“FACTOR VIII:C LEVELS AND MACROVASCULAR COMPLICATIONS IN DIABETES MELLITUS”

REG NO. BG0110006

Dissertation

Submitted to the
KLE University, Belgaum, Karnataka

In Partial Fulfillment
of the requirements for the degree of

M. D.
in
GENERAL MEDICINE

DEPARTMENT OF MEDICINE,
JAWAHARLAL NEHRU MEDICAL COLLEGE,
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ENDORSEMENT

This is to certify that the dissertation entitled **"FACTOR VIII:C LEVELS AND MACROVASCULAR COMPLICATIONS IN DIABETES MELLITUS"** is a bonafide research work done by **THE CANDIDATE REGISTER NUMBER BG0110006.**

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LIST OF ABBREVIATIONS USED

AD	-	Anno domini
AGE	-	Advanced glycosylated end products
AHF	-	Activated Hageman factor
ARIC	-	Atheroscelrosis risk in communities
ATP	-	Adenosine triphosphate
bp	-	Base pair
Ca2+	-	Calcium Ion
CAD	-	Coronary Artery Disease
cAMP	-	Cyclic adenosine monophosphate
C-IMT	-	Carotid intima media thickness
CVD	-	Cardiovascular Disease
DAN	-	Diabetic autonomic neuropathy
DCCT	-	Diabetes Control and Complication Trial
DM	-	Diabetes mellitus
DNA	-	De-oxyribo nucleic acid
ESRD	-	End stage kidney disease
g	-	Gram
GDM	-	Gestational diabetes mellitus
GLUT	-	Glucose transporter
h	-	Hour
Hb	-	Haemoglobin
HDL	-	High density Lipoprotein
HNF	-	Hepatocyte nuclear transcription factor
IFG	-	Impaired fasting glucose

IGT	-	Impaired glucose tolerance
IPD	-	In patient department
IRS	-	Insulin receptor substrate
IU/dL	-	International Units per deciliter
K+	-	Potassium Ion
LDL	-	Low density lipoprotein
Max	-	Maximum
mg	-	Milligrams
mg/dL	-	Milligrams per deciliter
mmol/L	-	Millimol per litre
MODY	-	Maturity onset diabetes of the young
NGT	-	Normal glucose tolerance
NO	-	Nitric oxide
OPD	-	Out patient department
PAI	-	Plasminogen activator inhibitor
PI3kinase	-	Phophatidyl inositol – 3 kinase.
PKC	-	Protein kinase C
PVD	-	Peripheral vascular disease
ROS	-	Reactive oxygen species
SD	-	Standard deviation
Min	-	Minimum
SUR	-	Sulfonyl urea receptor
T2 DM	-	Type 2 diabetes mellitus
TC	-	Total cholesterol
TG	-	Triglycerides

TGF β	-	Transforming growth factor β
VEGF A	-	Vascular endothelial growth factor A
vWF	-	Von Willerbrand factor
x^2	-	Chi-square

ABSTRACT

Background and objectives

The plasma levels of many clotting factors, including fibrinogen, factor VII, factor VIII:C, factor XI, factor XII, kallikrein and von Willebrand factor, are elevated in diabetes. The present study was undertaken to determine plasma activity of factor VIII:C in diabetics, with and without macrovascular complications.

Methodology

The present one year case control study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum from January 2011 to December 2011. A total 50 patients that is, 25 patients with DM but without macrovascular complications were enrolled in group B and 25 patients with DM and macrovascular complications were enrolled in group C. These patients were compared with 25 age matched individuals with no specific illness and who were non-diabetic (group A).

Results

In the present study the male to female ratio in group A, B and C was 3.16:1, 2.12:1 and 1.5:1 respectively. The mean age in group A was 55.20 ± 9.15 years. Similarly in group B the mean age was 55.44 ± 7.74 years and group C it was 55.40 ± 6.44 years ($p>0.05$). The factor VIII:C was significant raised in 56% of patients in group B and 88% of patients in group C compared to 12% in group A ($p< 0.001$). The mean factor VIII:C levels in group C were significantly high

(256.80±49.38) compared to group B (179.52±46.37) and group A (111.57±25.48) (p=0.001).

Conclusion and interpretation

The plasma activity of factor VIII:C was significantly high in diabetics with and without macrovascular complications when compared with healthy age matched controls. Within the diabetic subgroup, patients with macrovascular complications had significantly higher factor VIII:C levels compared to those without complications.

Keywords

Diabetes mellitus; Factor VIII:C; High density lipoprotein; Low density lipoprotein; Macrovascular complications; Serum cholesterol; Triglycerides;

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Chapter 1

Introduction

INTRODUCTION

Diabetes Mellitus has afflicted mankind since time immemorial. The implications of diabetes have prompted never ending search and research into intriguing pathogenesis of the metabolic illness and its several ensuing complications.

Diabetes Mellitus is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. The vast majority of cases of diabetes fall into two broad categories: those having little or no endogenous insulin secretory capacity (IDDM or type 1 DM) and those who retain endogenous insulin secretory capacity but have a combination of resistance to insulin action and an inadequate compensatory insulin secretory response (NIDDM, or Type 2 DM).[1,2]

Type 2 diabetes mellitus (DM) accounts for more than 90% of the diabetic population world wide. Rates of diabetes are increasing worldwide. The International Diabetes Federation predicts that the number of people living with diabetes will rise from 366 million in 2011 to 552 million by 2030.[3]

The top 10 countries in number of people with diabetes are currently India, China, the United States, Indonesia, Japan, Pakistan, Russia, Brazil, Italy, and Bangladesh. The prevalence of diabetes and its adverse health effects have risen more rapidly in South Asia than in any other region of the world.[4]

The prevalence of diabetes in India based on the Indian Council of Medical Research (ICMR) multicentric survey[5] was around two percent in urban India and one percent in rural India before 30 years. In just three decades, these prevalence rates have shot up to 12 to 16% in urban India and 3 to 8% in rural India, in adults over 20 years of age representing a 600 to 800% increase in prevalence rates of diabetes. Indeed, India is now referred to as the "Diabetic Capital" of the world.

The field of diabetology has seen new developments occurring at a bewildering pace. Diabetes mellitus is associated with an increased risk of atherosclerosis, and macrovascular complications are a major cause of morbidity and mortality in this disease.[6] It is estimated that, 80% of patients with diabetes mellitus die a thrombotic death[7] and 75% of these deaths are due to cardiovascular complications and remainder due to cerebrovascular events and peripheral vascular complications.[8]

Endothelial dysfunction is the earliest event that precedes the development and progression of diabetic vascular complications.[9] The pathogenesis of endothelial dysfunction in diabetes is complex. Multiple cellular and molecular mechanisms are involved in the development of diabetic dysfunctional endothelium (hyperglycemia, insulin resistance, impaired lipid metabolism and lipoproteins, oxidative stress).[6,10]

The plasma levels of some biomarkers may be measured as indirect indices of endothelial cell damage, activation and inflammation to assess endothelial function (nitric oxide, asymmetric dimethylarginine, endothelin-1,

von Willebrand factor [vWF], adhesion molecule, plasminogen activator inhibitor-1[PAI-1]).[11-13]

The plasma levels of many clotting factors, including fibrinogen, factor VII, factor VIII:C, factor XI, factor XII, kallikrein and von Willebrand factor, are elevated in diabetes. The fibrinolytic system is relatively inhibited as a consequence of an increase in plasminogen activator inhibitor type-1 levels.[7] This procoagulant state and hypofibrinolysis contributes to macrovascular and microvascular complications of diabetes mellitus.[14-15]

In 1980 a prospective study indicated factor VIII:C to be a risk factor for arterial disease[16] and other studies also suggested association of elevated factor VIII:C with both cardiac and cerebral vascular disease.[17-18]

Several studies showed that elevated plasma levels of factor VIII:C are associated with an increased risk of venous thrombosis[19,20] and the risk of recurrent venous thromboembolism.[21]

The association of factor VIII:C levels to arterial thrombosis is a subject currently undergoing active research worldwide. Several case control studies have reported association of factor VIII:C with coronary artery disease. This association was eliminated in ARIC study[22] but not in Caerphilly heart study,[23] leaving the possibility open that factor VIII:C and von Willebrand factor have an effect on cardiovascular risk. Regarding the risk of stroke, the ARIC study[22] showed that per SD increase in factor VIII:C and von Willebrand factor, the risk is increased 1.34 fold (95% CI 1.2 to 1.5) and 1.36 fold (95% CI 1.2 to 1.5), respectively.

A study showed raised levels of factor VIII:C are associated with risk of thromboembolism.[24] Mortality due to ischaemic heart disease is much lower in patients with hemophilia A than in general male population which may suggest that factor VIII:C is involved in the pathogenesis of arterial thrombosis.[25]

It can be derived from these studies[24,25] that factor VIII:C causes increased thrombin generation and increased formation of fibrin and platelet aggregates.

Based on these observations, assuming that diabetic patients may have an increased risk of thrombosis, if their coagulation factor VIII activity is elevated, the present study was undertaken to determine plasma activity of factor VIII in diabetics, with and without macrovascular complications, and to compare with controls and to investigate the association between plasma activity of factor VIII:C and diabetic macrovascular complications.

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Chapter 2

Objectives

OBJECTIVES

The objectives of the present study were;

1. To determine plasma activity of factor VIII:c in diabetics, with and without macrovascular complications, and to compare with controls.

2. To investigate the association between plasma activity of factor VIII:c and diabetic macrovascular complications.

Chapter 3

Review of Literature

REVIEW OF LITERATURE

Diabetes Mellitus

Diabetes mellitus refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of DM exist and are caused by a complex interaction of genetics and environmental factors. Depending on the etiology of the DM, factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production.[1]

The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system. DM is the leading cause of end-stage renal disease (ESRD), nontraumatic lower extremity amputations, and adult blindness. It also predisposes to cardiovascular diseases. With an increasing incidence worldwide, DM will be a leading cause of morbidity and mortality for the foreseeable future.[1]

History of Diabetes Mellitus

For 2,000 years diabetes has been recognized as a devastating and deadly disease. In the first century A.D. a Greek, Aretaeus, described the destructive nature of the affliction which he named "diabetes" from the Greek word for "siphon."[26]

Eugene J. Leopold in his text Aretaeus the Cappodacian describes Aretaeus' diagnosis "….for fluids do not remain in the body, but use the body

only as a channel through which they may flow out. Life lasts only for a time, but not very long. For they urinate with pain and painful is the emaciation. For no essential part of the drink is absorbed by the body while great masses of the flesh are liquefied into urine".[26]

Physicians in ancient times, like Aretaeus, recognized the symptoms of diabetes but were powerless to effectively treat it. Aretaeus recommended oil of roses, dates, raw quinces, and gruel. And as late as the 17th century, doctors prescribed gelly of viper's flesh, broken red coral, sweet almonds, and fresh flowers of blind nettles.[26]

In the 17th century a London physician, Dr. Thomas Willis, determined whether his patients had diabetes or not by sampling their urine. If it had a sweet taste he would diagnose them with diabetes mellitus- "honeyed" diabetes. This method of monitoring blood sugars went largely unchanged until the 20th century.[26]

In the early 20th century, diabetologists such as Dr. Frederick Allen prescribed low calorie diets-as little as 450 calories per day for his patients. His diet prolonged the life of people with diabetes but kept them weak and suffering from near starvation.[26]

In 1921, in Ontario, Canada, a young surgeon Frederick Banting, and his assistant Charles Best, kept a severely diabetic dog alive for 70 days by injecting it with a murky concoction of canine pancreas extract. With the help of Dr. Collip and Dr. Macleod, Banting and Best administered a more refined extract of insulin

to Leonard Thompson, a young boy dying of diabetes. Within 24 hours, Leonard's high blood sugars had dropped to near normal levels.[26]

Since insulin's discovery, medical breakthroughs continued to prolong and ease the lives of people with diabetes. In 1935 Roger Hinsworth discovered there were two types of diabetes: "insulin sensitive" (type I) and "insulin insensitive" (type II). By differentiating between the two types of diabetes, Hinsworth helped open up new avenues of treatment.[26]

Starting in the late 1930s, new types of pork and beef insulin were created to better manage diabetes. Protamine zinc insulin, a longer acting insulin, was created in 1936. In 1938 NPH insulin was marketed, and in 1952 Lente, containing high levels of zinc which promotes a longer duration of action was invented.[26]

In the 1950s, oral medications-sulfonylureas were developed for people with T2 DM.[26]

The HbA1c test was devised in 1979 in order to create a more precise blood sugar measurement. With the A1c, hemoglobin, the oxygen-carrying pigment in red blood cells, is used to track glucose changes over a period of four months, the life span of the cell. Hemoglobin links with the glucose in blood; the more glucose present, the greater amount of hemoglobin linked with glucose.[26]

The A1c became a standard measurement for blood sugar control in the comprehensive ten-year study from 1983 to 1993 the Diabetes Control and Complications Trial (DCCT). With the conclusion of the DCCT in 1993, studies

showed that people who were able to keep their blood glucose levels as close to normal as possible had less chance of developing complications associated with diabetes.[26]

Before this, many doctors had not put much emphasis on tight control of blood glucose levels. The common belief for decades was that diligent monitoring of blood sugars and intensive insulin therapy had little consequence for people with diabetes. Since the DCCT's findings, statistics have proven that tight blood glucose control can be extremely beneficial for people with diabetes.[26]

CLASSIFICATION OF DIABETES MELLITUS

DM is classified on the basis of the pathogenic process that leads to hyperglycemia, as opposed to earlier criteria such as age of onset or type of therapy. The two broad categories of DM are designated as;[1]

- Type 1
- Type 2

Both types of diabetes are preceded by a phase of abnormal glucose homeostasis as the pathogenic processes progresses. Type 1 diabetes is the result of complete or near-total insulin deficiency. Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production. Distinct genetic and metabolic defects in insulin action and/or secretion give rise to the common phenotype of hyperglycemia in type 2 DM and have important potential

therapeutic implications now that pharmacologic agents are available to target specific metabolic derangements. Type 2 DM is preceded by a period of abnormal glucose homeostasis classified as impaired fasting glucose (IFG) or impaired glucose tolerance (IGT).[1]

Table 1. Spectrum of glucose homeostasis and diabetes mellitus[1]

Type of diabetes	Normal glucose tolerance (NGT)	Impaired fasting glucose or impaired glucose tolerance	Hyperglycemia: Diabetes mellitus		
			Not insulin required	Insulin required for control	Insulin required for survival
Type 1					
Type 2					
Other Specific types					
Gestational diabetes					
Time (years)					
FPG (mg/dl)	< 100	100-125		≥ 126	
2-h plasma glucose (mg/dl)	< 140	140 – 199		≥ 200	

Etiologic classification of diabetes mellitus[1]

I. Type 1 diabetes (-cell destruction, usually leading to absolute insulin deficiency)

A. Immune-mediated

B. Idiopathic

II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretory defect with insulin resistance)

III. Other specific types of diabetes

A. Genetic defects of β-cell function characterized by mutations in :

1. Hepatocyte nuclear transcription factor (HNF) 4α maturity onset diabetes of young (MODY) 1
2. Glucokinase (MODY 2)
3. HNF – 1α (MODY 3)
4. Insulin promoter factor (IPF) 1 (MODY 4)
5. HNF – 1β (MODY 5)
6. Neuro D1 (MODY 6)
7. Mitochondrial deoxyribo nucleic acid (DNA)
8. Sub units of adenosine triphosphate (ATP) – sensitive potassium channel.
9. Proinsulin or insulin conversion

B. Genetic defects in insulin action.

1. Type A insulin resistance
2. Leprechaunism
3. Rabson-Mendenhall syndrome
4. Lipodystrophy syndromes.

C. Diseases of the exocrine pancreas – pancreatitis, pancreatectomy, neoplasia, cystic fibrosis, hemochromatosis, fibrocalculouspancreatopathy.

D. Endocrinopathies – acromegaly, Cushing's syndrome, glucagonoma, pheochromocytoma, hyperthyroidism, somatostatinoma, aldosteronoma

E. Drug or chemical induced – Vacor, pentamidine, nicotinic acid, glucocorticoids, thyroid hormone, diazoxide, beta-adrenergic agonists, thiazides, phenytoin, α– interferon, protease inhibitors, clozapine, beta blockers.

F. Infections – congenital rubella, cytomegalovirus, coxsackie.

G. Uncommon forms of immune-mediated diabetes – "stiff-man" syndrome, anti-insulin receptor antibodies.

H. Other genetic syndromes sometimes associated with diabetes – Down's syndrome, Klinefelter's syndrome, Turner's syndrome, Wolfram's syndrome, Friedreich's ataxia, Huntington's chorea, Laurence-Moon-Biedl syndrome, myotonic dystrophy, porphyria, Prader-Willi syndrome.

IV. Gestational diabetes mellitus (GDM)

EPIDEMIOLOGY

The worldwide prevalence of DM has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 177 million in 2000. Based on current trends, more than 360 million individuals will have diabetes by the year 2030. Although the prevalence of both type 1 and type 2 DM is

increasing worldwide, the prevalence of type 2 DM is rising much more rapidly because of increasing obesity and reduced activity levels as countries become more industrialized. This is true in most countries, and 6 of the top 10 countries with the highest rates are in Asia. The prevalence of type 2 DM and its harbinger, IGT, is highest in certain Pacific islands, intermediate in countries such as India and the United States, and relatively low in Russia. This variability is likely due to genetic, behavioral, and environmental factors.[1]

In India it is estimated that presently 19.4 million individuals are affected by this deadly disease, which is likely to go up to 57.2 million by the year 2025.[27]

The prevalence of diabetes is four to six fold lower in rural areas, which is probably attributed to a conventional lifestyle which has beneficial effect on glucose tolerance (IGT). National Urban Diabetes Survey done in six cities, found age standardized prevalence rates of 12% for diabetes; with a slight male preponderance and 14% for impaired glucose tolerance. Subjects under the age of 40 years, had a prevalence of five percent for DM and 13% prevalence of impaired glucose tolerance.[28]

Epidemiologic determinants and risk factors of type 2 diabetes[29]

Genetic Factors

- Genetic markers
- Family history
- "Thrifty gene(s)"

Demographic characteristics

- Sex
- Age
- Ethnicity

Behavioral and lifestyle-related risk factors

- Obesity (including distribution of obesity and duration)
- Physical inactivity
- Diet
- Stress

Metabolic determinants and intermediate-risk categories of type 2 diabetes

- Impaired glucose tolerance
- Insulin resistance
- Pregnancy-related determinants
    - Parity
    - Gestational diabetes
    - Diabetes in offspring of women with diabetes during pregnancy
    - Intrauterine malnutrition or overnutrition

DIAGNOSIS OF DIABETES

Criteria for the Diagnosis of Diabetes Mellitus[1]

- Symptoms of diabetes plus random blood glucose concentration more than or equal to 11.1 mmol/L (200 mg/dL)[a] *or*
- Fasting plasma glucose more than or equal to 7.0 mmol/L (126 mg/dL)[b] *or*
- Two-hour plasma glucose more than or equal to 11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test[c]

[a]Random is defined as without regard to time since the last meal.

[b]Fasting is defined as no caloric intake for at least 8 h.

[c]The test should be performed using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water; not recommended for routine clinical use.

In the absence of unequivocal hyperglycemia and acute metabolic decompensation, these criteria should be confirmed by repeat testing on a different day.

Glucose tolerance is classified into three categories based on the FPG:

1. FPG less than 5.6 mmol/L (100 mg/dL) is considered normal;
2. FPG equal to 5.6–6.9 mmol/L (100–125 mg/dL) is defined as IFG; and
3. FPG more than or equal to 7.0 mmol/L (126 mg/dL) warrants the diagnosis of DM.

Oral glucose tolerance test

The test uses the following procedures.

- It first employs an FPG test.
- A blood test is then taken two hours after drinking a 75 g anhydrous glucose solution.

Based on the OGTT, IGT is defined as plasma glucose levels between 7.8 and 11.1 mmol/L (140 and 199 mg/dL). Diabetes is defined when plasma glucose is more than 11.1 mmol/L (200 mg/dL), 2 h after a 75 g oral glucose load.

The current criteria, for the diagnosis of DM emphasize that the FPG is the most reliable and convenient test for identifying DM, in asymptomatic individuals. A random plasma glucose concentration more than or equal to 11.1 mmol/L (200 mg/dL) accompanied by classic symptoms of DM (polyuria, polydipsia, weight loss) is sufficient for the diagnosis of DM.

Hemoglobin A1C (HbA1c) was advocated as a diagnostic test for DM. There is a strong correlation between elevations in the plasma glucose and the A1C, the relationship between the FPG and the A1C in individuals with normal glucose tolerance or mild glucose intolerance and thus the use of the A1C is currently recommended to diagnose diabetes.[1]

PATHOPHYSIOLOGY

Insulin biosynthesis

Insulin is produced in the beta cells of the pancreatic islets. It is initially synthesized as preproinsulin. Subsequent proteolytic processing removes the amino terminal signal peptide, giving rise to proinsulin. Cleavage of an internal 31-residue fragment from proinsulin generates the C peptide and the A (21 amino acids) and B (30 amino acids) chains of insulin, which are connected by disulfide bonds. The mature insulin molecule and C peptide are stored together and co-secreted from secretory granules in the beta cells.[1]

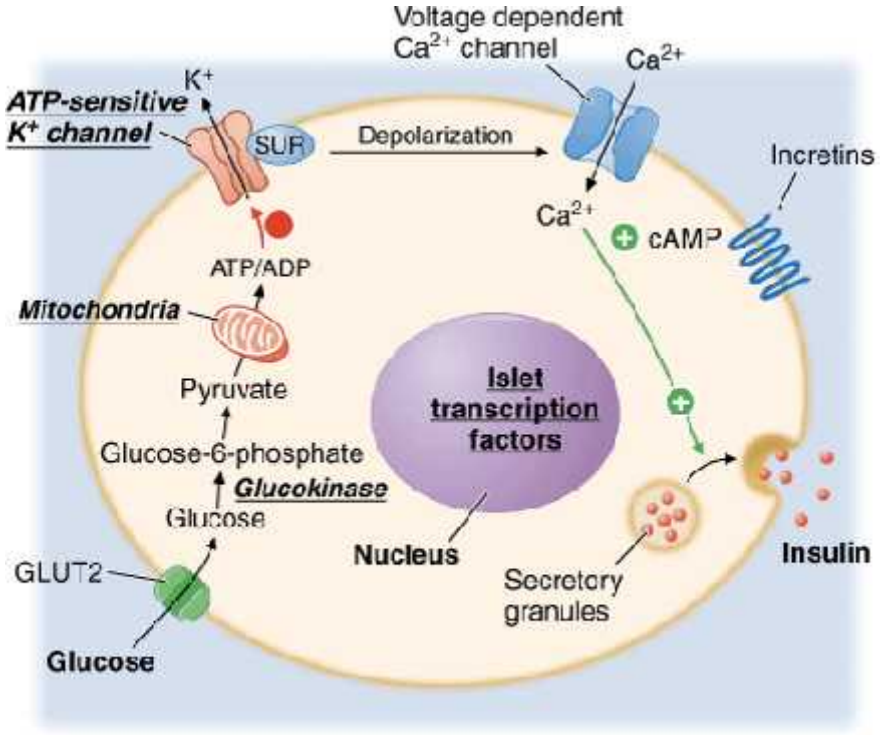


Figure 1. Diabetes and abnormalities in glucose-stimulated insulin secretion

Glucose and other nutrients regulate insulin secretion by the pancreatic beta cell. Glucose is transported by the GLUT2 glucose transporter; subsequent glucose metabolism by the beta cell alters ion channel activity, leading to insulin secretion. The SUR receptor is the binding site for drugs that act as insulin secretagogues. Mutations in the events or proteins underlined are a cause of maturity onset diabetes of the young (MODY) or other forms of diabetes.[1]

Secretion

Glucose is the key regulator of insulin secretion by the pancreatic beta cell, although amino acids, ketones, various nutrients, gastrointestinal peptides, and neurotransmitters also influence insulin secretion. Glucose levels > 3.9 mmol/L (70 mg/dL) stimulate insulin synthesis, primarily by enhancing protein translation and processing. Glucose stimulation of insulin secretion begins with its transport into the beta cell by the GLUT2 glucose transporter. Glucose phosphorylation by glucokinase is the rate-limiting step that controls glucose-regulated insulin secretion. Further metabolism of glucose-6-phosphate via glycolysis generates ATP, which inhibits the activity of an ATP-sensitive K^+ channel. This channel consists of two separate proteins: one is the binding site for certain oral hypoglycemics (e.g., sulfonylureas, meglitinides); the other is an inwardly rectifying K^+ channel protein. Inhibition of this K^+ channel induces beta cell membrane depolarization, which opens voltage-dependent calcium channels, and stimulates insulin secretion. Insulin secretory profiles reveal a pulsatile pattern of hormone release, with small secretory bursts occurring about every 10 min.[1]

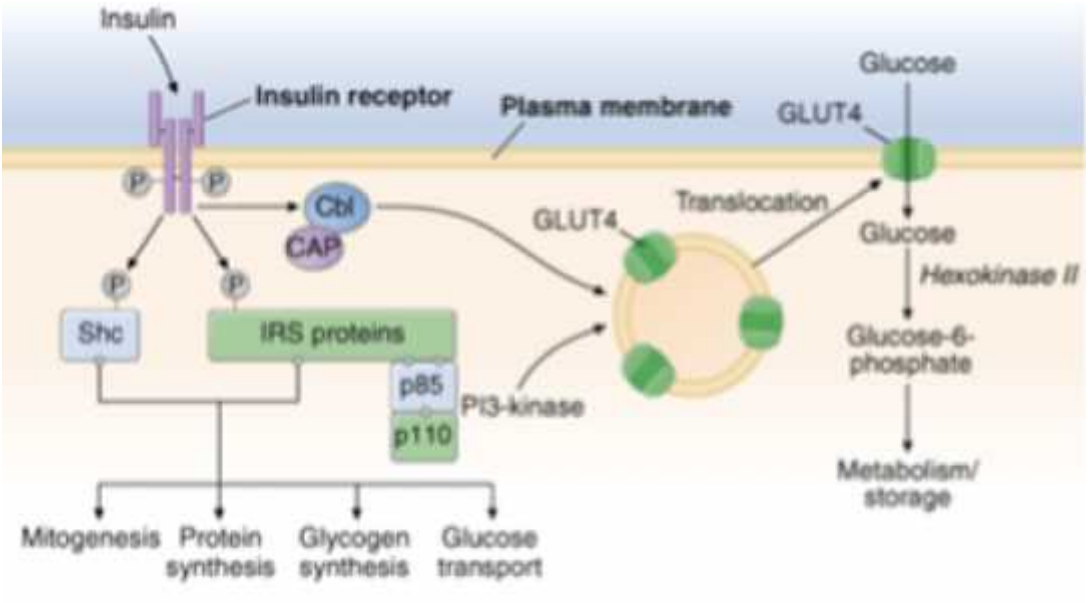


Figure 2. Insulin signal transduction pathway in skeletal muscle

The insulin receptor has intrinsic tyrosine kinase activity and interacts with insulin receptor substrates (IRS and Shc) proteins. A number of "docking" proteins bind to these cellular proteins and initiate the metabolic actions of insulin [GrB-2, SOS, SHP-2, p65, p110, and phosphatidylinositol-3'-kinase (PI-3-kinase)]. Insulin increases glucose transport through PI-3-kinase and the Cbl pathway, which promotes the translocation of intracellular vesicles containing GLUT4 glucose transporter to the plasma membrane.[1]

Action

Once insulin is secreted into the portal venous system, about 50% is degraded by the liver. Unextracted insulin enters the systemic circulation where it binds to receptors in target sites. Insulin binding to its receptor stimulates intrinsic tyrosine kinase activity, leading to receptor autophosphorylation and the

recruitment of intracellular signaling molecules, such as insulin receptor substrates (IRS). IRS and other adaptor proteins initiate a complex cascade of phosphorylation and dephosphorylation reactions, resulting in the widespread metabolic and mitogenic effects of insulin.[1]

Glucose homeostasis reflects a balance between hepatic glucose production and peripheral glucose uptake and utilization. Insulin is the most important regulator of this metabolic equilibrium, but neural input, metabolic signals, and other hormones result in integrated control of glucose supply and utilization. In the fasting state, low insulin levels increase glucose production by promoting hepatic gluconeogenesis and glycogenolysis and reduce glucose uptake in insulin-sensitive tissues, thereby promoting mobilization of stored precursors such as amino acids and free fatty acids. Glucagon, secreted by pancreatic alpha cells when blood glucose or insulin levels are low, stimulates glycogenolysis and gluconeogenesis by the liver and renal medulla. Postprandially, the glucose load elicits a rise in insulin and fall in glucagon, leading to a reversal of these processes.[1]

Type 2 Diabetes mellitus

Insulin resistance and abnormal insulin secretion are central to the development of type 2 DM. Although the primary defect is controversial, most studies support the view that insulin resistance precedes an insulin secretory defect but that diabetes develops only when insulin secretion becomes inadequate.[1]

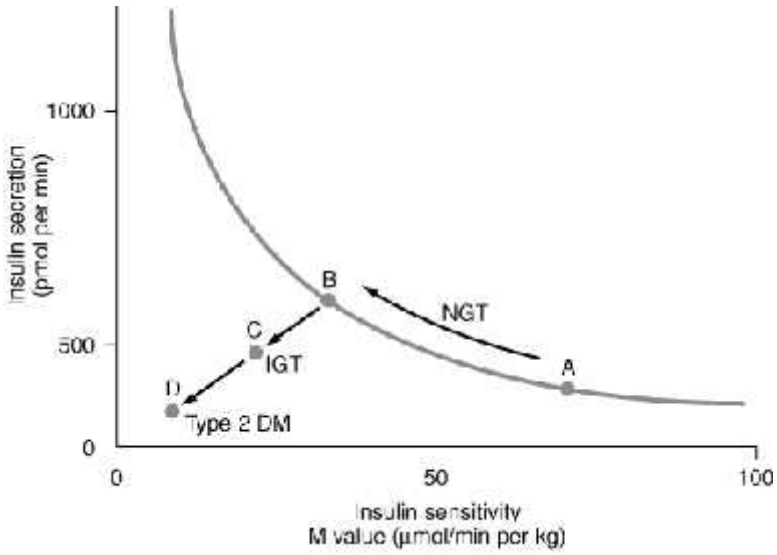


Figure 3. Metabolic changes during the development of type 2 diabetes mellitus

Insulin secretion and insulin sensitivity are related, and as an individual becomes more insulin resistant (by moving from point A to point B), insulin secretion increases. A failure to compensate by increasing the insulin secretion results initially in impaired glucose tolerance (IGT; point C) and ultimately in type 2 DM (point D).[1]

Pathophysiology of type 2 DM

Type 2 DM is characterized by impaired insulin secretion, insulin resistance, excessive hepatic glucose production, and abnormal fat metabolism. Obesity, particularly visceral or central, is very common in type 2 DM. In the early stages of the disorder, glucose tolerance remains near normal, despite insulin resistance, because the pancreatic beta cells compensate by increasing insulin output. As insulin resistance and compensatory hyperinsulinemia progress, the pancreatic islets in certain individuals are unable to sustain the

hyperinsulinemic state. IGT, characterized by elevations in postprandial glucose, then develops. A further decline in insulin secretion and an increase in hepatic glucose production lead to overt diabetes with fasting hyperglycemia. Ultimately, beta cell failure may ensue.[1]

Complications of type 2 diabetes mellitus[1]

Acute

- Diabetic Ketoacidosis
- Hyperglycemic Hyperosmolar State

Chronic

- Microvascular
    - Eye disease
        - Retinopathy (nonproliferative/proliferative)
        - Macular edema
    - Neuropathy
        - Sensory and motor (mono- and polyneuropathy)
        - Autonomic
    - Nephropathy
- Macrovascular
    - Coronary artery disease
    - Peripheral vascular disease
    - Cerebrovascular disease
- Other

- Gastrointestinal
- Genitourinary
- Dermatologic
- Cataracts
- Glaucoma
- Infectious
- Periodontal disease

Chronic complications

The risk of chronic complications increases as a function of the duration of hyperglycemia; they usually become apparent in the second decade of hyperglycemia. Since type 2 DM often has a long asymptomatic period of hyperglycemia, many individuals with type 2 DM have complications at the time of diagnosis.[1]

The microvascular complications of both type 1 and type 2 DM result from chronic hyperglycemia. Large, randomized clinical trials of individuals with type 1 or type 2 DM have conclusively demonstrated that a reduction in chronic hyperglycemia prevents or delays retinopathy, neuropathy, and nephropathy. Other incompletely defined factors may modulate the development of complications.[1]

Evidence implicating a causative role for chronic hyperglycemia in the development of macrovascular complications is less conclusive. However, coronary heart disease events and mortality are two to four times greater in patients with type 2 DM. These events correlate with fasting and postprandial

plasma glucose levels as well as with the A1C. Other factors like dyslipidemia and hypertension also play important roles in macrovascular complications.[1]

Mechanisms of complications

Four prominent theories, which are not mutually exclusive, have been proposed to explain how hyperglycemia might lead to the chronic complications of DM.[1]

One theory is that increased intracellular glucose leads to the formation of advanced glycosylation end products (AGEs) via the nonenzymaticglycosylation of intra- and extracellular proteins. Nonenzymaticglycosylation results from the interaction of glucose with amino groups on proteins. AGEs have been shown to cross-link proteins (collagen, extracellular matrix proteins), accelerate atherosclerosis, promote glomerular dysfunction, reduce nitric oxide synthesis, induce endothelial dysfunction, and alter extracellular matrix composition and structure. The serum level of AGEs correlates with the level of glycemia, and these products accumulate as glomerular filtration rate declines.[1]

A second theory is based on the observation that hyperglycemia increases glucose metabolism via the sorbitol pathway. Intracellular glucose is predominantly metabolized by phosphorylation and subsequent glycolysis, but when increased, some glucose is converted to sorbitol by the enzyme aldosereductase. Increased sorbitol concentration alters redox potential, increases cellular osmolality, generates reactive oxygen species, and likely leads to other types of cellular dysfunction.[1]

A third hypothesis proposes that hyperglycemia increases the formation of diacylglycerol leading to activation of protein kinase C (PKC). Among other actions, PKC alters the transcription of genes for fibronectin, type IV collagen, contractile proteins, and extracellular matrix proteins in endothelial cells and neurons.[1]

A fourth theory proposes that hyperglycemia increases the flux through the hexosamine pathway, which generates fructose-6-phosphate, a substrate for O-linked glycosylation and proteoglycan production. The hexosamine pathway may alter function by glycosylation of proteins such as endothelial nitric oxide synthase or by changes in gene expression of transforming growth factor β (TGF-β) or plasminogen activator inhibitor-1 (PAI-1).[1]

Growth factors appear to play an important role in DM-related complications, and their production is increased by most of these proposed pathways. Vascular endothelial growth factor A (VEGF-A) is increased locally in diabetic proliferative retinopathy and decreases after laser photocoagulation. TGF- β is increased in diabetic nephropathy and stimulates basement membrane production of collagen and fibronectin by mesangial cells. Other growth factors, such as platelet-derived growth factor, epidermal growth factor, insulin-like growth factor I, growth hormone, basic fibroblast growth factor, and even insulin, have been suggested to play a role in DM-related complications. A possible unifying mechanism is that hyperglycemia leads to increased production of reactive oxygen species or superoxide in the mitochondria; these compounds may activate all four of the pathways described above.[1]

Macrovascular complications

The pathophysiology of the link between diabetes and cardiovascular disease (CVD) is complex and multifactorial. Understanding these profound mechanisms of disease can help clinicians identify and treat CVD in patients with diabetes, as well as help patients prevent these potentially devastating complications. This article reviews the biological basis of the link between diabetes and CVD, from defects in the vasculature to the cellular and molecular mechanisms specific to insulin-resistant states and hyperglycemia. It concludes with a discussion of heart failure in diabetes, a clinical entity that demonstrates many of the mechanisms discussed.

Diabetes is a prime risk factor for cardiovascular disease (CVD). Vascular disorders include retinopathy and nephropathy, peripheral vascular disease (PVD), stroke, and coronary artery disease (CAD). Diabetes also affects the heart muscle, causing both systolic and diastolic heart failure. The etiology of this excess cardiovascular morbidity and mortality is not completely clear. Evidence suggests that although hyperglycemia, the hallmark of diabetes, contributes to myocardial damage after ischemic events, it is clearly not the only factor, because both pre-diabetes and the presence of the metabolic syndrome, even in normoglycemic patients, increase the risk of most types of CVD.[30]

In 2002, a survey[31] of people in the United States with diagnosed diabetes found that, surprisingly, 68% of patients did not consider themselves at risk for heart attack or stroke. In addition, only about half of patients surveyed reported

that their health care providers discussed the high risk of CVD in diabetes and what steps they could take to reduce that risk.

Health care providers are now focused on decreasing cardiovascular risk in patients with diabetes by treating dyslipidemia and hypertension and by improving glycemic control. Moreover, the American Diabetes Association/American College of Cardiology "Make the Link" public awareness campaign has improved knowledge related to CVD in patients with diabetes.[30]

However, managing cardiovascular risk factors in patients with diabetes does not eradicate these complications. We are only just beginning to understand the complex and multifactorial etiology of CVD in diabetes.

Macrovasculature

Atherosclerosis is the major threat to the macrovasculature for patients with and without diabetes. The general pathogenesis of atherosclerosis has been reviewed elsewhere, but several factors specific to diabetes are worth mentioning here. Clinically, dyslipidemia is highly correlated with atherosclerosis, and up to 97% of patients with diabetes are dyslipidemic. In addition to the characteristic pattern of increased triglycerides and decreased HDL cholesterol found in the plasma of patients with diabetes, abnormalities are seen in the structure of the lipoprotein particles. In diabetes, the predominant form of LDL cholesterol is the small, dense form. Small LDL particles are more atherogenic than large LDL particles because they can more easily penetrate and form stronger attachments to the arterial wall, and they are more susceptible to oxidation. Because less cholesterol is carried in the core of small LDL particles than in the core of large

particles, subjects with predominantly small LDL particles have higher numbers of particles at comparable LDL cholesterol levels.[32]

Oxidized LDL is pro-atherogenic because once the particles become oxidized they acquire new properties that are recognized by the immune system as "foreign." Thus, oxidized LDL produces several abnormal biological responses, such as attracting leukocytes to the intima of the vessel, improving the ability of the leukocytes to ingest lipids and differentiate into foam cells, and stimulating the proliferation of leukocytes, endothelial cells, and smooth muscle cells,[33] all of which are steps in the formation of atherosclerotic plaque. In patients with diabetes, LDL particles can also become glycated, in a process similar to the glycation of the protein hemoglobin (measured in the hemoglobin A_{1c} [A1C] assay). Glycation of LDL lengthens its half-life and therefore increases the ability of the LDL to promote atherogenesis. Paradoxically, however, glycation of HDL shortens its half-life and renders it less protective against atherosclerosis.[30]

Moreover, diabetic blood is more likely to be high in triglycerides. Hypertriglyceridemia in diabetes occurs, in part, because insulin action regulates lipid flux. Insulin promotes the activity of the enzyme lipoprotein lipase, which mediates free fatty acid uptake into adipose tissue (storage) and also suppresses the activity of the enzyme hormone-sensitive lipase, resulting in decreased release of free fatty acids into the circulation. Hypertriglyceridemia can lead to increased production of the small, dense form of LDL and to decreased HDL transport of cholesterol back to the liver.[30]

Dyslipidemia is only one mechanism by which diabetes promotes atherosclerosis; endothelial dysfunction often contributes. Healthy endothelium regulates blood vessel tone, platelet activation, leukocyte adhesion, thrombogenesis, and inflammation. The net effect of healthy endothelium is vasodilatory, anti-atherogenic, and anti-inflammatory.[30]

When these mechanisms are defective, the process of atherosclerosis is accelerated. Therefore, both insulin deficiency and insulin resistance promote dyslipidemia accompanied by increased oxidation, glycosylation, and triglyceride enrichment of lipoproteins. In addition, endothelial dysfunction is present, and all of these factors contribute to the increase in atherogenicity, and thus macrovascular disease, found in patients with diabetes.

Microvascular Disease

Typically, when we hear the term “microvascular disease” associated with diabetes, we think of retinopathy, nephropathy, and neuropathy. In addition, however, small vessels throughout the body are affected by diabetes, including those in the brain, heart, and peripheral vasculature. This small vessel damage is typically not related to atherosclerosis and is not predicted by lipid levels. Whereas atherosclerosis is the major threat to the macrovasculature, a variety of cellular and molecular mechanisms contribute to microvascular disease in diabetes.[30]

The microcirculation is regulated by central and local regulatory mechanisms. The central regulation is via autonomic sympathetic and parasympathetic nerves that reach the vascular smooth muscle. Local regulation

is carried out by substances produced by the endothelial cells and by local products of metabolism. The endothelium produces both vasodilators and vasoconstrictors. Normally, the vascular smooth muscle receives continuous regulatory nerve signals and a continual supply of vasodilating nitric oxide (NO) from the endothelium, as well as a continuous flow of metabolic products. These regulatory mechanisms adjust microvascular flow instantaneously to meet the metabolic needs of the tissue.[34]

Diabetes contributes to defects in the autonomic nervous system, the endothelium, and local metabolism, all of which can result in microvascular disease. Diabetic autonomic neuropathy (DAN) is one factor associated with impaired autoregulation of blood flow in a variety of vascular beds, including the skin and the heart.[35,36]

Patients with DAN have increased rates of sudden cardiac death as well as a higher overall cardiovascular mortality rate. These patients have been found to lack the normal cardiac flow reserve that is activated under conditions of increased demand for myocardial perfusion,[37] which may partially explain the high mortality rate in this population.

In addition to the dysregulation of vascular tone caused by DAN, subjects with diabetes have been found to have decreased bioavailability of NO, a potent vasodilator, as well as increased secretion of the vasoconstrictor endothelin-1. This resulting state of vasoconstriction has been found in subjects with the metabolic syndrome as well as those with diabetes.[38] In this situation, the vasculature is in a hyper-constricted state. Not only do hypertension and its

concomitant complications result from vasoconstriction, but blood flow is limited to respective tissues. Diabetes decreases NO bioavailability because of either insulin deficiency or defective insulin signaling (insulin resistance) in endothelial cells.[39] Hyperglycemia also acutely inhibits the production of NO in arterial endothelial cells.[40]

In a sense, the ultimate outcome of blood flow to tissues is the transport and exchange of substances between blood and tissue fluid. Thus, despite an appropriate amount of blood flow, any process that inhibits product exchange will impair the homeostasis of the tissue containing the vascular bed. Capillary basement membrane thickening associated with prolonged hyperglycemia is a structural hallmark of diabetic microvascular disease. Thickening of the basement membrane impairs the amount and selectivity of transport of metabolic products and nutrients between the circulation and the tissue.[41] In fact, in skeletal muscle of patients with type 2 diabetes, exercise-stimulated oxygen delivery from the capillaries is delayed, which may account in part for the poor exercise tolerance found in people with type 2 diabetes.[42]

Transport of substances from the circulation, across the microvessel wall, and into tissue interstitium is regulated by a variety of interdependent mechanisms, including pressure, flow, and size and charge specificity. Paradoxically, basement membrane thickening *increases* microvascular permeability because of alterations in the physical dimensions of the meshwork and changes in the normal electrical charge surrounding the pores between endothelial cells. These abnormalities allow for the transport of large molecules normally excluded from passage across the microvasculature. In clinical terms,

transcapillary leak of albumin in the kidney provides an important indicator of microvascular disease.[43]

The urine microalbumin test, initially indicated for the detection of early diabetic nephropathy, actually reflects the health of the entire microvasculature. Thus, a patient with a microalbuminuria not only has nephropathy, but also can be assumed to have widespread microvascular disease.[44]

Inflammation

Inflammation is a normal response to tissue injury or pathogen exposure and is a critical factor in the body's ability to heal itself or to fight off infection. The inflammatory response involves the activation of leukocytes (white blood cells) and is mediated, in part, by a family of cytokines and chemokines. Although inflammation is beneficial, if this response is chronically activated it can have a detrimental effect. Diabetes has long been considered a state of chronic, low-level inflammation,[45] and there is some evidence to suggest that this immune activation may precede insulin resistance in diabetic and pre-diabetic states and ultimately may be the factor that initially increases cardiovascular risk in these disease processes.[46]

Recent evidence suggests cross-talk between the molecular pathways involved in both inflammation and insulin signaling, and this cross-talk may provide clues to the strong relationship between insulin-resistant states (such as the metabolic syndrome and type 2 diabetes), inflammation, and CVD.[47]

As previously discussed, researchers have found a reduced production of the potent vasodilator NO and an increased secretion of the vasoconstrictor and growth factor endothelin-1 in subjects with the metabolic syndrome, and these abnormalities not only enhance vasoconstriction, but are associated with the release of pro-inflammatory cytokines.[48]

Proinflammatory cytokines cause or exacerbate injury by a variety of mechanisms including enhanced vascular permeability, programmed cell death (apoptosis), recruitment of invasive leukocytes, and the promotion of reactive oxygen species (ROS) production.[49]

Recently, a study[50] found serum sialic acid, a marker of low-grade inflammation,[45] to be strongly predictive of type 2 diabetes in 128 patients from the United Kingdom who were followed for a mean of 12.8 years. In addition to predicting type 2 diabetes, this marker also predicted cardiovascular mortality independent of other known risk factors for CVD, including pre-existing CVD.[50]

These observations have led investigators to suspect a common, unknown antecedent[51] and to consider chronic inflammation as one candidate for this precursor.

In addition to diabetes, obesity is associated with increased levels of a number of adipokines (cytokines released from adipose tissue), including tumor necrosis factor-α, interleukin 1β, interleukin 6, and plasminogen activator inhibitor 1 (PAI-1), all linked to the inflammatory response.[52] The levels of these pro-inflammatory cytokines typically increase as fat mass increases; however, one exception is the adipokine adiponectin, which has anti-inflammatory

properties and is decreased in obese subjects,[53] exacerbating the chronic inflammatory nature of obesity. In addition to their endocrine properties, these locally produced cytokines have been found to possess autocrine and paracrine properties that can influence neighboring tissues as well as the entire organism.

Hypercoagulability

In addition to affecting the leukocytes in the blood, diabetes is also related to a hypercoagulable state. The coagulability of the blood is crucially important in ischemic cardiovascular events because the majority of MI and stroke events are caused by the rupture of atherosclerotic plaque and the resulting occlusion of a major artery by a blood clot (thrombus).[30]

Up to 80% of patients with diabetes die a thrombotic death. Seventy-five percent of these deaths are the result of an MI, and the remainder are the result of cerebrovascular events and complications related to PVD.[54]

The first defense against a thrombotic event is the vascular endothelium. As previously discussed, diabetes contributes to widespread endothelial dysfunction. The endothelium and the components of the blood are intricately linked, such that clotting signals initiated in the endothelial cell can activate platelets and other blood components, and vice versa.[55]

Patients with diabetes exhibit enhanced activation of platelets and clotting factors in the blood. Increased circulating platelet aggregates, increased platelet aggregation in response to platelet agonists, and the presence of higher plasma levels of platelet coagulation products, such as beta-thromboglobulin, platelet

factor 4, and thromboxane B_2, demonstrate platelet hyperactivity in diabetes. Coagulation activation markers, such as prothrombin activation fragment 1+2 and thrombin–anti-thrombin complexes, are also elevated in diabetes. In addition, patients with diabetes have elevated levels of many clotting factors including fibrinogen, factor VII, factor VIII:C, factor XI, factor XII, kallikrein, and von Willebrand factor. Conversely, anticoagulant mechanisms are diminished in diabetes. The fibrinolytic system, the primary means of removing clots, is relatively inhibited in diabetes because of abnormal clot structures that are more resistant to degradation, and also because of an increase in PAI-1.[7]

Clinicians attempt to reverse this hypercoagulable state with aspirin therapy, widely recommended for use as primary prevention against thrombotic events in patients with diabetes. However, numerous studies have suggested that aspirin in recommended doses does not adequately inhibit platelet activity in patients with diabetes. This concept of "aspirin resistance" is controversial and has not been found consistently in all diabetic patient populations, but it may provide insight into the high rates of thrombotic events in diabetes even among those appropriately treated.[56]

Coagulation Factor VIII:C

Human factor VIII:C, also called anti-hemophilic factor, is a plasma glycoprotein that performs a substantial role in blood-clotting.[57] By being found in the blood plasma, factor VIII:C is able to get to any part of the body that may be bleeding. Without being localized in the blood, this factor would have to be expressed in every cell of the body to ensure bleeding could be stopped anywhere

it started. Human factor VIII:C amino acid sequence is 216aa long. This amino acid sequence is derived from an mRNA sequence containing 2536bp.[58]

However, the coding sequence for the protein is only 651bp long. This protein is not only found in humans though. Many other creatures, dogs and mice included, also use a form of factor VIII:C in blood-clotting. In these other organisms the amino acid sequence is conserved, but the cDNA and mRNA varies slightly.[58]

Blood Coagulation in mammals

Blood clotting in mammals results from the conversion of a soluble plasma protein, into an insoluble matrix of fibers (Fig. 1). Fibrinogen or factor I is the soluble plasma protein that is eventually converted into fibrin, the insoluble matrix.[58] The change of fibrinogen into fibrin requires many other components that all play a role in the change.One protein that acts on the fibrinogen is thrombin.

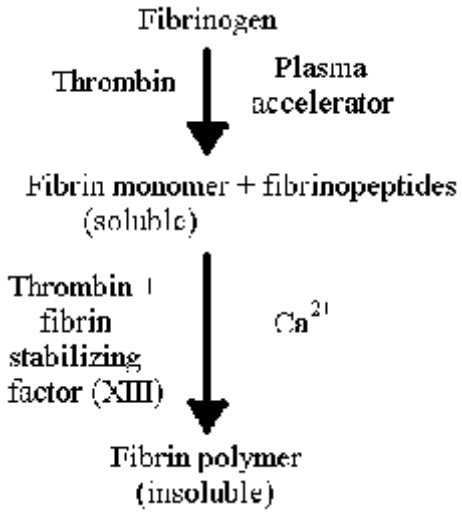


Figure 4. The formation of fibrin in human plasma[59]

However, thrombin is not found in normal circulating blood. Thrombin is generated from its precursor, prothrombin (factor II), by what has been described as a waterfall or cascade with each product triggering the activation of the next.[59]

In order for thrombin to be made, one factor, factor VIII:C, is required along with calcium and without factor VIII:C, thrombin cannot be made. Figure 2 displays the waterfall diagram of how thrombin is made with factor VIII:C interacting in the reaction sequence.

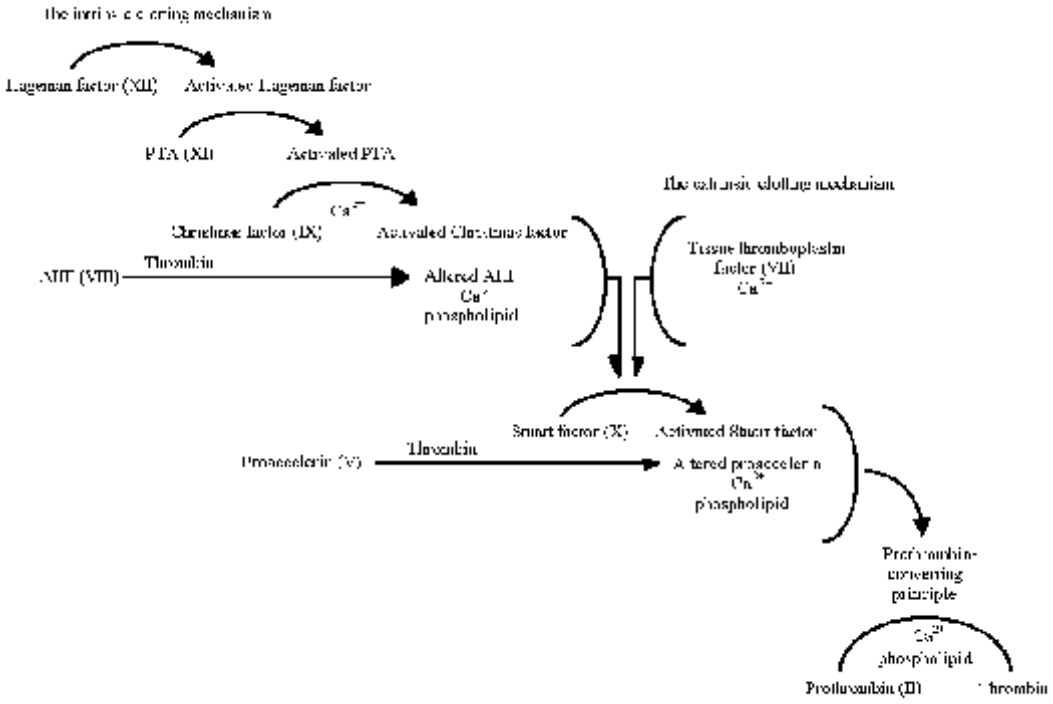


Figure 5. The mechanisms of normal coagulation. Factor VIII:C shown interacting to help form thrombin[59]

In order for factor VIII:C to form a functional protein, it must be activated by Christmas factor on the surface of phospholipids, along with calcium ions being present. However, Factor VIII:C circulates through the blood not as a solitary unit, but as a complex paired with von Willebrand factor.[57] As shown in

Figure 2 after factor VII is cleaved by thrombin, it binds with factor IX at either the surface of activated platelets or endothelial cells. Factor IX is then able to activate factor X, which in turn converts prothrombin to thrombin in the presence of factor V. With calcium present, the waterfall activation sequence is able to continue, however, without it, activated factor VIII:C is not produced. Without the activation of factor VIII:C, prothrombin is never changed into thrombin and clotting will not function properly.[57]

Imbalance of coagulation factor VIII:C activity

Increased (sustained rises) of factor VIII:C are seen during pregnancy, surgery chronic inflammation, malignancy, liver disease, hyperthyroidism, intravascular haemolysis and renal disease.[60,61] Factor VIII:C is also elevated in inflammatory conditions.[62]

Some of these haemostasis variables may also reflect the endothelial activation or dysfunction, associated with inflammation, since inflammatory mediators including TNF-α and interleukin-6 have been shown to cause endothelial dysfunction.[63,64]

Several cross-sectional studies[65-69] have suggested an association of Factor VIII:C or VWF with clinical atherosclerotic heart disease. Low factor VIII:C level protect against ischemic heart disease. Mortality due to IHD is much lower in patients with hemophilia A than in the general male population, which may suggest factor VIII:C is involved in the pathogenesis of arterial thrombosis.[25,70,71]

Body mass index (positively correlated with factor VIII:C levels and higher levels of glucose (DM), insulin, fibrinogen and triglycerides are also associated with increased factor VIII:C activity.[22,72,73]

High levels of factor VIII:C are a risk factor for thrombosis with a greater impact on venous than on arterial thrombosis. This risk is dose dependent for venous thrombosis and increased factor VIII:C activity account for 16% of all venous thrombotic events, whereas factor VIII:C activity explain 4% of all arterial events. High factor VIII:C levels may increase the risk of venous thrombosis via enhanced thrombin formation or through the induction of acquired APC resistance. The relationship between factor VIII:C activity and arterial thrombosis may be based on the combination of increased thrombin formation and increased platelet adhesion/aggregation, at sites of arterial wall damage.[74]

Risk factors for coronary heart disease (CHD), including prethrombotic changes in haemostasis, cluster with the insulin resistance (IR) syndrome. Multivariate genetic analysis performed on twins in the study on IR assessed by HOMA, fibrinogen, plasminogen activator inhibitor, tissue plasminogen activator, factor VIII:C, VWF, and factor XIIIB-subunit. Since IR and prethrombotic changes are features of diabetes and CHD, the finding of one set of pleotrophic genes warrants the identification of these common pathways, which may provide new avenues for treatment, and prevention of both diabetes and CHD.[75]

The role of hypertension, hypercholesterolemia and hypertriglyceridemia has been implicated in the pathogenesis of carotid atherosclerosis and roles for

hypertension and hyperglycemia in stroke were indicated. A positive association between factor VIII:C activity and carotid atherosclerosis was seen in Chinese population.[76]

Study on atherosclerosis risk in four US communities in which, they measured hemostatic variables in 12681 men and women aged 45 to 64 years showed factor VIII:C activity was 20% points higher in blacks than in whites, 4% points higher in women than in men. Von Willebrand Factor antigen was higher in blacks than in whites. It was lower in men than women among blacks. But slightly higher in men than in women among whites. aPTT decreased with age both in men and women. aPTT was shorter in women than in men. A striking association of coagulating factors with race and diabetes has been observed.[77]

The mean coagulation activities of factor VII and VIII were found to be higher in Americans than Japanese, suggesting the rational difference in coagulation factors activity.[78]

Ethnic Chinese population showed that elevation of coagulation factors differed in male and female diabetic patients. In sex differences, shorter aPTT and factor VIII:C showed higher level in male diabetic patients, whereas female diabetic patients had shorter aPTT, significant higher levels of fibrinogen, factor VII, factor VIII:C and plasminogen implied that female diabetic patients may have higher risk for CHD than male.[79]

People with diabetes been reported to have a raised factor VIII:C and VWF compared to non-diabetics. Factor VIII:C and VWF increases with each 5-

year increase in age. Significant associations were also found for women consuming ethanol, body mass index (not VWF in men) menopausal status (Factor VIII:C not VWF), insulin and triglycerides (not VWF in women). Since factor VIII:C and VWF are both pro-coagulants favouring clot formation and platelet adhesion higher levels might be expected to be a risk factor for thrombosis and atherosclerosis.[73]

Compared to age and sex matched normal subjects, plasma activity of factor VIII:C was reported to be significantly elevated in the diabetic patients. These elevated plasma levels of factor VIII:C /VWF have been linked to vascular endothelial injury.[80]

Diabetes, hypertension, body mass index and triglycerides are some factors known to be associated with perturbed endothelial function and vascular inflammation.[81,82]

High factor VIII:C levels may stimulate the formation of thrombin and thus result in increased platelet activation and fibrin formation, process that may contribute to the development of large occlusive thrombi from the microthrombi initially formed on the damaged endothelium, leading to stroke in subjects with presumed large vessel disease,[83] mainly the result of atherothromboembolism.[84]

Platelet aggregation and spontaneous thrombolytic activity were assessed in patients with non-insulin dependent diabetes. Suppressed thrombolysis was observed in diabetes. The relationship observed between increase in fibrinogen, fibrinopeptide and D-dimer aggregation has been equivocal. Elderly diabetic

males may have a prethrombotic state not because of platelet hyper-agreeability but because of suppressed thrombolytic activity.[85]

Eighty percent of patients with diabetes mellitus die a thrombotic death. Vascular endothelium, the primary defense against thrombosis, is abnormal in diabetes. Endothelial abnormalities play a role in the enhanced activation of platelets and clotting factors in diabetes. The plasma levels of many clotting factors like fibrinogen, factor VII, factor VIII:C, factor IX, factor XII, Kallikrein and VWF have been reported. The fibrinolytic system, primary means of removing clots, is relatively inhibited in diabetes due to abnormal clot structures that are more resistant to degradation and then increase in plasminogen activator inhibitor type I. Increased platelet hyperactivity was seen in diabetes suggesting the clinical observation that diabetes is a hypercoaguable state.[7]

The existence of a state of hypercoagulability in type I diabetes, may be related to poor glycemic control. The haemostatic disturbances precede demonstrable vascular complications.[86]

In prospective study, conducted on clotting parameters of children and adolescents with insulin dependent diabetes mellitus (type I diabetes) were compared with those of a healthy control group. There was no statistical difference for most of the coagulation parameters compared to control group, suggesting no hypercoagulable state was seen in paediatrics diabetes as it is described for adults with type I diabetes mellitus.[87]

Investigations were done to study the relationship between insulin resistance and haemostatic risk factor due to shared genes or environmental factors, in 107 monozygotic and 207 dizygotic twin pairs. The results showed haemostatic factors like fibrinogen, plasminogen activator inhibitor, tissue plasminogen activator, factor VIII:C, VWF and factor XIII-B subunit were independently influenced by genetic factors and environmental factors.[75]

Six male patients with insulin-dependent diabetes were examined for assays of factor VIII:C coagulant activity, VWF, ristocein co-factor, a PTT, and vasopressin, varying the blood glucose level at 5, 15 and 25 m mol/litre maintained for one hour by infusing insulin. It was observed the insignificant fall in factor VIII:C concentration with increase in blood glucose. The result indicates level of factor VIII:C seen in diabetes are not due to short-term increase in blood glucose and acute hyperglycemia does not promote procoagulant change in blood.[88]

In a study 23 patients with poorly glycemic-controlled non-insulin dependent diabetes mellitus without complications compared with 31 well-controlled diabetic patients without complication and 46 healthy normal subjects. Coagulability was seen to be enhanced in patients with poorly controlled diabetes mellitus compared with well-controlled diabetic patients and healthy normal subjects, on the basis of assessments of platelet dependent thrombin generation test. Good glycemic control may help to correct a hypercoagulable state in diabetic patients.[89]

45 diabetic patients and 45 matched controls were evaluated for haemostatic parameters, prothrombin time, PTT, thrombin time, coagulation factors assay II, VII, IX and plasma fibrinogen, ADP-induced platelet aggregation, protein C, alpha 2-antiplasmin, PAI and FDPs. Significantly elevated levels of PAI and alpha 2-antiplasmin together with low protein C level was seen. This could lead to prothrombotic tendency in insulin dependent diabetic patients. Non-insulin dependent diabetic patients with above-mentioned parameters, high levels of ADP-induced platelet aggregation and plasminogen activator may increase the risk of thrombotic complications.[90]

Healthy volunteers exposed to 24 hours of selective hyperinsulinaemia, selective hyperglycemia or combined, causes increases in factor VIII:C coagulant activity, plasma thrombin-antithrombin complexes, prothrombin fragment 1.2 and platelet CD40 ligand. The hyperinsulinaemia and hyperglycemia or combination of both create a pro-thrombotic state and in addition may be pro-inflammatory and pro-atherogenic because of the pro-inflammatory actions of CD40 ligand and tissue factor.[91]

A study from New Delhi, India investigated the haemostatic parameters and assessed their relationship with microvascular complications in type 2 diabetes mellitus. Coagulation and fibrinolysis parameters were measured in 60 type 2 diabetic patients (M:F 1:1) with (n=40) and without (n=20) diabetic microvascular complications and in 30 nondiabetic healthy subjects (M:F 1:1). The mean age of diabetic patients and healthy controls was 56.9±8.78 and 53.2±7.58 respectively (p=0.05). The plasma levels of PAI-1 (22.6±6.85 vs

44.8±20.8,p=0.00). However factor VIII:C activity did not differ significantly either between diabetics and health controls or between diabetics with and without complications. (97.75±5.05 vs 97.80±4.20; p=0.9).[8]

Atherosclerosis Risk in Communities Study (ARIC), a biethnic cohort of 12,330 men and women, 45–64 years of age, concluded that, factor VIII:C and other hemostasis variables are associated with the development of diabetes in middle-aged adults.[14]

Chapter 4

Methodology

METHODOLOGY

This one year case control study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

Study design

The study design was one year case control study.

Study period and duration

This study was conducted from January 2011 to December 2011.

Place

This study was conducted at Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum a teaching hospital attached to Jawaharlal Nehru Medical College, Belgaum.

Source of Data

Patients admitted under the Department of General Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum were studied as cases and those who were attending medicine OPD/ IPD for health check up aged between 40 to 70 years were enrolled as controls.

Sample size

A total of 75 patients that is, 25 non diabetic, 50 cases (25 diabetics without macrovascular complications and 25 diabetics with macrovascular complications) were selected for the study.

Sampling procedure

The sample size was calculated based on the formula as mentioned below.

$n = 4 \text{ X } p \text{ X } q / d^2$

Where p = Prevalence (50%)

q = 100 – p (100 – 50=50)

d = Absolute error 10

$n = 4 \text{ X } 50 \text{ X } 50 / 10^2$

Therefore, n = 75

Based on the above formula sample size was calculated as a total of 75 patients. Further, these patients were divided into three groups as follows:

Group A

Patients attending the medicine OPD or admitted in the medicine wards, for routine health check-up, having no specific illness and who are non-diabetic aged between 40 and 70 years.

Group B

Patients admitted to the medical wards with diabetes mellitus but with no macrovascular complications.

Group C

Patients admitted to the medical wards with diabetes mellitus with a history of past macrovascular disease such as myocardial infarction, cerebrovascular accident or peripheral vascular disease.

Sampling method

Simple random sampling was employed where every third patient who fulfils the inclusion criteria was included in the study.

Selection criteria

Inclusion Criteria

- Cases and controls aged between 40 to 70 years of age.
- Controls: Group A – Individuals with no specific illness and who are non-diabetic.
- Cases: Group B – Patients with DM but without macrovascular complications.
- Cases: Group C – Patients with DM and macrovascular complications.

Exclusion Criteria

- Patients with any acute illness.
- Diabetic patients with acute complications such as diabetic ketoacidosis.
- Pregnant women.
- Patients below 40 years of age and those above 70 years of age.
- Patients with Type 1 diabetes mellitus.

- Patients with associated collagen vascular diseases.

Ethical clearance

Prior to the commencement of the study, ethical clearance was obtained from the Ethical and Research Committee, Jawaharlal Nehru Medical College, Belgaum.

Informed Consent

All the cases and controls fulfilling selection criteria were explained about the purpose of study and a written informed consent was obtained before enrollment (Annexure I).

Method of collection of data

Demographic data such as age, sex, occupation, history regarding diabetes mellitus and complications were recorded. A thorough physical and clinical examination was conducted with special emphasis on central nervous system and cardiovascular system and the findings were recorded on a predesigned and pretested proforma (Annexure II).

Investigations

Investigations such as haemogram and lipid profile (total cholesterol, triglycerides, HDL, LDL) were done.

Based on NCEP (National Cholesterol Education Program) guidelines[92] normal values of lipid parameters were;

- Low density lipoprotein < 100 mg/dL.
- High density lipoprotein;
    - Female > 50 mg/dL.
    - Males > 40 mg/dL.
- Total Cholesterol < 200 mg/dL.
- Triglycerides < 150 mg/dL.

Factor VIII:C assay

The estimation of factor VIII:C assay was done by one stage assay of factor VIII:C method.[93] It consists of comparing the ability of dilutions of the patients plasma and of a standard plasma to correct the APTT of a plasma know to lack factor VIII:C, but containing all other factors for normal coagulation. The values between 50 to 150 were interpreted as normal.

Statistical analysis

The data obtained was coded and entered into Microsoft Excel Worksheet. The categorical data was expressed as rates, ratios and proportions. The continuous data was expressed as mean ± standard deviation (SD) and the comparison was done using ANOVA test. A probability value ('p' value) of less than or equal to 0.05 was considered as statistically significant.

Chapter 5

Results

RESULTS

The present one year case control study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. A total 50 patients and 25 age matched healthy controls were enrolled in the study.

For the analysis the patients were divided into three groups namely;

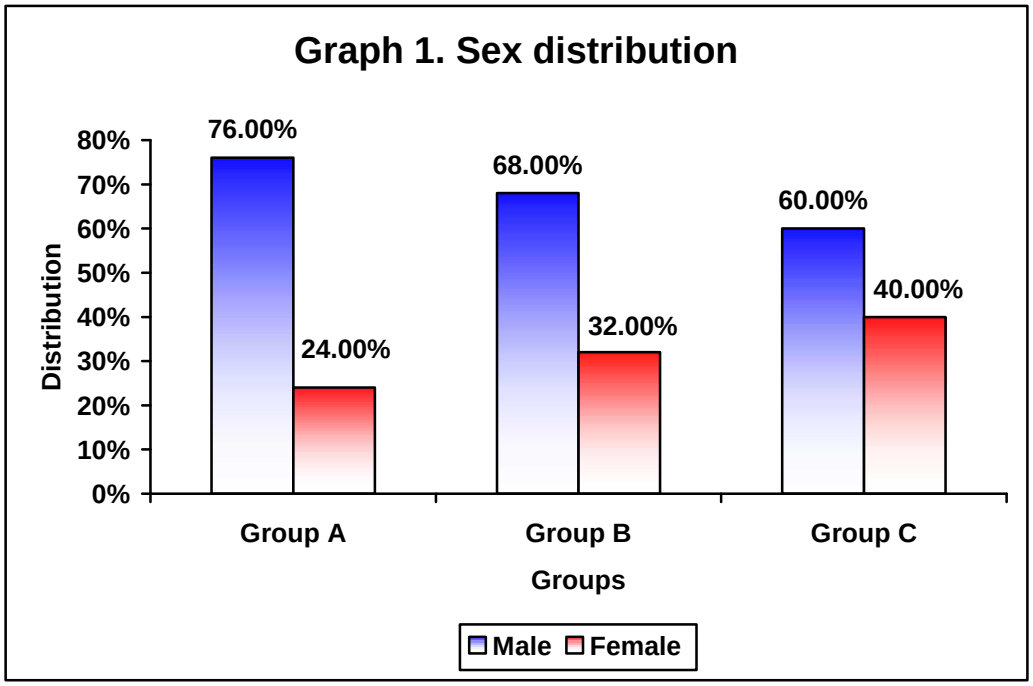
- Group A – Individuals with no specific illness and who are non-diabetic.
- Group B – Patients with DM but without macrovascular complications.
- Group C – Patients with DM and macrovascular complications.

The data obtained was tabulated into the Microsoft Excel Worksheet and analysed. The results obtained were tabulated as below.

Table 1. Sex distribution

Sex	Group A (n=25)		Group B (n=25)		Group C (n=25)	
	Number	**Percent**	**Number**	**Percent**	**Number**	**Percent**
Male	19	76.00	17	68.00	15	60.00
Female	6	24.00	8	32.00	10	40.00
Total	**25**	**100.00**	**25**	**100.00**	**25**	**100.00**

x^2 = 1.471 **DF = 2** **p = 0.479**

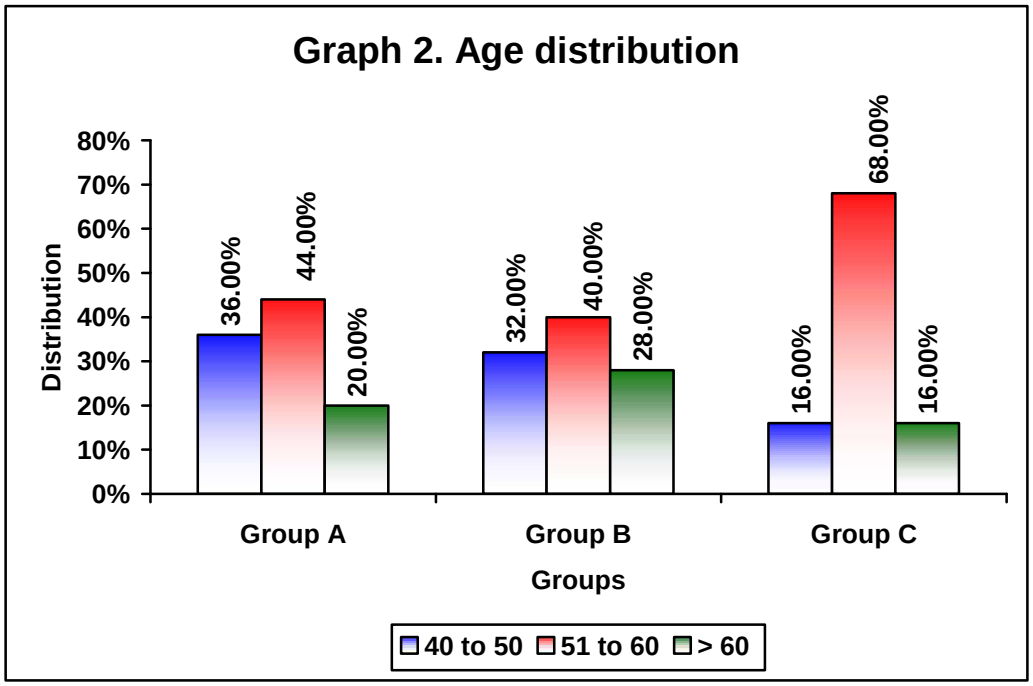


In the present study among all the three groups males out numbered females (Group A 76%; group B 68% and Group C 60%). The male to female ratio in group A, B and C was 3.16:1, 2.12:1 and 1.5:1 respectively.

Table 2. Age distribution

Age group (Years)	Group A (n=25)		Group B (n=25)		Group C (n=25)	
	Number	Percent	Number	Percent	Number	Percent
40 to 50	9	36.00	8	32.00	4	16.00
51 to 60	11	44.00	10	40.00	17	68.00
> 60	5	20.00	7	28.00	4	16.00
Total	**25**	**100.00**	**25**	**100.00**	**25**	**100.00**

x^2=5.138 DF = 4 p = 0.273



In the present study most of the patients were aged between 51 to 60 years (40% in group B and 68% in group C). Also, most of the controls in group A (44%) had age between 51 to 60 years. The age group was comparable in all the three groups.

Table 3. Mean age

Age (Years)	Group A	Group B	Group C
Mean	55.20	55.44	55.40
SD	9.15	7.74	6.44
Median	58.00	55.00	55.00
Min	40.00	43.00	41.00
Max	70.00	68.00	68.00

$F_{2,72}$=0.0007 **p=0.993**

In the present study the mean age in group A was 55.20 ± 9.15 years. Similarly in group B the mean age was 55.44 ± 7.74 years and group C it was 55.40 ± 6.44 years. The mean age of all three groups was comparable.

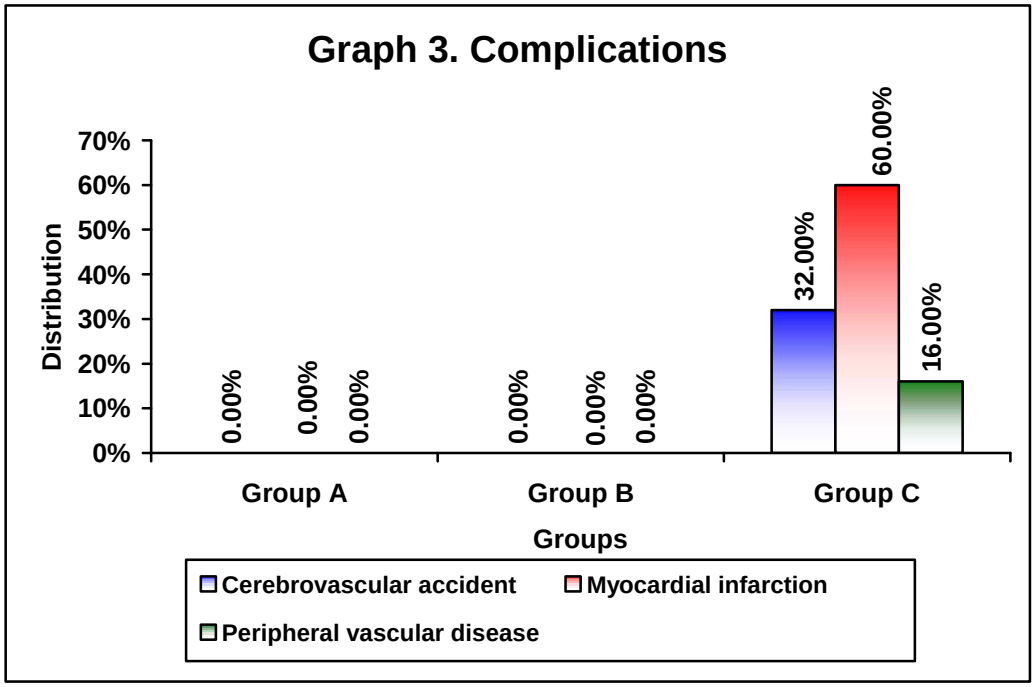
Table 4. History of hypertension

History	Group A (n=25)		Group B (n=25)		Group C (n=25)	
	Number	Percent	Number	Percent	Number	Percent
Yes	0	0.00	0	0.00	12	48.00
No	25	100.00	25	100.00	13	52.00
Total	**25**	**100.00**	**25**	**100.00**	**25**	**100.00**

In this study the history of hypertension was present 48% of patients in group C.

Table 5. Complications

Complications	Group A (n=25)		Group B (n=25)		Group C (n=25)	
	No.	%	No.	%	No.	%
Cerebrovascular accident	0	0.00	0	0.00	8	32.00
Myocardial infarction	0	0.00	0	0.00	15	60.00
Peripheral vascular disease	0	0.00	0	0.00	4	16.00
Total	**0**	**0.00**	**0**	**0.00**	**27**	**108.00**

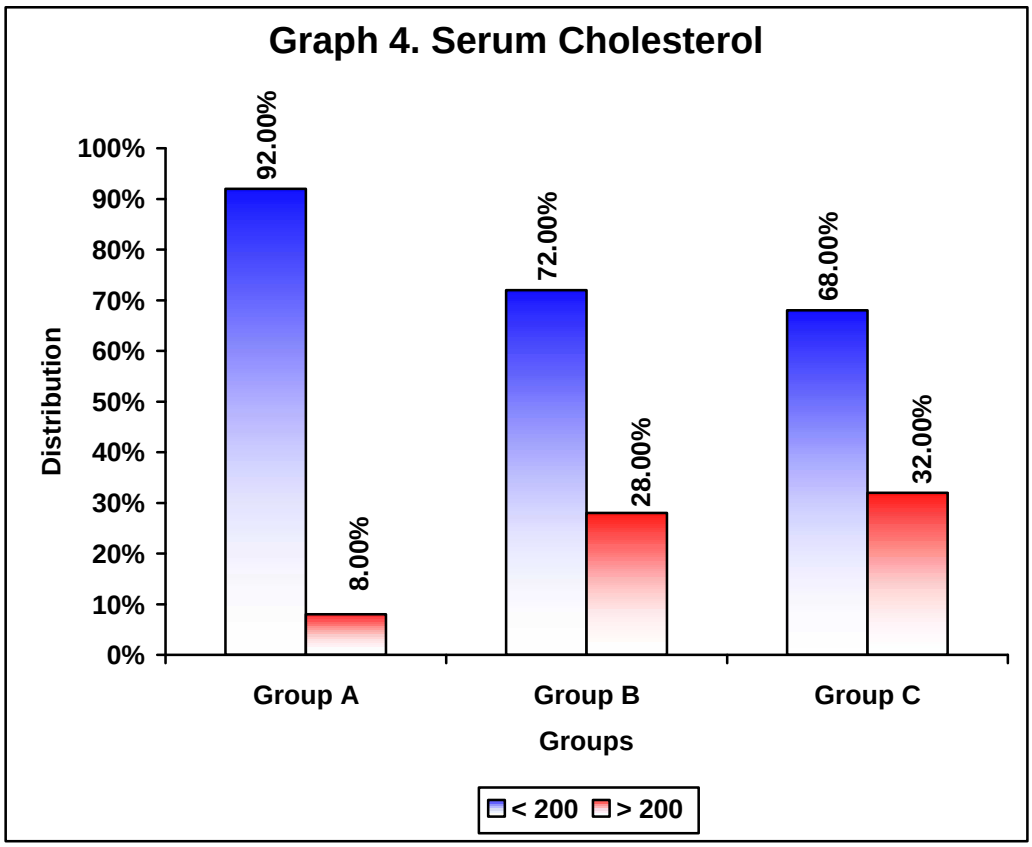


Among the patients in group C, 32% had cerebrovascular accidents, while 60% and 16% of patients had myocardial infarction and peripheral vascular disease, respectively.

Table 6. Serum Cholesterol

Serum cholesterol levels (mg/dL)	Group A (n=25)		Group B (n=25)		Group C (n=25)	
	No	**%**	**No**	**%**	**No**	**%**
< 200	23	92.00	18	72.00	17	68.00
> 200	2	8.00	7	28.00	8	32.00
Total	**25**	**100**	**25**	**100**	**25**	**100**

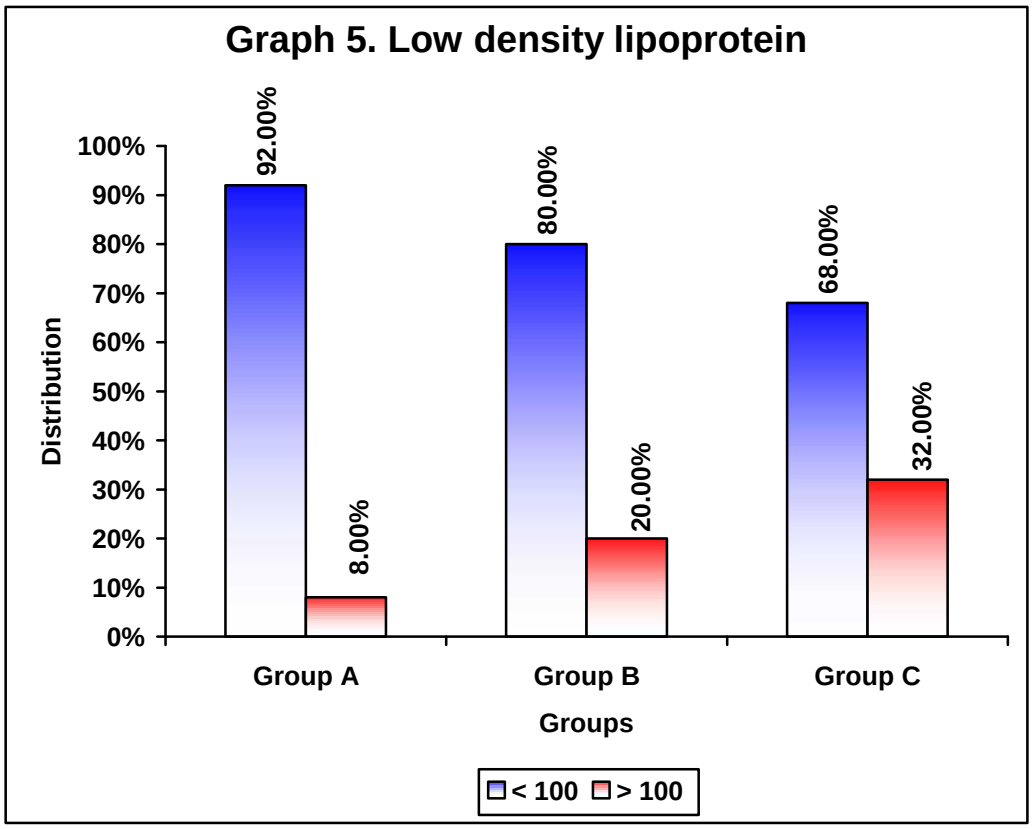
x^2=4.716 **DF=2** **p=0.095**



In the present study raised cholesterol levels were seen 28% of patients in group B and 32% of patients in group C.

Table 7. Low density lipoprotein

Low density lipoprotein (mg/dL)	Group A (n=25)		Group B (n=25)		Group C (n=25)	
	No	%	No	%	No	%
< 100	23	92.00	20	80.00	17	68.00
> 100	2	8.00	5	20.00	8	32.00
Total	**25**	**100**	**25**	**100**	**25**	**100**
x^2 = 4.500		DF = 2			p = 0.105	

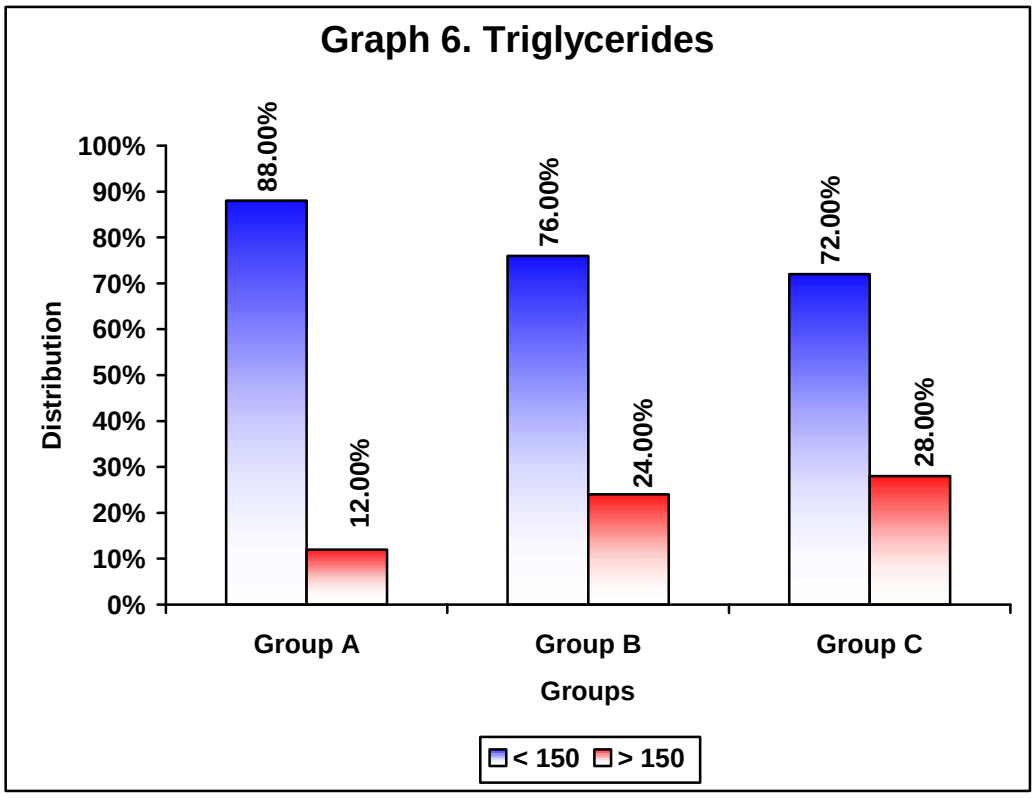


In this study among patients with group C, 32% had raised LDL levels and in group B same was noted among 20% of patients.

Table 8. Triglycerides

Triglyceride levels (mg/dL)	Group A (n=25)		Group B (n=25)		Group C (n=25)	
	No	**%**	**No**	**%**	**No**	**%**
< 150	22	88.00	19	76.00	18	72.00
≥ 150	3	12.00	6	24.00	7	28.00
Total	**25**	**100**	**25**	**100**	**25**	**100**

x^2=2.066 **DF=2** **p=0.356**

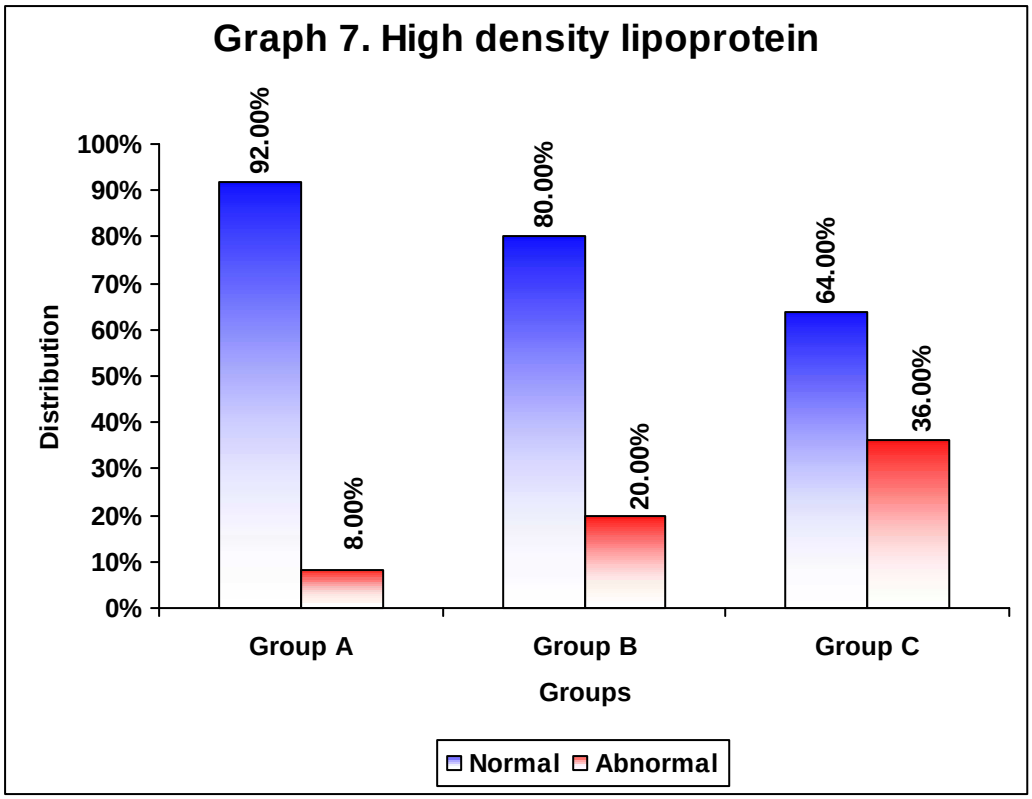


In this study among elevated triglycerides were noted among 24% and 28% of patients from group B and C respectively.

Table 9. High density lipoprotein

HDL Levels (mg/dL)	Group A (n=25)		Group B (n=25)		Group C (n=25)	
	No	**%**	**No**	**%**	**No**	**%**
Normal	23	92.00	20	80.00	16	64.00
Abnormal	2	8.00	5	20.00	9	36.00
Total	**25**	**100**	**25**	**100**	**25**	**100**

x^2 = 5.879 **DF = 2** **p = 0.053**

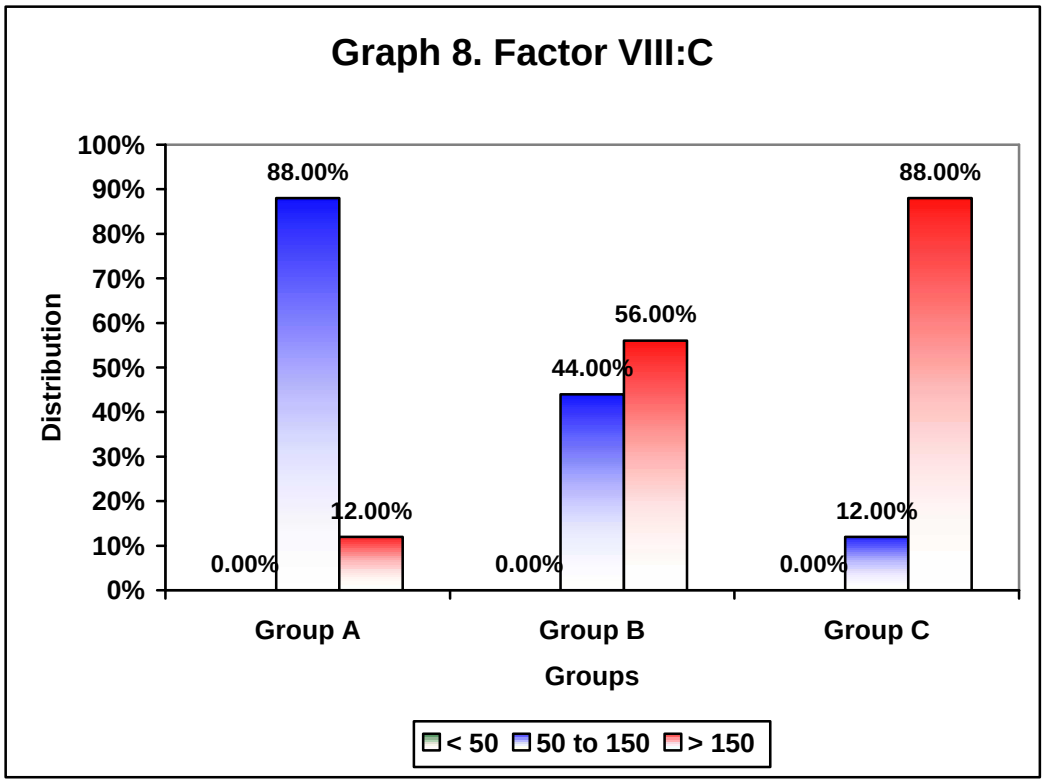


In the present study high density lipoprotein levels were low among 20% of patients in group B and 36% patients in group C.

Table 10. Factor VIII:C

Factor VIII	Group A (n=25)		Group B (n=25)		Group C (n=25)	
	Number	Percent	Number	Percent	Number	Percent
< 50	0	0.00	0	0.00	0	0.00
50 to 150	22	88.00	11	44.00	3	12.00
> 150	3	12.00	14	56.00	22	88.00
Total	**25**	**100.00**	**25**	**100.00**	**25**	**100.00**

x^2=29.167 **DF=2** **p < 0.001**



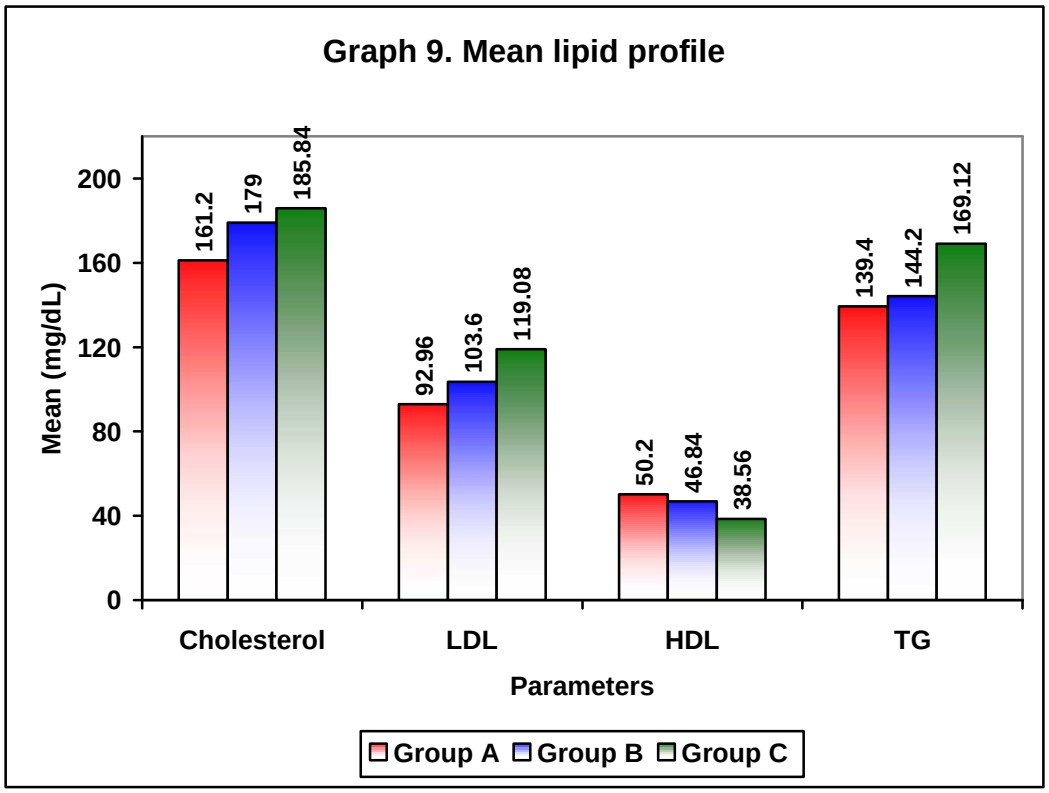
In the present study factor VIII was raised in 56% of patients in group B and 88% of patients in group C compared to 12% in group A.

Table 11. Mean lipid profile

Parameters	Group A (n=25)		Group B (n=25)		Group C (n=25)		F'	'p'
(mg/dL)	Mean	SD	Mean	SD	Mean	SD	value	value
Cholesterol	161.20	25.12	179.00	13.29	185.84	37.92	5.404	0.007
LDL	92.96	5.22	103.60	19.58	119.08	40.68	6.264	0.003
HDL	50.20	8.66	46.84	6.47	38.56	13.74	8.807	<0.001
TG	139.40	21.68	144.20	15.57	169.12	56.58	4.878	0.010

Probability value between the three groups

Parameters	'p' value		
	Group A and B	Group A and C	Group B and C
Cholesterol	0.073	0.006	1.000
LDL	0.468	0.002	0.122
HDL	0.729	<0.001	0.015



Overall, the mean cholesterol, low density lipoprotein, high density lipoprotein and triglyceride levels are as shown in table 11 and graph 10. The mean cholesterol, low density lipoprotein and triglyceride levels were significant high in group B and C compared to group A. Similarly the mean high density lipoprotein levels were significant less in group B and C compared to group A.

The comparison of mean cholesterol levels between group C and group A showed significant raised mean cholesterol levels among patients with group C (p=0.006) but no significant difference was observed when compared with group B (p=1.000). Similarly in patients with group B also no significant difference in cholesterol levels was noted when compared with group A (p=0.073).

The comparison of mean LDL levels between group C and group A showed significant rise in LDL levels in patients with group C (p=0.002) but no significant difference was observed when compared with group B (p=0.122). Similarly, in patients with group B also no significant difference in LDL levels was noted when compared with group A (p=0.468).

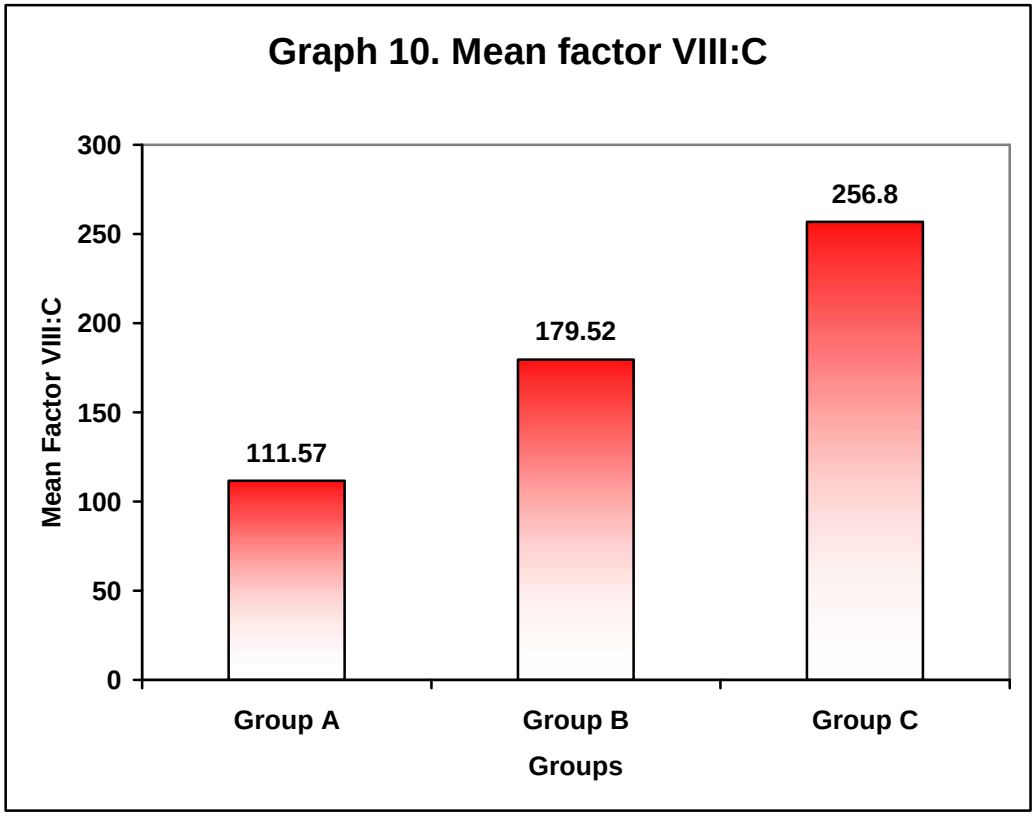
The comparison of mean HDL levels between group C and group A showed significant decline in HDL levels among patients with group C (p<0.001) and also when compared with group B (p=0.015). Whereas, in patients with group B no significant difference in HDL levels was noted when compared with group A (p=0.729).

Table 12. Mean factor VIII assay

Parameters	Group A (n=25)		Group B (n=25)		Group C (n=25)		'F'	'p'
	Mean	SD	Mean	SD	Mean	SD	value	value
Factor VIII	111.57	25.48	179.52	46.37	256.80	49.38	40.032	<0.001

Probability value between the three groups

	'p' value		
	Group A and B	Group A and C	Group B and C
Factor VIII:C	< 0.001	<0.001	0.010



In this study the mean factor VIII levels in group C were significantly high (256.80 ± 49.38) compared to group B (179.52 ± 46.37) and group A (111.57 ± 25.48).

The comparison of mean factor VIII:C levels between group C and group A showed statistically significant rise among patients with group C ($p<0.001$) and also when compared with group B ($p=0.010$). Similarly in patients with group B statistically significant rise in factor VIII:C levels was observed when compared with group A ($p=0.001$).

Chapter 6

Discussion

DISCUSSION

Diabetes mellitus is associated with an increased risk of atherosclerosis, and macrovascular complications are a major cause of morbidity and mortality in this disease.[6] Thrombosis is the cause of death in 80% of patients with diabetes.[7]

Endothelial dysfunction is the earliest event that precedes the development and progression of diabetic vascular complications.[9] The pathogenesis of endothelial dysfunction in diabetes is complex. Multiple cellular and molecular mechanisms are involved in the development of diabetic dysfunctional endothelium.[6,10]

The plasma levels of some biomarkers may be measured as indirect indices of endothelial cell damage, activation and inflammation to assess endothelial function (nitric oxide, asymmetric dimethylarginine, endothelin-1, von Willebrand factor, adhesion molecule, plasminogen activator inhibitor-1).[11-13]

The plasma levels of many clotting factors, including fibrinogen, factor VII, factor VIII:C, factor XI, factor XII, kallikrein and von Willebrand factor, are elevated in diabetes. The fibrinolytic system is relatively inhibited as a consequence of an increase in plasminogen activator inhibitor type-1 levels.[7] This procoagulant state and hypofibrinolysis contributes to macrovascular and microvascular complications of diabetes mellitus.[8,15]

A recent study[24] suggested that, raised levels of factor VIII:C are associated with risk of thromboembolism. Mortality due to ischaemic heart

disease is much lower (80% reduction) in patients with hemophilia A than in general male population which may suggest that factor VIII:C is involved in the pathogenesis of arterial thrombosis.[25] Hence from these studies[24,25] it can be inferred that, factor VIII:C causes increased thrombin generation and increased formation of fibrin and platelet aggregates. Based on these observations the present study was undertaken to determine plasma activity of factor VIII:C in diabetics, with and without macrovascular complications.

The present one year case control study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. A total 50 patients that is, 25 patients with DM (without macrovascular complications) were enrolled in group B and 25 patients with DM (with macrovascular complications) were enrolled in group C. These patients were compared with 25 age matched individuals with no specific illness and who were non-diabetic (group A).

In the present study among all the three groups males out numbered females (Group A 76%; group B 68% and Group C 60%). The male to female ratio in group A, B and C was 3.16:1, 2.12:1 and 1.5:1 respectively. Most of the patients were aged between 51 to 60 years (40% in group B and 68% in group C) and in group A 44% had age between 51 to 60 years. The mean age in group A was 55.20 ± 9.15 years. Similarly in group B the mean age was 55.44 ± 7.74 years and group C it was 55.40 ± 6.44 years suggesting that the demographic characteristics of the study population in all the three groups were comparable ($p>0.05$).

A study[8] by Madan R et al from New Delhi, India investigated the haemostatic parameters and assessed their relationship with microvascular complications in type 2 diabetes mellitus. Coagulation and fibrinolysis parameters were measured in 60 type 2 diabetic patients (M:F 1:1) with (n=40) and without (n=20) diabetic microvascular complications and in 30 nondiabetic healthy subjects (M:F 1:1). The mean age of diabetic patients and healthy controls was 56.9±8.78 and 53.2±7.58 respectively (p=0.05).

In the present study the history of hypertension was present in 48% of patients in group C. The history of cerebrovascular accident was present in 32%, myocardial infarction was noted among 60% and peripheral vascular disease in 16%.

In the present study the lipid abnormalities revealed raised cholesterol levels among 28% and 32% of patients in group B and group C respectively. The LDL levels were raised in 20% and 32% of patients in group B and group C respectively. Elevated triglycerides were noted among 24% and 28% of patients from group B and C respectively. The HDL levels were low among 20% of patients in group B and 36% patients in group C.

In this study overall, the mean cholesterol, low density lipoprotein and triglyceride levels were significantly high in group B and C compared to group A ($p<0.05$). Similarly the mean high density lipoprotein levels were significantly low in group B and C compared to group A ($p<0.001$).

The comparison of mean cholesterol levels between group C and group A showed significant raised mean cholesterol levels among patients with group C

(p=0.006) but no significant difference was observed when compared with group B (p=1.000). Similarly in patients with group B, no significant difference in cholesterol levels was noted when compared with group A (p=0.073).

The comparison of mean LDL levels between group C and group A showed significant rise in LDL levels in patients with group C (p=0.002) but no significant difference was observed when compared with group B (p=0.122). Similarly, in patients with group B, no significant difference in LDL levels was noted when compared with group A (p=0.468).

The comparison of mean HDL levels between group C and group A showed significant decline in HDL levels among patients with group C (p<0.001) and also when compared with group B (p=0.015). Whereas, in patients with group B no significant difference in HDL level was noted when compared with group A (p=0.729). These findings were in accordance with the lipid profile expected in diabetes.

The comparison of mean triglyceride levels between group C and group A showed significantly raised triglyceride levels among patients with group C and also when compared with group B (p=0.018). Whereas, in patients with group B no significant difference in triglyceride levels was noted when compared with group A (p=0.370).

A recent study[94] conducted in Allahabad, India found that serum total cholesterol, LDL cholesterol and triglycerides were significantly raised (p<0.0001) where as the level of HDL cholesterol was significantly lower (p<0.0001) in diabetic subjects as compared to controls.

Another recent study[95] conducted in Nigeria the total cholesterol, triglycerides, and low density lipoprotein of diabetics were significantly higher than those of the non-diabetics. The HDL though lower in diabetics, was not significantly different from that of non-diabetics.

Clotting factor abnormalities have been less extensively studied in the past.

The raised levels of factor VIII:C are associated with the risk of recurrent thromboembolism. The incidence of recurrent venous thromboembolism in a cohort was 10.6%. Patients with recurrence had higher mean plasma levels of factor VIII:C than those without recurrence (182 ± 66 vs 157 ± 54 IU/dL; p=0.009). the relative risk of recurrent thrombosis was 1.08 (95% CI; 1.04 to 1.12; p<0.001) for each increase of 10 IU/dL in plasma levels of factor VIII:C.[21]

The association of factor VIII:C levels with arterial thrombosis is a subject currently undergoing active research worldwide. Several case control studies have reported association of factor VIII:C with coronary heart disease.[96] After correction of the other associated risk factors this association was eliminated in the ARIC study[22] but not in the Caerphilly heart cardiovascular study[23] leaving the possibility open that factor VIII:C and vWF have an effect on cardiovascular risk. More recently, the prospective cardiovascular health study showed that, elevated factor VIII:C levels were associated with cardiovascular disease and mortality in elderly men.[97] Regarding the risk of stroke, the ARIC study showed that per SD increase in factor VIII:C and in vWF, the risk increased 1.34 fold (95% CI; 1.2 to 1.5) and 1.36 fold (95% CI; 1.2 to 1.5), respectively.[22]

The present study significantly showed higher factor VIII:C levels in diabetics and diabetics with macrovascular complications as compared to healthy controls. The factor VIII:C was raised in 56% of patients in group B and 88% of patients in group C compared to 12% in group A. This difference between group A, B and C with raised factor VIII:C was statistically significant ($p< 0.001$).

The mean factor VIII:C levels in group C were significantly high (256.80±49.38) compared to group B (179.52±46.37) and group A (111.57±25.48). The comparison of mean factor VIII:C levels between group C and group A showed statistically significant rise among patients with group C ($p<0.001$) and also when compared with group B ($p=0.010$). Similarly in patients with group B statistically significant rise in factor VIII:C levels was observed when compared with group A ($p=0.001$).

Elevated coagulation factor VIII activity is independent risk factor for thrombosis, with a greater impact on venous than arterial thrombosis.[19] A recent study[24] from Bosnia concluded that diabetic patients had the elevated plasma coagulation factor VIII activity and increased fibrinogen concentration thus an increased risk of thrombosis and vascular diseases..

Various other researchers have reported elevated factor VIII activity in diabetic patients, mostly in patients with DM type 2.[15,99] Studies[15,100] found no differences in plasma factor VIII activity between patients with DM type 2 and control. Both groups of researchers have studied coagulation and fibrinolysis parameters in type 2 diabetic patients with and without vascular complications.

Plasma levels of coagulation factors VII, factor VIII:C and fibrinogen had been found to be increased in diabetic patients, yet mechanisms leading to their high plasma levels are debatable. The mechanism by which factor VIII:C influence thrombotic risk has not yet been identified. The most factor VIII:C circulates as a complex with von Willebrand factor (vWf). The main function of factor VIII:C is to activate factor X functioning as a cofactor for activated factor IX in the presence of phospholipids and calcium. Elevated factor VIII:C induces the increase of thrombin formation and may contribute to the development of large occlusive thrombi. Factor VIII activity level above 100-150 IU/dL(100%-150%) increases 3-fold the risk of thrombosis while the level above 150 IU/dL (150%) increases the same risk 6-fold when compared with levels below 100%. Furthermore, each increase in factor VIII:C level with 10 IU/dL (10%) is associated with a 10% increase in the risk of the thrombotic event.[24]

A study[8] conducted by Madan R et al in New Delhi, India investigated the haemostatic parameters and assessed their relationship with microvascular complications in type 2 diabetes mellitus. The study reported that, factor VIII activity did not differ significantly either between diabetics and healthy controls or between diabetics with and without complications. (97.75±5.05 vs 97.80±4.20; p=0.9). However this study did not take into account macrovascular complications of diabetes.

Atherosclerosis Risk in Communities Study (ARIC), a biethnic cohort of 12,330 men and women, 45–64 years of age, concluded that, factor VIII:C and other hemostatic variables are associated with the development of diabetes in

middle-aged adults. Though the findings of this study were comparable with the present study, there were variations between the two studies regarding sample size and study design as well the other parameters.[14]

Chapter 7

Conclusion

CONCLUSION

Based on the results of the present study it may be concluded that, the plasma activity of factor VIII:C was significantly high in diabetics with and without macrovascular complications when compared with healthy age matched controls.

Also, within the diabetic subgroup, patients with macrovascular complications had significantly higher factor VIII:C levels compared to those without complications.

Chapter 8

Summary

SUMMARY

The plasma levels of many clotting factors, including fibrinogen, factor VII, factor VIII:C, factor XI, factor XII, kallikrein and von Willebrand factor, are elevated in diabetes. The present study was undertaken to determine plasma activity of factor VIII:C in diabetics, with and without macrovascular complications, and to compare with controls and to investigate the association between plasma activity of factor VIII:C and diabetic macrovascular complications.

This one year case control study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum from January 2011 to December 2011. A total 50 patients that is, 25 patients with DM but without macrovascular complications were enrolled in group B and 25 patients with DM and macrovascular complications were enrolled in group C. These patients were compared with 25 age matched individuals with no specific illness and who were non-diabetic (group A).

In the present study the male to female ratio in group A, B and C was 3.16:1, 2.12:1 and 1.5:1 respectively. Most of the patients were aged between 51 to 60 years (40% in group B and 68% in group C) and in group A 44% had age between 51 to 60 years. The mean age in group A was 55.20 ± 9.15 years. Similarly in group B the mean age was 55.44 ± 7.74 years and group C it was 55.40 ± 6.44 years suggesting that the demographic characteristics of the study population in all the three groups were comparable ($p>0.05$). The history of hypertension was present in 48% of patients in group C. The history of

cerebrovascular accident was present in 32%, myocardial infarction was present in 60% and peripheral vascular disease in 16%. The factor VIII:C was significant raised in 56% of patients in group B and 88% of patients in group C compared to 12% in group A ($p< 0.001$). The mean factor VIII:C levels in group C were significantly high (256.80±49.38) compared to group B (179.52±46.37) and group A (111.57±25.48) ($p=0.001$).

The plasma activity of factor VIII:C was significantly high in diabetics with and without macrovascular complications when compared with healthy age matched controls. Within the diabetic subgroup, patients with macrovascular complications had significantly higher factor VIII:C levels compared to those without complications.

Chapter 9

Bibliography

BIBLIOGRAPHY

1. Fauci AS, Kasper DS, Longo DL, Braunwald E, Hauser SL, Jameson JL, et al. Harrison's principles of internal medicine. United States; McGraw Hill: 2008.

2. American Diabetes Association. Clinical practice recommendations 2007. Diabetes Care 2007;30:S4.

3. One adult in ten will have diabetes by 2030. International Diabetes Federation. 2011. Available from: URL: Available at http://www.idf. org/media-events/press-releases/2011/diabetes-atlas-5th-edition Access date: 08.07.2011.

4. Ghaffar A, Reddy KS, Singhi M. Burden of non-communicable diseases in South Asia. BMJ 2004;328(7443):807-10.

5. Tripathy BB, Chandalia HB. RSSDI:Textbook of diabetes mellitus.2nd ed., New Delhi: Jaypee Brothers; 2008.

6. Pandoli A, De Filips 1. EA. Chronic hyperglycemia and nitric oxide bioavailability play a pivotal role in pro-atherogenic vascular modiications. Genes Nutr 2007;2:195-208.

7. Carr ME. Diabetes mellitus:A hypercoagulable state. J Diabetes Complications 2001;15:44-54.

8. Madan R, Gupt B, Saluja S, Kansra UC, Tripathi BK, Guliani BP. Coagulation profile in diabetes and its association with diabetic microvascular complications. J Assoc Physicians India. 2010;58:481-4.

9. De Vriese AS, Verbeuren TJ, Van De Voorde J, Lameire NH, Vanhoutte PM. Endothelial dysfunction in diabetes. Br J Pharmacol 2000;130:963-74.

10. Guerci B, Bohme P, Kearney-Schwartz A, Zannad F, Drouin P. Endothelial dysfunction and type 2 diabetes. Diabetes Metab 2001;27:436-47.

11. Hamilton SJ, Chew GT, Watts GF. Therapeutic regulation of endothelial dysfunction in type 2 diabetes mellitus. Diabetes Vasc Dis Res 2007;4:89–102

12. Thorand B, Baumert J, Chambless L, Meisinger C, Kolb H, Döring A, et al. The MONICA/ KORA Study Group. Elevated markers of endothelial dysfunction predict type 2 diabetes mellitus in middle-aged men and women from the general population. Arterioscler Thromb Vasc Biol 2006;26:398-405.

13. Babic N, Huskic J, Nakas-Icindic E, Tihic-Kapidzic S, Music M. New information about von Willebrand`s factor? Health Med 2010;4:187-94.

14. Duncan BB, Schmidt MI, Offenbacher S, Wu KK, Savage PJ, Heiss G. Factor VIII and other hemostasis variables are related to incident diabetes

in adults. The Atherosclerosis Risk in Communities (ARIC) Study. Diabetes Care. 1999;22(5):767-72.

15. Dunn EJ, Grant PJ. Type 2 diabetes: An atherothrombotic syndrome. Curr Mol Med 2005;5:323-32.

16. Koster T, Blann AD, Briet E, Vandenbroucke JP, Rosendaal FR. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. Lancet 1995;345:152-5.

17. O'Donnell J, Laffan M Elevated plasma factor VIII levels-a novel risk factor for venous thromboembolism. Clin Lab 2001;47(1-2):1-6.

18. Kraaijenhagen RA, in't Anker PS, Koopman MMW, Reitsma PH, Prins MH, Van Den Ende A, Büller HR. High plasma concentration of factor VIIIc is a major risk factor for venous thromboembolism. Thromb Haemost 2000;83:5-9.

19. Kamphuisen PW, Eikenboom CJ, Bertina RM. Elevated factor VIII levels and the risk of thrombosis. Arterioscler Thromb Vasc Biol 2001;21:731-8.

20. Mettinger KL. A study of hemostasis in ischemic cerebrovascular disease, I: Abnormalities in factor VIII and antithrombin. Thromb Res 1982;26:183-92.

21. Kyrle PA, Minar E, Hirschl M, Bialonczyk C, Stain M, Schneider B, et al. High plasma levels of factor VIII and the risk of recurrent venous thromboembolism. N Engl J Med 2000;343:457–62.

22. Folsom AR, Wu KK, Rosamond WD, Sharett AR, Chambless LE. Prospective study of haemostatic factors and incidence of coronary artery disease: The Atherosclerosis Risk in Communities (ARIC) study. Circulation 1997;96:1102-1108.

23. Rumley A, Lowe GD, Sweetnam PM, Yarnell JW, Ford RP. Factor VIII, von Willebrand factor and the risk of major ischaemic heart disease in the Caerphilly Heart Study. Br J Haematol. 1999;105(1):110-6.

24. Babić N, Dervisević A, Huskić J, Musić M. Coagulation factor VIII activity in diabetic patients. Med Glas Ljek komore Zenicko-doboj kantona. 2011;8(1):134-9.

25. Rosendaal Fr, Varekamp I, Smith C, Brocker-Vriends AH, van Dijck H, Vandenbroucke JP et al. Mortality and causes of death in Dutch haemophiliacs 1973-1986;Br J Haematol 1989;71:71-6.

26. Sattley M. The history of diabetes. 1996; Available from: URL: http://www.diabeteshealth.com/read/2009/11/23/6456/the-history-of-diabetes-/ Access date; 18.09.2010.

27. Pradeepa R. Epidemiology of diabetes in India – Current perspective and future projection. J Indian Med Assoc 2002; 100(3): 144-8.

28. Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, Winter PD. Fetal and infant growth and impaired glucose tolerance at age 64. BMJ 1991; 303(6809): 1019-22.

29. Kroneberg HM, Melmed S, Polonsky KS, Larsen PR. Williams textbook of endocrinology. 11th ed., Philadelphia Pa: Saunders, Elsevier; 2008.

30. Dokken B. The pathophysiology of cardiovascular disease and diabetes:beyond blood pressure and lipids. Diabetes Spectrum. 2008; 21(3):160-5.

31. Merz CN, Buse JB, Tuncer D, Twillman GB. Physician attitudes and practices and patient awareness of the cardiovascular complications of diabetes. J Am Coll Cardiol 2002;40:1877-81.

32. Rosenson RS. Clinical role of LDL and HDL subclasses and apolipoprotein measurement. ACC Curr J Rev 2004;33–7.

33. Chan AC. Vitamin E and atherosclerosis. J Nutr 1998;128:1593–96.

34. Lewis DH. The effect of trauma on the regulation of the microcirculation. Pathophysiol 1998;5(Suppl 1):191.

35. Smith SE, Smith SA, Brown PM. Cardiac autonomic dysfunction in patients with diabetic retinopathy. Diabetologia 1981;21:525–8.

36. Ewing DJ, Campbell D, Clarke BF. The natural history of diabetic autonomic neuropathy. Q J Med 1980;49:95–108.

37. Taskiran M, Fritz-Hansen T, Rasmussen V, Larsson HBW Hilsted J. Decreased myocardial perfusion reserve in diabetic autonomic neuropathy. Diabetes 2002;51:3306–10.

38. Koh KK, Han SH, Quon MJ. Inflammatory markers and the metabolic syndrome. J Am Coll Cardiol 2005;46:1978–85.

39. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813–20.

40. Williams SB, Goldfine AB, Timimi FK, Ting HH, Roddy MA, Simonson DC, et al. Acute hyperglycemia attenuates endothelium-dependent vasodilation in humans in vivo. Circulation 1998;97:1695–701.

41. Hayden MR, Sowers JR, Tyagi SC. The central role of vascular extracellular matrix and basement membrane remodeling in metabolic syndrome and type 2 diabetes:the matrix preloaded. Cardiovasc Diabetol 2005;4:9–29.

42. Bauer TA, Reusch JEB, Levi M, Regensteiner JG. Skeletal muscle deoxygenation after the onset of moderate exercise suggests slowed microvascular blood flow kinetics in type 2 diabetes. Diabetes Care 2007; 30:2880–85.

43. Tooke JE. Microcirculation and diabetes. British Med Bull 1989;45:206–23.

44. Weir M. Microalbuminuria and cardiovascular disease. Clin J Am Soc Nephrol 2007;2:581–90.

45. Pickup JC, Mattock MB, Chusney GD, Burt D. NIDDM as a disease of the innate immune system: Association of acute-phase reactants and interleukin-6 with metabolic syndrome X. Diabetologia 1997;40:1286–92.

46. Festa A, D'Agostino R, Howard G, Mykkänen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome. Circulation 2000;102:42–7.

47. Kim J, Koh KK, Quon MJ. The union of vascular and metabolic actions of insulin in sickness and in health. Arterioscler Thromb Vasc Biol 2005;46:1978–85.

48. Woods M, Mitchell JA, Wood EG, Barker S, Walcot NR, Rees GM, et al. Endothelin-1 is induced by cytokines in human vascular smooth muscle cells: Evidence for intracellular endothelin-converting enzyme. Mol Pharmacol 1999;55:902–9.

49. Chung KF, Barnes PJ. Cytokines in asthma. Thorax 1999;54:825–57.

50. Pickup JC, Mattock MB. Activation of the innate immune system as a predictor of cardiovascular mortality in type 2 diabetes mellitus. Diabet Med 2003;20:723–6.

51. Jarrett RJ, Shipley MJ. Type 2 (non-insulin-dependent) diabetes mellitus and cardiovascular disease: Putative association via common antecedents; Further evidence from the Whitehall Study. Diabetalogia 1988;31:737–40.

52. Trayhurn P, Wood IS. Signalling role of adipose tissue: Adipokines and inflammation in obesity. Biochem Soc Trans 2005;33:1078–81.

53. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Comm 1999;257:79–83.

54. Gu K, Cowie CC, Harris ML. Mortality in adults with and without diabetes in a national cohort of the US population, 1971–1993. Diabetes Care 1998;21:1138–45.

55. Weyrich AS, Prescott SM, Zimmerman GA. Platelets, endothelial cells, inflammatory chemokines, and restenosis: Signaling in the vascular play book. Circulation 2002;106:1433–5.

56. Evangelista V, Totani L, Rotondo S, Lorenzet R, Tognoni G, De Berardis G, et al. Prevention of cardiovascular disease in type 2 diabetes: how to improve the clinical efficacy of aspirin. Thromb Haemost 2005;93:8–16.

57. Pratt KP, Shen BW, Takeshima K, Davie EW, Fujikawa K, Stoddard BL. Structure of the C2 domain of human factor VIII at 1.5 Å resolution. Nature 1999;402:439-441.

58. Coagulation Factor VIII. 2003. Available from:URL:http://www.bio.davidson.edu/courses/molbio/molstudents/spring2003/woodw/hemoa.html Access Date:10.06.2012

59. Ratnoff OD, Bennett B. The Genetics of Hereditary Disorders of Blood Coagulation. Science. 1973;179:1291-8.

60. Holmberg L, Nilsson IM. AHF related protein in clinical praxis. Scand J Haematol 1974;12:221-31.

61. Bloom Al. The biosynthesis of factor VIII. Clin Haematol 1979;8:53-77.

62. Mathonnet F, Mazancourt PD, Denninger MH, Morot M, Benattar N, Giudicelli Y. Role of factor VIII on activated protein C resistance ratio in inflammatory diseases. Br J Haematol 1996;95:423-5.

63. Bhagat K, Moss R, Collier J, Vallance P. Endothelial "stunning" following a brief exposure to endotoxin: A mechanism to link infection and infarction? Cardiovasc Res 1996;32:822-9.

64. Wang P, Ba ZE, Chaudhry IH. Administration of tumor necrosis factor-alpha in vivo depresses endothelium-dependent relaxation. Am J Physiol 1994;266:H2535-41.

65. Egeberg O. Clotting factor levels in patients with coronary atherosclerosis. Scand J Clin Lab Invest 1962;14:253-8.

66. Cooperberg AA, Teitelbaum JI. The concentration of anti-hemophilic globulin (AHG) in patients with coronary artery disease. Ann Int Med 1961;54:899-907.

67. Bern MM, Cassani MP, Horton J, Rand L, Davis G. Changes of fibrinolysis and Factor VIII coagulant, antigen, and ristocetin co-factor in diabetes mellitus and atherosclerosis. Thrombo Res 1980;19:831-9.

68. Rak K, Beck P, Udvardy M, Pfliegler G, Misz M, Boda Z. Plasma levels of beta-thromboglobulin and Factor VIII-related antigen in diabetic children and adults. Thrombo Res 1983;29:155-62.

69. Sugrue DD, Trayner I, Thompson GR, Vere VJ, Imeson JD, Stirling Y, et al. Coronary artery disease and haemostatic variables in heterozygous familial hypercholesterolaemia. Br Heart J 1985;53:265-8.

70. Study on the Development and Function of a Monoclonal Antibody to FVIII-C2 Domain Inhibit FVIII Procoagulant Activity. Available from: URL: http://www.tumorres.com/brain-tumor/2910.htm Access Date: 12.06.12.

71. Triemstra M, Rosendaal Fr, Smith C, van der Ploeg HM, Briet E. Mortality in patients with hemophilia:Changes in a Dutch population from 1986 to 1992 and 1973 to 1986. Ann Intern Med 1995;123:823-7.

72. Conlan MG, Folsom AR, Pinch A, Davis CE, Sorlie P, Marcucci G, et al. Associations of factor VIII and von Willebrand factor with age, race, sex and risk factors for atherosclerosis: The Atherosclerosis Risk in Communities (ARIC) study. Thromb Haemost 1993;70:380-5.

73. Cushman M, Yanez D, Psaty BM, Fried LP, Heiss G, Lee M, et al. Association of fibrinogen and coagulation factors VII and VIII with

cardiovascular risk factors in the elderly: The Cardiovascular Health Study: Cardiovascular Health Investigators. Am J Epidemiol 1996;143: 665-76.

74. Kamphuisen PW, Jeroen CJ, Elkenboom, Bertina RM. Elevated factor VIII levels and the risk of thrombosis: Brief review. Arteriosclerosis, thrombosis and Vascular Biology 2001;21:731.

75. de Lange M, Snieder H, Ariens RA, Andrew T, Grant PJ, Spector TD. The relation between insulin resistance and hemostasis: Pleotropic genes and common environment. Twin Res 2003;6 (2):152-61.

76. Pan WH, Bai CH, Chen JR, Chiu HC. Associations between carotid atherosclerosis and high factor VIII activity, dyslipidaemia and hypertension. Stroke 1997;28 (1):88-94.

77. Aaron R. Folsom, Kenneth K, Wu KK, Maureen G. Conlan, Andrea Finch, Davis CE et al. Distribution of haemostatic variables in blacks and whites: Population reference values from the atherosclerosis risk in communities (ARIC) study. Ethnicity Dis 1992;2:35-46.

78. Iso H, Folsom AR, Wu KK, Finch A, Munger RG, Sato S et al. Hemostatic variables in Japanese and Caucasian men. Plasma fibrinogen, Factor VIIC, Factor VIIIC, and von Willebrand factor and their relations to cardiovascular disease risk factors. Am J Epidemiol 1989;130(5): 925-34.

79. Chan P, Pan WH. Coagulation activation in type 2 diabetes mellitus: The higher coronary risk of female diabetic patients. Diabetic Medicine 1995;12:504-7.

80. Vukowich TC, Schernthaner G, Knobl PN, Hay U. The effect of near normoglycemic control on plasma factor VIII/ von Willebrand factor and fibrin degradation products in insulin-dependent diabetic patients. J Clin Endocrinol Metab 1989;69 (1):84-9.

81. Blann A. Von Willebrand factor and the endothelium in vascular disease. Br J Biomed Sci 1993;50:125-34.

82. Pottinger BE, Read RC, Paleolog EM, Higgins PG, Pearson JD. Von Willebrand factor in an acute phase reactant in man. Thromb Res 1989;53:387-94.

83. Jager A, van Hinsberg VWM, Kostense PJ, Emeis JJ, Yudkin JS, Nijpels G, et al. von Willebrand factor, C-reactive protein, and 5-year mortality in diabetic and non-diabetic subjects. Arterioscler Thromb Vasc Biol 1999;19:3071-8.

84. Badimon L, Badimon JJ, Chesebro JH, Fuster V. von Willebrand factor and cardiovascular disease. Thromb Haemost 1993;70:111-8.

85. Nakajima S, Noguchi T, Taka T, Ueda T, Kaizu K, Fukamizu M et al. A global platelet test of thrombosis and thrombolysis detects a prothrombotic state in some patients with non-insulin dependent diabetes and in some patients with stroke. Platelets 2000;11(8):459-66.

86. El Khawand C, Lavenne, Jamart J, Donckler J, Chaolain B, Lavenne E, et al. Hemostasis variables in type I diabetic patients without demonstrable vascular complications. Diabetes care 1993;16 (8):1137-45.

87. Zeiltier P, Thiede A, Muller HL. Prospective study on plasma clotting parameters in diabetic children: No evidence for specific changes in coagulation system. Exp Clin Endocrinol Diabetes 2001;109 (3):146-50.

88. Grant PJ, Stickland MH, Davies JA, Prentice Cr. Effect of controlled hyperglycemia on Factor VIII concentrations in insulin dependent diabetes mellitus. Thromb Res 1998;50 (1):157-62.

89. Aoki I, Shimoyama K, Aoki N, Homori M, Yanagisawa A, Nakahara K et al. Platelet-dependent thrombin generation in patients with diabetes mellitus: Effects of glycemic control on coagulability in diabetes. J Am Coll Cardiol 1996;27 (3):560-6.

90. Fattah MA, Shaheen MH, Mahfour MH. Disturbances of haemostasis in diabetes mellitus. Dis Markers 2003-2004;19 (6):251-8.

91. Vaidyula VR, Rao AK, Mozzoli M, Homko C, Cheung P, Boden G. Effects of hyperglycemia and hyperinsulinaemia on circulating tissue factor procoagulant activity and platelet CD40 ligand. Diabetes 2006;55 (1):202-8.

92. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on

Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001; 285: 2486–97.

93. Dacie and Lewis. Practical haematology. Investigation of haemostasis. 9th ed., London: Churchill Livingstone; 2001.

94. Smith S, Lall AM. A Study on Lipid Profile Levels of Diabetics *and* Non-Diabetics among Naini Region of Allahabad, India. Turk J Biochem 2009;33(4):138-41.

95. Osadolor B, Olaiya MB, Amegor OF. Lipid Profiles of Diabetic and Non-Diabetic Patients Attending the University Teaching Hospital, Ado Ekiti, Nigeria. International J Natural Applied Sci 2009;5(3):271-5.

96. Stormorken H, Erikssen J. Plasma antithrombin III and factor VIII antigen in relation to angiographic findings, angina and blood groups in middle-aged men. Thromb Haemost. 1977;38(4):874-8.

97. Tracy RP, Arnold AM, Ettinger W, Fried L, Meilahn E, Savage P. The relationship of fibrinogen and factors VII and VIII to incident cardiovascular disease and death in the elderly: results from the cardiovascular health study. Arterioscler Thromb Vasc Biol. 1999;19(7):1776-83.

98. Babić N, Dervisević A, Huskić J, Musić M. Coagulation factor VIII activity in diabetic patients. Med Glas Ljek komore Zenicko-doboj kantona. 2011;8(1):134-9.

99. Coca M, Cucuianu M, Hancu N. Effect of abdominal obesity on prothrombitic tendency in type 2 diabetes. Behavior of clotting factors VII and VIII, ibrinogen, and von Willebrand factor. Rom J Intern Med 2005;43:115-26.

100. Erem C, Hacıhasanoglu A, Çelik Ş, Ovalı E, Ersöz HÖ, Ukinç K, Deger O, Telatar M. Coagulation and fibrinolysis parameters in Type 2 diabetic patients with and without diabetic vascular complications. Med Princ Pract 2005;14:22–30.

Annexures

Annexure I

ANNEXURE I – CONSENT FORM

"FACTOR VIII:C LEVELS AND MACROVASCULAR COMPLICATIONS IN DIABETES MELLITUS"

Objective and purpose of the study:

This research is intended to investigate the association between plasma activity of Factor VIII and diabetic macrovascular complications. The principal investigator of the study is Dr. **** ****** under the guidance of Dr. **** ******. My co-operation will be of great help to patients with diabetes mellitus by helping them detect if they are at risk of complications and thus in guiding to institute an aggressive therapy.

Procedure:

If you agree to be part of the research study you will be asked the relevant history and will be subjected to relevant clinical examination and investigations. You will also have to give blood and undergo ultra sonographic examination for the necessary investigations

Risk and Benefits:

The only risk and possible discomfort you might get is while taking blood from your arm for the investigations. It may cause swelling, pain, redness, bruising or infection (rarely happens) at the site from where the blood is drawn.

Alternatives

Taking part in this study is voluntary. You may choose not to take part in this study, or if you decide to take part now, you can later change your mind and withdraw from the study. Your decision will not change the present or future health care or other services that you receive. The study doctor or sponsor may you're your participation in this study any time. If you choose not to take part in the study you will receive the standard treatment for patients with your condition.

Privacy and Confidentiality

All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study may be published but your identity will be confidential in any publication.

Institution / Sponsor's policy

Does not apply to this research

Financial incentives for participation

You will not be paid / offered any gifts /incentives for participating in the study.

Authorization to publish the results

The results of the study would be forwarded to the KLE University, Belgaum as part of requirement towards the completion of MD degree, review and publishing.

If I have any questions about your rights as a participant you may call Principal and Chairman, J.N.M.C Ethical Committee for Human Research phone number **** ******.

Consent Statement

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any of my legal rights by signing this form. My signature below indicates that I have read, or it has been read to me, this entire consent form, and have had all my questions answered.

In case of the queries during study or in future you may contact following person

Principal investigator : Dr. ***** *******

Guide : Dr. ***** *******

Name of the Participant: _____

Signature / Thumb print _____

Name of the Witness _____

Signature _____

Name of the investigator _____

Signature _____

Date: Place:

Annexures

Annexure II

ANNEXURE II – PROFORMA

Case No:

NAME:

AGE/SEX:

IP No.

Significant Past Medical History.

-History and duration of diabetes mellitus.

-History of hypertension and duration.

Reason For Admission And Clinical Findings

-Cerebrovascular Accident

-Myocardial Infarction

-Peripheral Vascular Disease.

-Fundus Examination

Investigations

-Hemoglobin

- Total Leucocyte Count

-Lipid Profile

-Factor VIII Assay

-Carotid Intima Medial Thickness

Annexures

Annexure III

ANNEXURE III – KEY TO MASTER CHART

-	– Absent
+	– Present
dL	– Deci Liter
DM	– Diabetes mellitus
F	– Female
gm%	– Gram percent
HTN	– High density Lipoprotein
M	– Male
mg	– Milligram
mm	– Millimeter

MASTER CHART

Serial number	In patient number	Sex	Age (Years)	History				Clinical findings			Investigations								
				DM		HTN							Lipid profile						
				History	Duration (Years)	History	Duration (Years)	Cerebrovascular accident	Myocardial infarction	Peripheral vascular disease	Haemoglobin (gm%)	Total leucocyte count (/mm3)	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Triglycerides (mg/dL)	Factor VIII assay	Carotid intima medial thickness
1	416897	M	60	-	-	-	-	-	-	-	15	5400	##	45	90	##	154	-
2	416605	M	58	-	-	-	-	-	-	-	12.5	6400	##	46	90	##	102	-
3	416221	M	65	-	-	-	-	-	-	-	12	14500	##	48	90	##	142	-
4	416025	M	60	-	-	-	-	-	-	-	14.6	5600	##	42	96	##	130	-
5	419522	M	70	-	-	-	-	-	-	-	13	5600	##	41	##	##	104.2	-
6	413719	M	52	-	-	-	-	-	-	-	13	6800	##	56	87	##	131	-
7	413874	M	60	-	-	-	-	-	-	-	12.8	9000	##	53	92	##	111	-
8	405016	M	57	-	-	-	-	-	-	-	15.9	10800	##	49	92	##	114	-
9	404481	F	60	-	-	-	-	-	-	-	11.6	6500	##	56	91	##	79	-
10	403978	F	46	-	-	-	-	-	-	-	13	7000	##	62	86	##	155	-
11	403171	M	43	-	-	-	-	-	-	-	15	9000	##	46	96	##	122	-
12	402201	F	44	-	-	-	-	-	-	-	11.8	4200	##	53	84	##	89	-
13	349358	M	46	-	-	-	-	-	-	-	13	6500	##	53	94	##	67	-
14	409774	M	59	-	-	-	-	-	-	-	12.4	6400	##	50	90	##	102	-
15	409581	M	48	-	-	-	-	-	-	-	16	4700	##	32	96	##	106.9	-
16	409488	M	60	-	-	-	-	-	-	-	12.8	9000	##	42	94	##	111	-
17	409483	M	40	-	-	-	-	-	-	-	12.6	7800	##	49	##	##	95	-
18	407946	F	60	-	-	-	-	-	-	-	11.6	6500	##	51	95	##	79	-
19	408176	F	65	-	-	-	-	-	-	-	12.6	6100	##	29	96	##	122	-
20	404899	M	65	-	-	-	-	-	-	-	12	14500	##	61	91	##	158	-
21	405254	F	45	-	-	-	-	-	-	-	16	4700	##	59	84	##	66	-
22	405493	M	58	-	-	-	-	-	-	-	14	6300	##	63	98	##	96	-
23	418232	M	70	-	-	-	-	-	-	-	13	5600	##	59	96	##	104.2	-
24	415799	M	40	-	-	-	-	-	-	-	15	9000	##	58	98	##	122	-
25	417776	M	49	-	-	-	-	-	-	-	14	6800	##	52	90	42	127	-

MASTER CHART

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MASTER CHART

Serial number	In patient number	Sex	Age (Years)	History				Clinical findings			Investigations								
				DM		HTN							Lipid profile						
				History	Duration (Years)	History	Duration (Years)	Cerebrovascular accident	Myocardial infarction	Peripheral vascular disease	Haemoglobin (gm%)	Total leucocyte count (/mm3)	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Triglycerides (mg/dL)	Factor VIII assay	Carotid intima medial thickness
1	417218	M	68	+	-	-	-	-	-	-	12.5	8100	##	49	96	##	226	-
2	416221	M	65	+	-	-	-	-	-	-	11.8	7800	##	48	94	##	98	-
3	415830	F	62	+	-	-	-	-	-	-	14.9	12000	##	46	94	##	146	-
4	403552	M	55	+	-	-	-	-	-	-	12	15000	##	38	93	##	142	-
5	403978	F	46	+	-	-	-	-	-	-	10.6	8100	##	54	96	##	243	-
6	403171	M	43	+	-	-	-	-	-	-	13	11300	##	39	91	##	164	-
7	409844	M	50	+	-	-	-	-	-	-	12.6	10000	##	46	92	##	162	-
8	409929	M	55	+	-	-	-	-	-	-	9.3	7200	##	43	##	##	203	-
9	412143	F	64	+	-	-	-	-	-	-	14.5	11500	##	53	96	##	148	-
10	412091	F	68	+	-	-	-	-	-	-	12.4	7000	##	48	98	##	141	-
11	409468	M	52	+	-	-	-	-	-	-	15	7300	##	45	91	##	140	-
12	409929	M	55	+	-	-	-	-	-	-	12	15000	##	35	93	##	142	-
13	412143	F	48	+	-	-	-	-	-	-	10.6	8100	##	54	98	##	243	-
14	411914	M	62	+	-	-	-	-	-	-	14.9	12000	##	32	95	##	146	-
15	407746	F	60	+	-	-	-	-	-	-	11.1	8600	##	55	97	##	243	-
16	403847	M	59	+	-	-	-	-	-	-	11.4	12000	##	42	92	##	250	-
17	409468	M	52	+	-	-	-	-	-	-	12.7	13200	##	46	98	##	143	-
18	405254	F	45	+	-	-	-	-	-	-	13.5	8100	##	59	92	##	262	-
19	402505	M	55	+	-	-	-	-	-	-	9.9	13700	##	49	97	##	220	-
20	402311	M	58	+	-	-	-	-	-	-	11	12600	##	49	94	##	141	-
21	414600	F	58	+	-	-	-	-	-	-	12.7	13200	##	56	96	##	193	-
22	349358	M	66	+	-	-	-	-	-	-	13.1	11200	##	48	##	##	225	-
23	2106934	M	43	+	-	-	-	-	-	-	13	11300	##	46	##	##	164	-
24	419647	M	49	+	-	-	-	-	-	-	11	12600	##	45	##	##	141	-
25	420205	M	48	+	-	-	-	-	-	-	12.6	10000	##	46	##	##	162	-

MASTER CHART

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MASTER CHART

Serial number	In patient number	Sex	Age (Years)	History				Clinical findings			Investigations								
				DM		HTN							Lipid profile						
				History	Duration (Years)	History	Duration (Years)	Cerebrovascular accident	Myocardial infarction	Peripheral vascular disease	Haemoglobin (gm%)	Total leucocyte count (/mm3)	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Triglycerides (mg/dL)	Factor VIII assay	Carotid intima medial thickness
1	413196	F	59	+	20	+	20	-	+	-	13.6	7800	##	19	93	##	196	-
2	413786	M	54	+	10	-	-	-	+	-	16.1	13900	##	42	84	##	194	-
3	414534	M	58	+	6	+	6	-	+	-	9.1	9100	##	31	89	##	198	-
4	414583	F	58	+	8	+	-	-	+	-	14.6	8600	##	54	98	##	175	-
5	411810	M	55	+	20	+	2	-	+	-	11.9	10700	##	42	98	##	184	-
6	349358	F	54	+	-	-	-	-	-	+	11.6	6300	##	52	97	##	281	-
7	415343	F	50	+	-	-	-	-	+	-	13.4	22500	##	51	91	##	274	-
8	403836	F	65	+	-	-	-	-	-	+	13.5	10100	##	50	90	##	183	-
9	439682	M	55	+	-	-	-	-	+	-	14.4	11700	##	43	92	##	248	-
10	461382	M	54	+	-	-	-	-	+	-	11.8	13500	##	42	##	##	241	-
11	473358	M	60	+	-	-	-	-	-	-	13.5	11400	##	30	92	##	208	-
12	403376	F	45	+	-	+	-	-	+	-	11	11400	##	56	##	##	169	-
13	472926	M	54	+	-	-	-	-	+	-	8.2	7100	##	48	93	##	205	-
14		M	45	+	-	-	-	-	+	-	12.1	10400	##	16	98	##	205	-
15	403454	M	60	+	-	-	-	-	+	-	12.9	10300	##	45	##	##	288	-
16	473572	M	41	+	-	+	-	+	+	-	16	16000	##	23	86	##	220	1.2
17	457381	M	52	+	-	+	-	+	-	-	12.4	6500	##	41	##	##	147	0.8
18	464840	M	52	+	-	-	-	+	-	-	11.4	9500	##	48	96	##	142	0.9
19	417396	F	52	+	-	+	-	+	-	-	12.7	10200	##	21	##	##	235	1.1
20	412163	M	56	+	-	+	-	+	+	-	13.9	12000	##	47	97	##	143	0.9
21	406436	F	65	+	-	+	-	+	-	-	13	14200	##	53	##	##	184	1.0
22	408806	M	57	+	-	-	-	+	-	-	14.2	14600	##	15	##	##	210	1.1
23	414279	F	52	+	3	-	-	-	+	-	14.7	8200	##	23	80	##	176	1
24	442716	M	64	+	-	+	-	+	-	+	12.9	10300	##	19	##	##	198	0.9
25	417935	F	68	+	-	+	-	-	-	+	11.6	7600	##	53	92	##	226	1

MASTER CHART

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MASTER CHART - GROUP A

Serial number	In patient number	Sex	Age (Years)	History		Clinical findings			Investigations						
											Lipid profile				
				Diabetes mellitus	Hypertension	Cerebrovascular accident	Myocardial infarction	Peripheral vascular disease	Haemoglobin (gm%)	Total leucocyte count (/mm3)	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Triglycerides (mg/dL)	Factor VIII assay
1	416897	M	60	-	-	-	-	-	15	5400	##	45	90	##	154
2	416605	M	58	-	-	-	-	-	12.5	6400	##	46	90	##	102
3	416221	M	65	-	-	-	-	-	12	14500	##	48	90	##	142
4	416025	M	60	-	-	-	-	-	14.6	5600	##	42	96	##	130
5	419522	M	70	-	-	-	-	-	13	5600	##	41	##	##	104.2
6	413719	M	52	-	-	-	-	-	13	6800	##	56	87	##	131
7	413874	M	60	-	-	-	-	-	12.8	9000	##	53	92	##	111
8	405016	M	57	-	-	-	-	-	15.9	10800	##	49	92	##	114
9	404481	F	60	-	-	-	-	-	11.6	6500	##	56	91	##	79
10	403978	F	46	-	-	-	-	-	13	7000	##	62	86	##	155
11	403171	M	43	-	-	-	-	-	15	9000	##	46	96	##	122
12	402201	F	44	-	-	-	-	-	11.8	4200	##	53	84	##	89
13	349358	M	46	-	-	-	-	-	13	6500	##	53	94	##	67
14	409774	M	59	-	-	-	-	-	12.4	6400	##	50	90	##	102
15	409581	M	48	-	-	-	-	-	16	4700	##	32	96	##	106.9
16	409488	M	60	-	-	-	-	-	12.8	9000	##	42	94	##	111
17	409483	M	40	-	-	-	-	-	12.6	7800	##	49	##	##	95
18	407946	F	60	-	-	-	-	-	11.6	6500	##	51	95	##	79
19	408176	F	65	-	-	-	-	-	12.6	6100	##	29	96	##	122
20	404899	M	65	-	-	-	-	-	12	14500	##	61	91	##	158
21	405254	F	45	-	-	-	-	-	16	4700	##	59	84	##	66
22	405493	M	58	-	-	-	-	-	14	6300	##	63	98	##	96
23	418232	M	70	-	-	-	-	-	13	5600	##	59	96	##	104.2
24	415799	M	40	-	-	-	-	-	15	9000	##	58	98	##	122
25	417776	M	49	-	-	-	-	-	14	6800	##	52	90	42	127

MASTER CHART - GROUP B

Serial number	In patient number	Sex	Age (Years)	History		Clinical findings			Investigations						
											Lipid profile				
				Diabetes mellitus	Hypertension	Cerebrovascular accident	Myocardial infarction	Peripheral vascular disease	Haemoglobin (gm%)	Total leucocyte count (/mm3)	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Triglycerides (mg/dL)	Factor VIII assay
1	417218	M	68	+	-	-	-	-	12.5	8100	##	49	96	##	226
2	416221	M	65	+	-	-	-	-	11.8	7800	##	48	94	##	98
3	415830	F	62	+	-	-	-	-	14.9	12000	##	46	94	##	146
4	403552	M	55	+	-	-	-	-	12	15000	##	38	93	##	142
5	403978	F	46	+	-	-	-	-	10.6	8100	##	54	96	##	243
6	403171	M	43	+	-	-	-	-	13	11300	##	39	91	##	164
7	409844	M	50	+	-	-	-	-	12.6	10000	##	46	92	##	162
8	409929	M	55	+	-	-	-	-	9.3	7200	##	43	##	##	203
9	412143	F	64	+	-	-	-	-	14.5	11500	##	53	96	##	148
10	412091	F	68	+	-	-	-	-	12.4	7000	##	48	98	##	141
11	409468	M	52	+	-	-	-	-	15	7300	##	45	91	##	140
12	409929	M	55	+	-	-	-	-	12	15000	##	35	93	##	142
13	412143	F	48	+	-	-	-	-	10.6	8100	##	54	98	##	243
14	411914	M	62	+	-	-	-	-	14.9	12000	##	32	95	##	146
15	407746	F	60	+	-	-	-	-	11.1	8600	##	55	97	##	243
16	403847	M	59	+	-	-	-	-	11.4	12000	##	42	92	##	250
17	409468	M	52	+	-	-	-	-	12.7	13200	##	46	98	##	143
18	405254	F	45	+	-	-	-	-	13.5	8100	##	59	92	##	262
19	402505	M	55	+	-	-	-	-	9.9	13700	##	49	97	##	220
20	402311	M	58	+	-	-	-	-	11	12600	##	49	94	##	141
21	414600	F	58	+	-	-	-	-	12.7	13200	##	56	96	##	193
22	349358	M	66	+	-	-	-	-	13.1	11200	##	48	##	##	225
23	2106934	M	43	+	-	-	-	-	13	11300	##	46	##	##	164
24	419647	M	49	+	-	-	-	-	11	12600	##	45	##	##	141
25	420205	M	48	+	-	-	-	-	12.6	10000	##	46	##	##	162

MASTER CHART - GROUP C

Serial number	In patient number	Sex	Age (Years)	History		Clinical findings			Investigations						
											Lipid profile				
				Diabetes mellitus	Hypertension	Cerebrovascular accident	Myocardial infarction	Peripheral vascular disease	Haemoglobin (gm%)	Total leucocyte count (/mm3)	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Triglycerides (mg/dL)	Factor VIII assay
1	413196	F	59	+	+	-	+	-	13.6	7800	##	19	93	##	296
2	413786	M	54	+	-	-	+	-	16.1	13900	##	42	84	##	294
3	414534	M	58	+	+	-	+	-	9.1	9100	##	31	89	##	298
4	414583	F	58	+	+	-	+	-	14.6	8600	##	54	98	##	275
5	411810	M	55	+	+	-	+	-	11.9	10700	##	42	98	##	184
6	349358	F	54	+	-	-	-	+	11.6	6300	##	52	97	##	291
7	415343	F	50	+	-	-	+	-	13.4	22500	##	51	91	##	274
8	403836	F	65	+	-	-	-	+	13.5	10100	##	50	90	##	283
9	439682	M	55	+	-	-	+	-	14.4	11700	##	43	92	##	248
10	461382	M	54	+	-	-	+	-	11.8	13500	##	42	##	##	241
11	473358	M	60	+	-	-	-	-	13.5	11400	##	30	92	##	298
12	403376	F	45	+	+	-	+	-	11	11400	##	56	##	##	269
13	472926	M	54	+	-	-	+	-	8.2	7100	##	48	93	##	275
14	462827	M	45	+	-	-	+	-	12.1	10400	##	16	98	##	255
15	403454	M	60	+	-	-	+	-	12.9	10300	##	45	##	##	288
16	473572	M	41	+	+	+	+	-	16	16000	##	23	86	##	280
17	457381	M	52	+	+	+	-	-	12.4	6500	##	41	##	##	147
18	464840	M	52	+	-	+	-	-	11.4	9500	##	48	96	##	142
19	417396	F	52	+	+	+	-	-	12.7	10200	##	21	##	##	235
20	412163	M	56	+	+	+	+	-	13.9	12000	##	47	97	##	143
21	406436	F	65	+	+	+	-	-	13	14200	##	53	##	##	284
22	408806	M	57	+	-	+	-	-	14.2	14600	##	15	##	##	280
23	414279	F	52	+	-	-	+	-	14.7	8200	##	23	80	##	276
24	442716	M	64	+	+	+	-	+	12.9	10300	##	19	##	##	298
25	417935	F	68	+	+	-	-	+	11.6	7600	##	53	92	##	266