.9	2.3	136	99	29	88	107
25	81	100	7.06	8.8	2.5	201	246	45	110	156
26	88	92	7.73	8.8	2.5	172	177	52	84	120
27	82	96	6.47	8.9	2.2	153	102	41	92	112
28	98	102	7.14	8.9	2.4	212	137	44	141	168
29	87	102	6.64	9.3	2.3	136	112	44	69	92
30	207	326	9.24	9	2	174	89	42	114	132
31	81	108	7.81	9.4	2.4	177	92	46	113	131
32	66	104	7.39	9.2	2.3	164	115	52	89	112
33	84	97	6.55	8.5	2.2	210	177	50	124	160
34	68	111	7.39	8.8	2.4	293	280	50	188	243
35	80	109	8.73	8.8	2.2	142	84	49	76	93
36	72	124	9.07	9.8	2.1	197	165	44	119	153
37	82	108	6.39	8.9	2.2	191	122	48	119	143

Master Chart - Controls

SI No	FBS (mg/dl)	PPBS (mg/dl)	HbA1c %	Ca (mg/dl)	Mg (mg/dl)	TC (mg/dl)	TAG (mg/dl)	HDLc (mg/dl)	LDL (mg/dl)	Non HDLc (mg/dl)
38	82	100	6.47	9	2.3	117	80	44	58	73
39	90	100	5.46	8.8	2.3	156	164	37	86	119
40	92	120	7.73	9.6	2.3	169	70	46	104	123
41	215	304	9.07	9.2	2	203	221	37	121	166
42	296	324	9.82	9.8	2.1	236	195	38	159	198
43	80	87	6.97	9.6	2.3	168	157	37	100	131
44	153	268	9.82	10.2	2.2	213	151	55	128	158
45	122	246	8.5	9	2.1	195	167	39	122	156
46	176	339	9.2	9.4	2	186	132	37	123	149
47	100	208	7.6	8.8	2.3	208	123	40	143	168
48	170	245	8.73	8.9	2.1	154	194	42	73	112
49	88	212	9.7	9.2	2.3	143	195	39	65	104
50	143	358	9.24	9.4	2.2	215	153	33	151	182
51	271	306	8.73	8.8	2.1	128	183	41	50	87
52	190	367	9.07	9.2	2.2	120	75	43	62	58
53	103	165	7.6	8.9	2	153	139	39	86	114
54	113	171	8.5	9.1	2.2	145	261	36	56	109
55	153	225	8.3	9.2	2.4	166	137	39	100	127