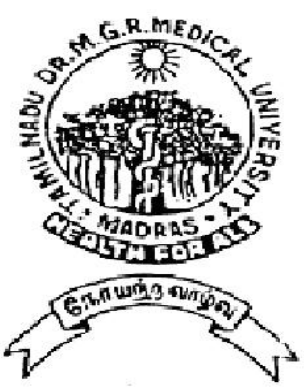
POST PRANDIAL HYPERTRIGLYCERIDEMIA AS A RISK FACTOR FOR MACROVASCULAR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS

Dissertation Submitted for

MD Degree (Branch I) General Medicine
March 2009



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CERTIFICATE

This is to certify that this dissertation titled "**Post Prandial Hypertriglyceridemia as a Risk Factor for Macrovascular Complications in Type 2 Diabetes Mellitus**" submitted by **DR.G.RANGANATHAN** to the faculty of General Medicine, The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the requirement for the award of MD degree branch I General Medicine, is a bonafide research work carried out by him under our direct supervision and guidance.

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I**, Dr.G.Ranganathan,** solemnly declare that the dissertation titled "**Post Prandial Hypertriglyceridemia as a Risk Factor for Macrovascular Complications in Type 2 Diabetes Mellitus"** has been prepared by me. This is submitted to **The Tamil Nadu Dr. M.G.R. Medical University, Chennai,** in partial fulfillment of the regulations for the award of MD degree (branch I) General Medicine.

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ACKNOWLEDGEMENT

At the outset, I thank our Dean **Dr. S.M.SHIVAKUMAR, M.S.,** for permitting me to use the facilities of Madurai Medical College and Government Rajaji Hospital to conduct this study.

I wish to express my respect and sincere gratitude to my beloved teacher and Head of the Department of Medicine, **PROF.A.AYYAPPAN, M.D.,** for his valuable guidance and encouragement throughout the study and also during my post graduate course. I owe my sincere thanks to him.

I also owe my sincere thanks to my beloved unit chief and my guide **PROF. S.VADIVELMURUGAN, M.D.,** for his guidance and support throughout the conduct of the study and also during my post graduate course.

I express my special thanks to the Professor and Head, Department of Diabetology, **PROF.A.J.ASIRVATHAM, M.D., D.Diab**, for his valuable guidance.

I sincerely thank the Professor and Head, Department of Cardiology, **PROF.S.PALANICHAMY,M.D.,D.M,** for his valuable support.

I am greatly indebted to my beloved teachers, Dr**. M.Kamaraj M.D., Dr. Daniel. K. Moses M.D., Dr.D.D.Venkatraman M.D., Dr.M.Muthiah, M.D., Dr. V.T.Premkumar M.D.**

I also wish to express my respect and sincere gratitude to my beloved Professor **Dr. P.Thirumalaikolundusubramanian M.D., Dr.Nalini Ganesh M.D., Dr. P.Selvaraj M.D.** I owe them a lot and sincerely thank them.

I am extremely thankful to my unit Assistant Professors **Dr.A.Senthamarai,M.D., Dr.Sheela Ganesh,M.D., Dr.R.Sundaram,M.D.**

My sincere thanks to my former Assistant Professors **Dr.R.Balajinathan,M.D., Dr.David Pradeep Kumar,M.D., Dr.Dharmaraj M.D., Dr. Senthil, M.D., and Dr.Ramakrishnan, M.D.,** for their constant encouragement, timely help and critical suggestions throughout the study and also for making my stay in the unit both informative and pleasurable.

I profusely thank the Biochemistry, Diabetology and Cardiology Departments for their cooperation and support.

I extend my thanks to my family and friends have stood by me during my times of need. Their help and support have been invaluable to the study.

Finally, I thank all the patients, who form the most integral part of the work, were always kind and cooperative. I pray for their speedy recovery and place this study as a tribute to them.

CONTENTS

Abbreviations

ADP – Adenosine Dinucleotide Phosphate

ATP – Adenosine Trinucleotide Phosphate

BG – Blood Glucose

BMI – Body Mass Index

CAD- Coronary Artery Disease

CVD – Cardiovascular Disease

CVA- Cerebro Vascular Accident

DM- Diabetes Mellitus

DNA – Deoxyribonucleic acid

FPG – Fasting Plasma glucose

HDL- High Density Lipoprotein

LDL- Low Density Lipoprotein

Lp(a)- Lipoprotein(a)

NAD – Nicotinamide Adenine Dinucleotide

NCEP- National Cholesterol Education Programme

PPG – Post Prandial Glucose

TAG - Triacylglycerol

TC- Total Cholesterol

TRL – Triglyceride rich lipoprotein

VLDL- Very Low Density Lipoprotein

vWF – Von Willebrand Factor

INTRODUCTION

India is frequently referred to as the diabetic capital of the world. Diabetes mellitus is widely prevalent in our country and its incidence is rising in alarming proportions. The worldwide prevalence of diabetes 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, >360 million individuals worldwide will have diabetes by the year 2030. Although the prevalence of both type 1 and type 2 diabetes is increasing worldwide, the prevalence of type 2 diabetes is rising much more rapidly because of increasing obesity and reduced activity levels as countries become more industrialized. Worldwide estimates project that in 2030 the greatest number of individuals with diabetes will be 45–64 years of age .

According to the Diabetes Atlas published by the International Diabetes Federation (IDF), there are an estimated 40 million persons with diabetes in India in 2007 and this number is predicted to rise to almost 70 million people in 2025 by which time every fifth diabetic subject in the world would be an Indian. Diabetes is a major cause of mortality, but several studies indicate that diabetes is likely underreported as a cause of death. A recent estimate suggested that diabetes was the fifth leading cause of death worldwide and was responsible for almost 3 million deaths annually (1.7–5.2% of deaths worldwide).

The real burden of the disease is however due to its micro and macro vascular complications which lead to increased morbidity and mortality. Atherosclerotic vascular diseases, particularly Coronary artery diseases (CAD), are leading causes of morbidity and mortality amongst diabetics. Interestingly apart from the known risk factors for CAD i.e., smoking, hypertension and obesity, current knowledge suggests the possible role of hypertriglyceridemia as an important risk factor for atherogenesis in diabetics. The 'response- to- injury' hypothesis of atherosclerosis states that the initial damage affects the arterial endothelium, leading to endothelial dysfunction. It has been hypothesized that hypertriglyceridemia induces endothelial dysfunction through the production of oxidative stress. This process may involve the over generation of superoxide anion, which in turn inactivates nitric oxide (NO). There is need for a simple investigation which can detect endothelial dysfunction at a much earlier date. Recently, much attention has been paid to the evidence that postprandial hypertriglyceridemia is an important contributing factor for the development of atherosclerosis in diabetes and trials are underway to determine if targeting triglycerides can halt the progress of atherosclerosis in diabetes.

REVIEW OF LITERATURE

DIABETES MELLITUS

Diabetes mellitus (DM) refers to a group of metabolic disorders which have a common denominator namely hyperglycemia. Factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production. 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.[1]

Classification

DM is classified on the basis of the pathogenic process that leads to hyperglycemia. The two broad categories of DM are designated type 1 and type 2.

- 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. Type 2 DM is preceded by a period of abnormal glucose homeostasis classified as impaired fasting glucose (IFG) or impaired glucose tolerance (IGT).

ETIOLOGIC CLASSIFICATION OF DIABETES MELLITUS

I. Type 1 diabetes (β-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 beta cell function characterized by mutations in

1. Hepatocyte nuclear transcription factor (HNF) 4 (MODY 1)
2. Glucokinase (MODY 2)
3. HNF-1 (MODY 3)
4. Insulin promoter factor-1 (IPF-1; MODY 4)
5. HNF-1 (MODY 5)
6. NeuroD1 (MODY 6)
7. Mitochondrial DNA
8. Subunits of 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, fibrocalculous pancreatopathy, mutations in carboxyl ester lipase.

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, protease inhibitors, clozapine

F. Infections—congenital rubella, cytomegalovirus, coxsackie

G. Uncommon forms of immune-mediated diabetes—"stiff-person" 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)

Glucose intolerance may develop during pregnancy. Insulin resistance is related to the metabolic changes of late pregnancy and the increased insulin requirements may lead to IGT. GDM occurs in ~4% of pregnancies in the United States; most women revert to normal glucose

tolerance post-partum but have a substantial risk (30–60%) of developing DM later in life. [1]

Diagnosis of diabetes mellitus

The National Diabetes Data Group and World Health Organization (WHO) have issued certain diagnostic criteria:

- Symptoms of diabetes plus random blood glucose concentration 11.1 mmol/L (200 mg/dL)[a] (or)
- Fasting plasma glucose 7.0 mmol/L (126 mg/dL)[b] (or)
- Two-hour plasma glucose 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 75gms anhydrous glucose dissolved in water; not recommended for routine clinical use.

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

(1) FPG < 5.6mmol/L (100 mg/dL) is considered normal

(2) FPG = 5.6–6.9 mmol/L (100–125 mg/dL) is defined as IFG

(3) FPG ≥ 7.0 mmol/L (126 mg/dL) warrants the diagnosis of DM.

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

Some individuals have both IFG and IGT. Individuals with IFG and/or IGT, recently designated 'pre-diabetes' by the American Diabetes Association (ADA), are at substantial risk for developing type 2 DM (25–40% risk over the next 5 years) and have an increased risk of cardiovascular disease.[2]

Risk Factors for Type 2 Diabetes Mellitus

1. Family history of diabetes (i.e., parent or sibling with type 2 diabetes)
2. Obesity (BMI >25 kg/m^2)
3. Habitual physical inactivity
4. Race/ethnicity (e.g., African American, Latino, Native American, Asian American, Pacific Islander)
5. Previously identified IFG or IGT
6. History of GDM or delivery of baby >4 kg (>9 lb)
7. Hypertension (blood pressure >140/90 mmHg)
8. HDL cholesterol level <35 mg/dL (0.90 mmol/L) and/or a triglyceride level >250 mg/dL (2.82 mmol/L)
9. Polycystic ovary syndrome or acanthosis nigricans
10. History of vascular disease[1]

Pathophysiology

Type 2 DM is characterized by impaired insulin secretion, insulin resistance, excessive hepatic glucose production, and abnormal fat metabolism. Obesity, particularly visceral or central (as evidenced by the

hip-waist ratio), 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.[3]

Abnormal Muscle and Fat Metabolism in diabetes

Insulin resistance, the decreased ability of insulin to act effectively on target tissues (especially muscle, liver, and fat), is a prominent feature of type 2 DM and results from a combination of genetic susceptibility and obesity. Insulin resistance is relative, however, since supernormal levels of circulating insulin will normalize the plasma glucose. Insulin resistance impairs glucose utilization by insulin-sensitive tissues and increases hepatic glucose output; both effects contribute to the hyperglycemia. Increased hepatic glucose output predominantly accounts for increased FPG levels, whereas decreased peripheral glucose usage results in postprandial hyperglycemia. In skeletal muscle, there is a greater impairment in non oxidative glucose usage (glycogen formation) than in oxidative glucose metabolism through glycolysis. Glucose

metabolism in insulin-independent tissues is not altered in type 2 DM. Hyperglycemia has been quoted to be the central process in the pathogenesis of the complications of diabetes.

Increased Hepatic Glucose and Lipid Production

In type 2 DM, insulin resistance in the liver reflects the failure of hyperinsulinemia to suppress gluconeogenesis, which results in fasting hyperglycemia and decreased glycogen storage by the liver in the postprandial state. Increased hepatic glucose production occurs early in the course of diabetes, though likely after the onset of insulin secretory abnormalities and insulin resistance in skeletal muscle. As a result of insulin resistance in adipose tissue and obesity, free fatty acid (FFA) flux from adipocytes is increased; leading to increased lipid synthesis, especially very low density lipoprotein (VLDL) and triglyceride (TGL) in hepatocytes. This lipid storage or steatosis in the liver may lead to non alcoholic fatty liver disease and abnormal liver function tests. This is also responsible for the dyslipidemia found in type 2 DM namely elevated TGL, reduced HDL, and increased small dense LDL (low density lipoprotein) particles. Both hyperglycemia and hyperlipidemia are important consequences of the disease that can trigger several inflammatory processes to produce larger damage at a later date.[1]

Diabetic Dyslipidemia

Individuals with DM may have several forms of dyslipidemia. The most common pattern of dyslipidemia is hypertriglyceridemia and reduced HDL cholesterol levels. DM itself does not increase levels of LDL, but the small dense LDL particles found in type 2 DM are more atherogenic because they are more easily glycated and susceptible to oxidation. All forms of dyslipidemia are more common in type 2 DM than in type 1 DM.[2] Recent studies indicate that hyperlipidemia could have an additive effect with hyperglycemia in diabetes and its complications.

Post prandial metabolic dysregulation

Two important processes involved in the development of atherosclerosis, the underlying cause of cardiovascular disease, are inflammation and coagulation activation. Although the exact mechanisms underlying atherogenesis are still not completely understood, vascular endothelial dysfunction is generally believed to be the starting point.

Under normal conditions, the multiple functional characteristics of the endothelium, including regulation of the vascular tone, thrombogenesis, vascular wall permeability, and cell growth, collectively protect the vascular system. Adequate production of nitric oxide has a pivotal role in the majority of these processes. Since endothelial functions cannot be measured directly, several indirect methods have been

developed to estimate these functions. However, only recently, the excessive and prolonged metabolic disturbances occurring in the postprandial state in type 2 diabetic subjects have regained interest as potential cardiovascular disease risk factors. The so-called traditional risk factors cannot fully explain the excessive cardiovascular disease risk of type 2 diabetic patients. Numerous studies indicate that postprandial metabolic derangements, most notably hyperglycemia and hypertriglyceridemia, which are exaggerated and prolonged in type 2 diabetes, are important cardiovascular disease risk factors since they induce oxidative stress and endothelial dysfunctions.

More than two thirds of patients with CAD have abnormal glucose metabolism. Most of them have impaired glucose tolerance in spite of a normal fasting glucose level. Postprandial glucose levels have been shown to have continuous, linear and direct relationship with cardiovascular and total mortality.[4,5] Starting at only 80 mg/dl postprandial or post-challenge glycaemia, the cardiovascular risk begins to increase linearly and enhances by 58% as it reaches to 140 mg/dl - the point at which patients are traditionally classified as glucose intolerant or pre-diabetic.[6] Coutinho et al showed a significant relationship between hyperglycemia and cardiovascular events.[7]

Postprandial hyperglycemia leads to hyperlipemia, manifested as elevated levels of TGL, chylomicrons, and remnant lipoproteins. These

elevated and protracted post meal lipid levels are common manifestations of insulin resistance and the metabolic syndrome. Already over 200 years ago, William Heberden made the first observation regarding a postprandial effect on the circulation of blood.[8] In the year 1979, Zilversmit postulated that atherosclerosis is a postprandial phenomenon. He proposed that postprandial accumulation of TRLs resulted from a reduction in the rate of clearance of TGL rich dietary remnant particles at the endothelial surface and promoted the development of endothelial dysfunction.[9] Ever since, a large body of evidence has accumulated indicating a relation between postprandial dysmetabolism, especially hyperglycaemia and hypertriglyceridemia, and the risk of cardiovascular disease.

POSTPRANDIAL HYPERGLYCAEMIA

Recent studies demonstrate that hyperglycemia induces an overproduction of superoxide by the mitochondrial electron-transport chain. Superoxide overproduction is accompanied by increased NO generation, due to endothelial NO synthase (eNOS) and inducible NO synthase (iNOS) uncoupled state, a phenomenon favouring the formation of the strong oxidant peroxynitrite, which in turn damages DNA. DNA damage is an obligatory stimulus for the activation of the nuclear enzyme poly (ADP-ribose) polymerase. Poly(ADP-ribose) polymerase activation in turn depletes the intracellular concentration of its substrate NAD^+, slowing the rate of glycolysis, electron transport, and ATP formation and produces an ADP ribosylation of the GAPDH (glyceraldehyde-3-phosphate dehydrogenase). These processes result in acute endothelial dysfunction in diabetic blood vessels that, convincingly, contributes to the development of CAD[10].

Several epidemiological studies have shown an association between two hour glucose concentrations after a 75 g glucose load (2h PPG) and the occurrence of cardiovascular disease in the general population. Thalhammer et al have shown that chronic hyperglycaemia has been associated with impaired endothelial function.[11]

Ceriello and co-workers have shown that postprandial hyperglycaemia is accompanied by several alterations of the coagulation

system.[12,13] Gresele et al showed that an oral glucose load in both healthy and type 2 diabetes patients caused a shortening of the half life of fibrinogen and an increase in plasma fibrinopeptide A and the fragments of prothrombin and factor VII.[14] In addition, acute, short term hyperglycaemia resulted in a transient hyper-reactivity of platelets to high shear stress, combined with a significant rise of plasma vWF in patients with type 2 diabetes. Taken together, these findings suggest that hyperglycaemia may induce a hypercoagulable state.

In a study done by Esposito et al in 2002, healthy subjects and those with impaired glucose tolerance were administered consecutive pulses of intravenous glucose. They found increased circulating cytokine concentrations (interleukin-6 and tumour necrosis factor-α) to a greater extent than during similar blood glucose levels which were kept stable during a hyperglycaemic clamp. This effect was more pronounced in subjects with impaired glucose tolerance.[15] The same study showed changes in interleukin-6 (but not tumour necrosis factor-α) plasma concentrations in type 2 diabetic patients after a carbohydrate meal.[16] Thus, blood glucose excursions may induce a proinflammatory response.

Shizukuda et al have demonstrated the cytotoxic effects of hyperglycemia in their study done in the year 2002.[17] Several other in vitro studies demonstrated cytotoxic effects of high glucose levels in various cell types. Of interest is the demonstration by Risso and

colleagues that intermittent high glucose levels induced more apoptosis than constant corresponding glucose levels in human umbilical vein endothelial cells.[18]

Four main molecular mechanisms underlying the hyperglycaemia-induced vascular damage have recently been reviewed, all of which are the result of intracellular hyperglycaemia.[10] These include-

1. Increased polyol pathway influx;
2. Increased advanced glycation end-product formation;
3. Activation of protein kinase C isoforms;
4. Increased hexosamine pathway flux.

These seemingly different mechanisms are the result of a common single pathway, that is, overproduction of superoxide by the mitochondrial electron transport chain. This hyperglycaemia-induced oxidative stress ultimately results in modification of intracellular proteins resulting in an altered function, DNA damage, activation of the transcription factor nuclear factor-κB, causing abnormal changes in gene expression, decreased production of nitric oxide, and increased expression of cytokines, growth factors and procoagulant and proinflammatory molecules.[10] Taken together, post load or postprandial glucose levels are associated with enhanced risk of cardiovascular disease.

Most epidemiological studies addressing the contribution of post load glucose levels to cardiovascular disease risk, especially the early

ones, did not take into account the earlier mentioned classical risk factors, such as dyslipidaemia. These data indicate that post load glucose may not be an independent cardiovascular disease risk factor but rather a risk marker, suggestive of underlying other metabolic disturbances, such as insulin resistance and dyslipidaemia that may have an even greater impact on cardiovascular disease risk.

POSTPRANDIAL HYPERTRIGLYCERAEMIA

The role of elevated triglyceride (TGL) levels in the pathogenesis of atherosclerotic cardiovascular disease has remained a controversial issue. TGL, the major lipids in chylomicrons, and very-low-density lipoprotein (VLDL) particles, are closely related to the metabolism of other lipoproteins, including high-density lipoprotein (HDL) particles.

Increased serum TGL levels are associated with at least four pathogenic conditions: decreased serum HDL cholesterol levels, increased remnant lipoproteins, increased small dense low-density lipoprotein (LDL), and increased thrombogenesis, all of which are believed to expedite atherosclerosis. Even though considerable evidence supports the view that elevated TGL level is an independent risk factor for coronary artery disease (CAD), adjustment for covariates frequently weakens or eliminates the predictive significance of TGL.

TABLE 1: Hypertriglyceridemia (according to the NECP adult treatment panel III)

TGL Level	TGL Category
<150 mg/dl	Normal TGL
150–199 mg/dl	Borderline TGL
200–499 mg/dl	High TGL
≥500 mg/dl	Very high TGL

TGL : triglyceride

In the insulin-resistant state the production of VLDL by the liver is inappropriately high. Together with a reduced lipoprotein lipase activity this results in high triglyceride concentrations, especially in the postprandial state. The large amount of TRLs (triglyceride-rich lipoproteins) and their prolonged residence time in the circulation may lead to increased exchange of the core lipid cholesteryl ester for triglycerides between TRL, LDL and HDL particles mediated by cholesteryl ester transfer protein. This process enriches LDL and HDL with triglyceride, and these particles are subsequently more readily hydrolysed by hepatic lipase resulting in smaller, denser LDL particles and lower concentrations of HDL.[2]

These abnormalities may explain the characteristic diabetic dyslipidaemia, which is now recognised to be very atherogenic.[19] Already in 1959, an association between plasma triglyceride concentrations and incident coronary heart disease was reported by Albrink et al.[20]

Vogel et al demonstrated impaired endothelial function in healthy subjects without risk factors for cardiovascular disease when subjected to a single high fat meal.[21] This suggests that a fat challenge may induce several abnormalities independent of the underlying disorder.

Murphy et al showed that doses of 20 g fat were capable of eliciting an insulin mediated release of lipoprotein lipase, an enzyme that catalyzes plasma TAG clearance. Thus there is impaired postprandial clearance of TAG in diabetic and prediabetic individuals either due to insulin resistance or decreased insulin secretion.[22]

A recently performed meta analysis including data of 57,000 subjects from 17 studies demonstrated that fasting triglyceride concentrations were an independent risk factor for cardiovascular disease, also when adjusted for HDL cholesterol.[23] An increase in plasma triglyceride by 1 mmol/l was associated with a relative risk of 1.3 for men and 1.8 for women.

In general practice, serum lipid concentrations including triglycerides are measured in the morning after an overnight fast. However, the fasting value should be considered the nadir of the 24 hour TGL profile and could therefore be misleadingly low. In the past few years several clinical studies have suggested that high postprandial TRL may be related to coronary heart and/or carotid artery disease in non diabetic and diabetic subjects.[24, 25, 26]

The Physician Health study, including 14,916 men aged 40–84 years, with a follow up of seven years, showed that the non-fasting triglyceride concentrations strongly predicted incident myocardial infarctions, with a relative risk of 1.40 (95% confidence interval 1.10 to 1.77) per 1.13 mmol/l increase. This study suggests that random or postprandial triglyceride concentrations are an important indicator of cardiovascular disease risk. [25]

Although fasting triglycerides are the most important determinant of postprandial triglycerides, it may be argued that in insulin resistant subjects with a delayed postprandial TRL clearance, non fasting TGL should be used to approximate overall TGL exposure. [27]

In male patients after a myocardial infarction, Karpe and co-workers found that the progression of coronary lesions over five years was related to the postprandial plasma levels of small chylomicron remnants. [28,29]

In line with these findings are coronary angiography data described by Mero et al, which suggest that especially small chylomicron remnants are implicated in the progression of CAD.[30]

Postprandial coagulation activation by TRL was demonstrated by several investigators, however the underlying mechanism(s) are not fully understood.[31,32] A study performed by Silveira et al suggests an important role for the intrinsic coagulation pathway, based on in vivo activation of

factor XI by triglyceride.[31] Other prothrombotic changes occurring with an oral fat load are increased plasmin activator inhibitor-1 (PAI-1) activity and PAI-1 antigen.[33] Postprandial lipaemia enhanced platelet P-selectin expression without affecting other markers of platelet activation.

Madhu et al studied the association between postprandial lipemia and endothelial dysfunction in type 2 DM and the association was found to be significant irrespective of the fasting triglyceride levels. This was independent of glycemic control and insulin sensitivity but was related to the interaction of diabetic state and obesity.[34, 35]

The effect of a high fat meal (50 g of fat) on cytokine concentrations, reflecting the inflammatory state, was studied by Nappo et al in healthy and type 2 diabetic patients. In healthy subjects, significant correlations were found between postprandial triglyceride and tumour necrosis factor-α levels, whereas in diabetic patients also a positive correlation between postprandial plasma triglyceride and interleukin-6 concentrations was observed. Antioxidant supplementation lowered the rise of the cytokines, suggesting that the cytokine response to triglycerides was mediated by oxidative stress.[16]

Ryu et al showed that postprandial triglyceride levels are also directly related to angiographic progression of coronary and carotid atherosclerosis.[36] MARS study showed that lowering levels of elevated

triglycerides by 20% to 40% reduces CAD rates by approximately 30% to 40%.[37]

This concept has also been proven in many diet related studies which suggest that targeting postprandial triglyceride may, in fact reduce the cardiovascular risk in these patients.[38,39] The amount and type of carbohydrate consumed in a meal is a major determinant of the post-prandial glucose surge. Diets with high glycemic index and low in fibre increase the risk of both cardiovascular disease and type 2 diabetes. Dietary fibre is effective at delaying gastric emptying, slowing digestion, and reducing post-prandial excursions of both glucose and fat. Minimally processed plant-based foods are natural sources of soluble and insoluble fibre that improve post-prandial dysmetabolism, reduce oxidant stress and inflammation, and lower the risks of CAD and diabetes. Consumption of nuts decreases meal-induced oxidative protein damage because they lower post-prandial oxidative stress and additionally provide antioxidants.[40] Epidemiologic studies consistently indicate that consumption of nuts at least five times per week reduces CAD and diabetes risks by 30%. Protein of high biological quality such as egg whites, fish, skinless poultry breast meat, and whey protein (or other non fat dairy protein) when eaten with meals dampen down postprandial inflammation and can help prevent obesity.

To summarise, historically, in diabetic patients, most emphasis was laid on hyperglycaemia, whereas recent evidence demonstrates the importance of dyslipidemia, in particular hypertriglyceridemia, as a cardiovascular disease risk factor. Much importance has been given to increased LDL levels in diabetes, which is assumed to be the most important pathogenetic factor in the development of macro vascular events and aggressive LDL lowering strategies with statins form an integral part of treatment protocols in diabetes. Although at present, epidemiological and long term intervention studies are largely lacking, in vivo data convincingly show an association between postprandial TRL and indicators of cardiovascular disease. Similar to postprandial hyperglycaemia, both in vivo and in vitro studies indicate that (postprandial) increases in triglycerides are proinflammatory, prothrombotic, and adversely affect several endothelial functions, by inducing oxidative stress. Therefore, it is feasible that prolonged postprandial hypertriglyceridemia leads to an atherogenic environment in vivo. However, as for postprandial hyperglycemia the evidence for postprandial hypertriglyceridemia as independent in cardiovascular disease is still scanty.

Although the beneficial effect of therapy targeting postprandial dysmetabolism still needs to be established, studies assessing the true atherogenic exposure of the vascular system in high risk patients should

perhaps abandon the classical glucose centred view and use physiological tests combining glucose and lipid loads. Most studies mentioned earlier demonstrated the effects of postprandial dysmetabolism on a single and rather artificial challenge, like a liquid 75 g glucose or liquid fat load. In daily life, most meals consumed are mixed and of solid consistence.

Ceriello and co-workers showed a cumulative adverse effect of postprandial hypertriglyceridemia and hyperglycaemia on endothelial function.[41] The effect of a single component challenge possibly underestimates the real life postprandial dysmetabolic state and therefore the use of standardised mixed meal containing of at least 75 g of carbohydrates and 50 g of fat in future postprandial (intervention) studies is recommended.[42]

AIMS AND OBJECTIVES

The aims of the study were-

1. To assess the value of postprandial hypertriglyceridemia as a marker of macro vascular events in type 2 diabetes mellitus.

2. To compare postprandial with fasting triglyceride levels and standard lipid ratios in predicting macro vascular complications.

MATERIALS AND METHODS

THE STUDY GROUP

The study was conducted on patients attending the out patient department of Government Rajaji Hospital, Madurai. Approval from the hospital ethical committee was obtained.

STUDY DESIGN

The study was a case control study conducted for a period of one year between July 2007- June 2008

Inclusion criteria

- Patients with new onset diabetes mellitus were included in the study.
- Twenty two healthy controls without diabetes or its complications were also included in the study for comparison.

Exclusion criteria

- Patients on lipid lowering agents.

Diagnosis of Type 2 diabetes mellitus was made by clinical details and routine blood investigations including fasting and postprandial blood sugar values. The WHO criteria were employed for the diagnosis of diabetes mellitus.[2]

The presence of absence of macro vascular complications was made on the basis of the following:

1. Clinical features suggestive of macro vascular events like CAD, stroke, hypertension and peripheral vascular disease.
2. Electrocardiogram
3. Echocardiogram.

METHODS

After the diagnosis of Type 2 diabetes mellitus and its complications, patients were divided into 3 groups based on the presence or absence of complications as follows-

Group I: Controls

Group II: Type 2 DM cases without Macro vascular Complications

Group III: Type 2 DM cases with Macro vascular Complications

The study groups were also divided into three categories based on the body mass index as follows[1]-

Obesity: BMI >30kg/m^2

Overweight: BMI >25.0-29.9kg/m^2

Normal weight: BMI 18.5-24.9kg/m^2

Underweight: BMI <18.5kg/m^2

BMI=Weight (in Kg)/ Height (in meter^2)

All the selected patients were subjected to a high fat meal which consisted of whipped cream (containing 75 grams of fat, five grams of

carbohydrate and 6 grams of protein per square meter of body surface area). For lipid analysis, blood samples were collected after 8 hours of fasting, two hours and four hours of postprandial state after giving high fat meal. Serum was separated and stored in the refrigerator. From the serum, total Cholesterol, HDL and Triglycerides were estimated separately by using ENZYMATIC COLORIMETRIC METHOD.

From the above values, LDL-C was estimated by using **FRIEDEWALD formula**

LDL-C= Total Cholesterol - (HDL+TGL/5)

The enzymatic method was used to estimate serum cholesterol values. Due to the specificity of enzymes, the enzymatic method is the most accurate and reference method for the estimation of Cholesterol.

Dyslipidemia was defined by one or more of the following-

1. Total Cholesterol (TC) >200mg/dl
2. HDL Cholesterol <35mg/dl
3. LDL Cholesterol >100mg/dl
4. Triglycerides >150mg/dl

The standard lipid ratios (TC/HDL, LDL/HDL) were also calculated.

The information collected regarding all the selected cases were recorded in a Master Chart. Data analysis was done with the help of computer using **Epidemiological Information Package (EPI 2002).**

Using this software, range, frequencies, percentages, means, standard deviations, chi square and ‘p’ values were calculated. Kruskul Wallis chi-square test was used to test the significance of difference between quantitative variables. A 'p' value less than 0.05 is taken to denote significant relationship.

RESULTS AND STATISTICAL ANALYSIS

EPIDEMIOLOGY

Majority of the patients were from in and around Madurai city. The total number of patients included in the study was 68. Twenty two controls were also included in the study for comparative analysis.

Among the total of 68 Type 2 diabetes mellitus patients, **44 diabetic patients [Female (F)-18; Male (M)-26]** had no evidence of macro vascular complications (**Group-II**), whereas **24 diabetic patients (F-14; M-10)** had evidence of macro vascular complications (**Group-III**).

Out of the 22 controls (group I), 10 were female and 12 were male. They had no evidence of diabetes or its complications after clinical and laboratory evaluation.

The age of the controls ranged from 36 to 60 years with a mean age of 51.2$\pm$7.9 years. The age of the patients in group II ranged from 32-62 years with a mean of 50.4$\pm$9.4 years, while that of group III ranged from 45-67 years with a mean of 56.4$\pm$6.6 years. Twenty two patients in group II (50%) and 11 patients in group III (45.8%) were in the age group of 51-60 years. The age distribution of the patients is shown in table 2.

TABLE 2: AGE DISTRIBUTION

Age group	Group I		Group II		Group III	
	No.	%	No.	%	No.	%
Up to 40 yrs	3	13.6	7	15.9	-	-
41-50	7	31.8	12	27.3	7	29.2
51-60	12	54.3	22	50	11	45.8
Above 60 yrs	-	-	3	6.8	6	25
Total	22	100	44	100	24	100
Range	**36-60**		**32-62**		**45-67**	
Mean	**51.2**		**50.4**		**56.4**	
S.D.	**7.9**		**9.4**		**6.6**	

The age groups of the cases and controls were comparable and there was no statistical difference (p=0.0788).

TABLE 3: SEX DISTRIBUTION

	Group I		Group II		Group III	
	No.	**%**	**No.**	**%**	**No.**	**%**
Males	12	54.5	26	59.1	10	41.7
Females	10	45.5	18	40.9	14	58.3
Total	22	100	44	100	24	100

It was observed that the female: male ratio was almost equal in groups I (1:1.2), a slightly higher ratio in group III (1.4:1) and lower in group II (1:1.4).

Patients and controls were classified as overweight, normal weight and underweight according to the body mass index. Out of 22 controls, 14 (63.6%) were in the normal weight patients as compared to 3 (13.6%) in the overweight patients. In group II, 31 out of 44 (70.5%) were in the normal weight patients as compared to 10 (22.7%) in the overweight patients. In group III, 15 out of 24 (62.5%) were normal weight patients compared to 6(25%) overweight patients. The results are shown in table 4.

TABLE 4: BODY MASS INDEX

BMI	**Group I**		**Group II**		**Group III**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Underweight (<20)	5	22.7	3	6.8	3	12.5
Normal weight (20-25)	14	63.6	31	70.5	15	62.5
Overweight (>25)	3	13.6	10	22.7	6	25
Range	16.9-29		19.4		18.8-30.4	
Mean	22.6		24		23.7	
S.D.	3.1		3		3.1	
'p'	**0.6126** **Not Significant**					

The BMI of the three groups was comparable and there was no statistical difference (p=0.6126).

BIOCHEMICAL ANALYSIS

Analysis of lipid profile was done in relation to the three study groups. The results are summarised in table 5.

TABLE 5: LIPID PROFILE

Parameter	Group I		Group II		Group III	
	No.	**%**	**No.**	**%**	**No.**	**%**
Total Cholesterol						
Normal (< 200)	18	81.8	19	43.2	10	41.7
High (> 200)	4	18.2	25	56.8	4	58.3
Range	150-265		150-280		160-270	
Mean	188.6		208.2		212.5	
S.D.	27.7		31.8		31	
'p'	**0.0202 Significant**					

The prevalence of hypercholesterolemia was more in group II and III than controls (57.5% in diabetics compared to 18.2% in controls) and the difference was statistically significant (p=0.0202). There was no significant difference in HDL or LDL values between the three groups.

Fasting (0 hr) and postprandial blood sugar values (2 and 4 hours) were analyzed in the three groups. The mean FPG in group I was 81.1±12.7 mg/dl compared to 128.5±32.6 mg/dl in group III. On the other hand mean 2 hour PPG values in group I were 125.3±8.5 mg/dl as opposed to 202.6±45.1 mg/dl in group III. The results are summarised in table 6.

TABLE 6: BLOOD SUGAR VALUES AT 0, 2, 4 hrs

Blood Sugar at	**Group I**		**Group II**		**Group III**		
	No.	**%**	**No.**	**%**	**No**	**%.**	
0 hour							
Normal	22	100	16	36.4	5	20.8	
High	-	-	28	63.6	19	79.2	
2 hours							
Normal	22	100	7	15.9	1	4.2	
High	-	-	37	84.1	23	95.8	
4 hours							
Normal	22	100	6	13.6	2	8.3	
High	-	-	38	86.4	22	91.9	
	Mean	**S.D.**	**Mean**	**S.D**	**Mean**	**S.D.**	**'p'**
0 hour	81.1	12.7	115.5	29.4	128.5	32.6	**0.0001 Significant**
2 hours	125.3	8.5	173.8	30.7	202.6	45.1	**0.0001 Significant**
4 hours	86.1	17.4	148.9	43	170.6	39.3	**0.0001 Significant**

All the three values were more in the groups II and III. The difference between the three groups was statistically significant (p=0.0001).

Fasting TGL levels (0 hr) and post load TGL levels (2 and 4 hours) were analysed. The mean fasting TGL values were 121±19.8mg/dl in group I, 156±63.1mg/dl in group II, 184±68.7mg/dl in group III. The mean 4-hrs post load TGL values were 131.5±29.4mg/dl in group I, 217±96.1mg/dl in group II, 264±101.7mg/dl in group III. TGL values remained persistently elevated in 65.9% (n=29) of patients in group II and 83.3% (n=20) of patients in group III compared to only 18.2% (n=4) of patients in group I.

TABLE 7: TGL levels at 0, 2 and 4 hours

	Group I		**Group II**		**Group III**		
TGL at	**No.**	**%**	**No.**	**%**	**No**	**%.**	
0 hour							
Normal	22	100	30	68.2	12	50	
High	-	-	14	31.8	12	50	
2 hours							
Normal	18	81.8	15	34.1	4	16.7	
high	4	18.2	29	65.9	20	83.3	
4 hours							
Normal	18	81.8	15	34.1	4	16.7	
high	4	18.2	29	65.9	20	83.3	
	Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**'p'**
0 hour	121	19.8	156.4	63.1	184.8	68.7	**0.0027 Significant**
2 hours	140.4	19.8	209.7	86.3	258.2	87.4	**0.0001 Significant**
4 hours	131.5	29.4	217	96.1	264.5	101.7	**0.0001 Significant**

The difference was statistically significant, indicating that postprandial hypertriglyceridemia (p=0.0027), may be more important in macro vascular complications than fasting TGL (p=0.0001).

Table 8 shows the baseline characteristics of the three groups. Age, BMI, LDL, and HDL cholesterol values were comparable.

TABLE 8: BASELINE CHARACTERISTICS OF PATIENTS

	GROUP I (n=22)	**GROUP II (n=44)**	**GROUP III (n=24)**	**'P' value**
Age(yrs)	51.2±7.9	50.4±9.4	56.4±6.6	0.0788(NS)
Male/Female(%)	54.5/45.5	59.1/40.9	41.7/58.3	-
BMI(kg/m^2)	22.6±3.1	24±3	23.7±3.	0.6126(NS)
FPG (mg/dl)	81.1±12.7	115.5±29.4	128.5±32.6	**0.0001(S)**
FTGL(mg/dl)	121±19.8	156.4±63.1	184.8±68.7	**0.0027(S)**
LDL(mg/dl)	112.3±30.3	122.6±33.6	126.8±27.7	0.1311(NS)
HDL(mg/dl)	44.8±8.1	47±10.7	45.2±10.8	0.5372(NS)
TC(mg/dl)	188.6±27.7	208.2±31.8	212.5±31	**0.0202(S)**

Values are mean±S.D unless specified. FTGL=fasting triglyceride

NS=not significant; S=significant

Hypertriglyceridemia at four hours after fat meal was compared between the three groups. There was significant difference in values between **groups I and III (p=0.0001), groups I and II (p=0.0006), groups I, II and III (p=0.0001).** Table 9 shows the comparative analysis of hypertriglyceridemia in relation to various groups.

TABLE 9: HYPERTRIGLYCERIDEMIA IN VARIOUS GROUPS

Hypertriglyceridemia	Group I(22)		Group II(44)		Group III(24)	
	No.	**%**	**No.**	**%**	**No.**	**%**
Present (TGL >150)	4	18.2	29	65.9	20	83.3
Absent (TGL <150)	18	81.8	15	34.1	4	16.7
TGL at 4 hours						
Range	62-192		88-392		80-398	
Mean	131.5		217		264	
S.D.	29.4		96.1		101.7	
'p' value for						
1. Group I & II	**0.0006 Significant**					
2. Group I & III	**0.0001 Significant**					
3. Group II & III	0.2122 Not Significant					
4. Groups I,II&III	**0.0001 Significant**					

These results indicate that persistent hypertriglyceridemia at four hours post load was seen more in patients with macro vascular complications.

Mean cholesterol values were higher in the patients with hypertriglyceridemia (215±33.2mg/dl) than in patients without hypertriglyceridemia (189.1±21.7mg/dl).

TABLE 10: HYPERTRIGLYCERIDEMIA AND TOTAL CHOLESTEROL

	Total Cholesterol						'p'
	Normal		Abnormal		Mean	S.D.	
	No	%	No	%			
Present (TGL>150)(53)	18	34	35	66	215.4	33.2	**0.0002 Significant**
Absent (TGL<150)(37)	29	78.4	8	21.6	189.1	21.7	

High cholesterol values correlated significantly with high TGL values (p=0.0002).

Correlation between 4-hr post load hypertriglyceridemia and blood sugar was analyzed. Patients with hypertriglyceridemia had higher mean glucose values (2-hr PPG 188.2±40.7mg/dl, 4-hr PPG 160±42.6mg/dl) than those who did not have hypertriglyceridemia (2-hr PPG 143±28mg/dl, 4-hr PPG 109±40.5mg.dl). The results are summarized in table 11.

TABLE 11: HYPERTRIGLYCERIDEMIA AND BLOOD SUGAR

Hypertriglyceridemia	**Fasting B.S.**		**2 hours B.S.**		**4 hours B.S.**	
	Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Present (TGL >150)	120.5	34.1	188.2	40.7	160.4	42.6
Absent (TGL <150)	96.4	23.5	143	28	109.1	40.5
'p'	**0.0004 Significant**		**0.0001 Significant**		**0.0001 Significant**	

It was found that hypertriglyceridemia correlated significantly with both fasting and postprandial blood sugar values. The 4-hr PPG correlated better with hypertriglyceridemia (p=0.0001) than fasting blood sugar (p=0.0004). From these results, it is seen that 4-hr post prandial hyperglycemia and hypertriglyceridemia correlated significantly in the presence of macro vascular complications.

The 4-hr post load hypertriglyceridemia was analyzed in relation to gender. Although the prevalence of hypertriglyceridemia was almost equal among males (49.1%) and females (50.9%), the mean TGL values were higher in females (234.9±107.1) than in males (185.9±84.8). The results are summarized in table 12.

TABLE 12: HYPERTRIGLYCERIDEMIA AND GENDER

Hypertriglyceridemia	**Gender**			
	Male		**Female**	
	No.	**%**	**No.**	**%**
Present (TGL >150)(53)	26	49.1	27	50.9
Absent (TGL <150)(37)	22	59.5	15	40.5
Mean (TGL at 4 hrs)	185.9		234.9	
S.D.	84.8		107.1	
'p'	**0.0281**			
	Significant			

The difference was found to be statistically significant (p=0.0281).

Correlation between post load hypertriglyceridemia and body mass index was analyzed. TGL values were high in 15 patients (28.3%) in the overweight group compared to three patients (5.7%) in the underweight group.

TABLE 13: HYPERTRIGLYCERIDEMIA AND BMI

Hyper triglyceridemia	BMI								'p'
	Under weight		Normal weight		Over weight		Mean	S.D.	
	No.	%	No.	%	No.	%			
Present (TGL >150)(53)	3	5.7	35	66	15	28.3	24.4	3.1	**0.0124 Significant**
Absent (TGL <150)(37)	8	21.6	25	67.6	4	10.8	22.4	2.8	

The correlation was statistically significant (p=0.0124).

The 4-hr hypertriglyceridemia was compared with standard lipid ratios. Although there was no significant correlation between triglyceride levels and standard lipid ratios, a slightly higher TC/HDL and LDL/HDL ratios were seen in patients with hypertriglyceridemia than those without it. The results are shown in tables 14 and 15.

TABLE 14: HYPERTRIGLYCERIDEMIA AND TC/HDL RATIO

Hyper triglyceridemia	**TC/ HDL ratio**						**'p'**
	Normal		**Abnormal**		**Mean**	**S.D.**	
Present (TGL >150)(53)	33	62.3	20	37.7	4.9	1.35	0.8328 Not significant
Absent (TGL <150)(37)	23	62.2	14	37.8	4.25	0.75	

It was observed that the mean TC/HDL ratios were 4.9±1.35 in the patients with hypertriglyceridemia compared to 4.25±0.75 in patients without it.

TABLE 15: HYPERTRIGLYCERIDEMIA AND LDL/HDL RATIO

Hyper triglyceridemia	LDL/ HDL ratio						'p'
	Normal		Abnormal		Mean	S.D.	
Present (TGL >150)(53)	41	77.4	12	22.6	2.91	0.93	0.4151 Not significant
Absent (TGL <150)(37)	32	86.5	5	13.5	2.53	0.73	

The mean LDL/HDL ratio was 2.91±0.93 in patients with hypertriglyceridemia in comparison with patients without hypertriglyceridemia where the ratio was 2.53±0.73. The correlation was not significant statistically.

DISCUSSION

Meal absorption is a complex phenomenon and postprandial hyperlipidemia and hyperglycemia are simultaneously present the post absorptive phase, particularly in patients with type 2 diabetes mellitus and IGT. In non diabetic subjects, there is evidence that postprandial hypertriglyceridemia is a risk factor for CVD, whereas in diabetic subjects, postprandial hyperglycemia has been recently proposed as an independent risk factor for CVD with hyperlipidemia as an emerging risk factor. The distinct role and relative importance of these two factors in the pathogenesis of CVD in diabetes is a matter of debate.

This study was done to highlight postprandial hypertriglyceridemia as a significant risk factor for vascular events. Fasting triglycerides, representing triglyceride metabolism under relaxation, have not generally been accepted as an independent risk factor for atherosclerosis including coronary artery disease. Individuals with normal fasting triglyceride levels exhibit highly varying postprandial triglyceride concentrations in the postprandial hours of a fatty test meal. Therefore, postprandial lipemia, representing triglyceride metabolic capacity under challenge, is considered to be more informative for assessing the role of triglyceride metabolism in the development of atherosclerosis. Consequently, a

number of case–control studies showed impaired triglyceride metabolic capacity, defined as increased and prolonged postprandial hypertriglyceridemia, to be closely linked to the presence of CAD.

Many of the observations made in our study correlated well with previous studies.

Most diabetic patients in this study were in the normal weight group (70.5% in uncomplicated diabetes and 60.5% in complicated diabetes). According to Indian data, almost 80% of type 2 DM patients in India are normal weight as compared to 60-80% of diabetics from west. The findings from our study correlated well with previous data from India as shown in the following table.

TABLE 16: PROFILE OF DIABETIC PATIENTS FROM INDIA (past observations)

Centre	Obese	Non obese	Lean
Cuttack[43]	7.8	65.8	26.4
Hyderabad[44]	25.4	56.7	17.9
Madras[45]	32.9	63.5	3.5
Our study	**23.8**	**66.5**	**9.7**
Values in percentage			

The mean fasting triglyceride levels in our study were 121±19.8mg/dl in the control group, 156.4±63.1mg/dl in uncomplicated

diabetes and 184.8±68.7mg/dl in complicated diabetes. This is in concordance with various Indian studies done in the past to ascertain triglyceride levels in normal individuals as shown in table 16.

TABLE 17: MEAN TRIGLYCERIDE LEVELS IN NORMAL INDIAN URBAN POPULATION

First Author	Year	Age-Group	Place	Sample Size	Triglycerides mg/dl
Gandhi BM	1982	20-70	Delhi	200	124.0±29
Vasisth S	1990	30-70	Delhi	186	128.1±30
Reddy KS	1992	25-64	Delhi	1581	110.2±45
Gopinath N	1994	25-64	Delhi	1345	131.0±54
Gupta R	1997	20-80	Jaipur	199	126.1±55
Gupta R	2002	20-80	Jaipur	1123	144.6±70

In the present study, the incidence of baseline hypercholesterolemia (p=0.0202) and hypertriglyceridemia (p=0.0027) was more in the diabetic patients than in controls and more so in complicated diabetes. This has been well emphasized in literature. Diabetic patients invariably have dyslipidemia sometime in the course of the disease.[2] This was also observed in studies done by Syvanne et al.[46], West et al[47] and Fontbonne et al[48] in patients with diabetes and impaired glucose tolerance. There was good correlation noted between insulin resistance and plasma TGL concentration, as TGL may influence an early

step in the insulin action pathway; alternatively, insulin resistance may cause hypertriglyceridemia.[49]

A study conducted by Rajmohan et al in South Indian type 2 diabetic subjects revealed that the prevalence of CAD was significantly higher among patients with isolated hypercholesterolemia, isolated high LDL, and isolated low high-density lipoprotein levels compared to normolipidemic individuals, but not in those with isolated hypertriglyceridemia.[50] In contrast, Anderson et al showed that CAD was higher in patients with isolated hypertriglyceridemia.[51] In our study, fasting cholesterol levels correlated significantly with triglyceride levels ($p=0.0002$) and both were high in patients with vascular events. Another observation made in this study was that there was no significant difference in the LDL or HDL cholesterol values between the three groups and triglycerides were elevated even with other lipoprotein measures in the normal range. This can be explained by the fact in that diabetic patients, altered morphology of the lipoprotein structure may contribute more to atherogenesis rather than the absolute value (small dense atherogenic LDL).[1,2]

All the patients who had fasting triglyceridemia also had elevated postprandial TGL. This is in accordance with various studies in the past which have made the observations that elevated postprandial TGL levels have been seen in persons with fasting hypertriglyceridemia.[46] There was

also good correlation between fasting (p=0.0006) and postprandial blood sugar (p=0.0001) and postprandial triglyceridemia in patients with vascular events. This has been noted in the previous study done by Ceriello et al where they proved the independent and cumulative role of postprandial hypertriglyceridemia and hyperglycemia in the causation of endothelial dysfunction in diabetes.[41]

It was seen in our study that persistent triglyceridemia at four hours post fat meal was seen in 83.3% of diabetic patients with macro vascular complications and 65.9% of patients without complications, whereas it was seen only in 18.2% of controls (p=0.0001). It is in concordance with various studies in the past which have also proved a consistent relationship between macro vascular complications in diabetes and postprandial lipids. Patsch et al.[53] showed that increased postprandial TGL levels were independently predictive of severe CAD. The Atherosclerosis Risk in Communities (ARIC) trial showed similar observations. [54] Another study done by Patsch et al also demonstrated that postprandial TGL is important for the propensity for atherosclerosis.[55] Golay et al have found in their study that postprandial lipids are frequently being neglected as important determinants of coronary events in patients with type 2 diabetes mellitus.[56]

It was found in our study that postprandial (post load) triglyceridemia (p=0.0027) correlated better that fasting triglyceridemia

with vascular events (p=0.0001). Gambhir et al (1999) from Noida, India have shown that there is significant elevation of triglycerides in diabetics in the postprandial state which are further associated with increased oxidative stress.

It has been shown in past studies that there is some evidence for TGL as an independent risk factor in certain subgroups, for example, women 50–69 years of age[47] and men with low total cholesterol levels.[57] A meta-analysis of 17 population-based prospective studies, which included 46000 men and 11000 women, revealed a 30%; and 75%; increased risk of CAD, respectively, for TGL levels.[58] In our study also, women had significantly higher levels of postprandial triglycerides as compared to men, especially in complicated diabetes (p=0.0281).

Some studies have indicated that there is an increased risk of CAD in the presence of TGL levels $\geq$204 mg/dl when the ratio of LDL-cholesterol to HDL-cholesterol exceeds five.[59, 60] Though standard lipid ratios did not correlate well with postprandial triglyceride levels in our study, the mean lipid ratios (TC/HDL, LDL/HDL) were found to be higher in patients with hypertriglyceridemia (4.9, 2.91) than those without it (4.25, 2.53).

The above observations demonstrate a good relationship between postprandial lipemia and atherosclerosis, and suggest that postprandial TGL levels may be a better indicator of atherogenicity than fasting levels.

Therefore, postprandial triglyceride measurements, especially in high risk groups may give important information for risk assessment and planning of treatment strategies for prevention of vascular complications.

However, the protocol for measuring postprandial hypertriglyceridemia has to be formulated, and it is necessary to formulate precise guidelines on the time intervals for measuring postprandial TGL, and the fat load to be used. The normal cut-off values for TGL also have to be internationally standardized. If these guidelines are established, simple measurement of post load triglyceride and subsequent dietary or pharmacological intervention may help to detect or prevent endothelial dysfunction in diabetes, thus alleviating the mortality and morbidity associated with the disease.

Summary

The study “postprandial hypertriglyceridemia as a risk factor for macro vascular complications in type 2 diabetes mellitus” was a case control study conducted on patients visiting the out patient department of Government Rajaji Hospital, Madurai. Sixty eight patients with type 2 diabetes mellitus and 22 healthy controls were included in the study. Selected patients and controls underwent clinical and biochemical evaluation including blood sugar, lipid profile and cardiac function to detect the presence of macro vascular complications of diabetes. Selected patients and subjects were subjected to a high fat meal and plasma triglycerides were measured two and four hours after fat challenge. It was observed that post load hypertriglyceridemia correlated better than fasting triglycerides in patients with macro vascular complications. It was also observed that persistent postprandial hypertriglyceridemia was observed in diabetic patients with complications.

Conclusions

1. Dyslipidemia was seen in significant proportion of diabetic patients, especially those with complications.

2. Persistent and significant post load hypertriglyceridemia was observed in new diabetic patients with macro vascular complications compared to controls; hence it is a useful marker for predicting vascular complications in type 2 diabetes mellitus.

3. Postprandial hypertriglyceridemia was more significant than fasting triglyceridemia in complicated diabetes.

4. Hypertriglyceridemia was observed independent of LDL, HDL and standard lipid ratios in patients with vascular disease. Hence it may represent an independent risk factor for vascular events in diabetes.

5. Although the emphasis in recent times is mainly on LDL cholesterol in type 2 diabetes and metabolic syndrome, postprandial TGL increase may be equally or more important than LDL cholesterol in causation of vascular events.

6. Detecting and correcting early postprandial hypertriglyceridemia with the help of a standardized fat challenge test may be a useful therapeutic option in halting endothelial dysfunction and hence macro vascular complications in diabetes.

APPENDIX

Bibliography

1. Harrison's Principles of Internal Medicine, 17th edition.

2. Joslins diabetes, 14th edition.

3. DeFronzo RA. Pathogenesis of type 2 (non-insulin-dependent) diabetes mellitus: a balanced overview. Diabetologia 1992; 35:389 –97.

4. Ceriello A .The post-prandial state and cardiovascular disease:relevance to diabetes mellitus. Diabetes Metab Res Rev 2000; 16:125 –32.

5. Heine RJ, Dekker JM. Beyond postprandial hyperglycaemia: metabolic factors associated with cardiovascular disease. Diabetologia 2002; 45: 461 – 75.

6. Haffner SM. The importance of hyperglycemia in the nonfasting state to the development of cardiovascular disease. Endocr Rev 1998; 19:583 –92.

7. Coutinho M, Gerstein HC, Wang Y, et al. The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. Diabetes Care 1999; 22:233 –40.

8. Heberden W. Some account of a disorder of the breast.Med Trans 1772; 2:59 –67.

9. Zilversmit DB. Atherogenesis:a postprandial phenomenon. Circulation 1979; 60:473 –85.

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

11. Thalhammer C, Balzuweit B, Busjahn A, et al. Endothelial cell dysfunction and arterial wall hypertrophy are associated with disturbed carbohydrate metabolism in patients at risk for cardiovascular disease. Arterioscler Thromb Vasc Biol 1999; 19:1173 –9.

12. Ceriello A .Coagulation activation in diabetes mellitus:the role of hyperglycaemia and therapeutic prospects. Diabetologia 1993; 36:1119 –25.

13. Ceriello A, Giacomello R, Stel G, et al. Hyperglycemia-induced thrombin formation in diabetes. The possible role of oxidative stress. Diabetes

1995; 44:924 –8

14. Gresele P, Guglielmini G, De Angelis M, et al. Acute, short-term hyperglycemia enhances shear stress-induced platelet activation in patients with type II diabetes mellitus. J Am Coll Cardiol 2003; 41:1013 –20.

15. Esposito K , Nappo F, Marfella R, et al. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans. Circulation 2002; 106:2067 –72.

16. Nappo F, Esposito K, Cioffi M, et al. Postprandial endothelial function in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals. J Am Coll Cardiol 2002; 39:1145 –50.

17. Shizukuda Y, Reyland ME, Buttrick PM. Protein kinase C-delta modulates apoptosis induced by hyperglycemia in adult ventricular myocytes. Am J Physiol Heart Circ Physiol 2002; 282:H1625 –34.

18. Risso A, Mercuri F, Quagliaro L, et al. Intermittent high glucose enhances apoptosis in human umbilical vein endothelial cells in culture. Am J Physiol Endocrinol Metab 2001; 281:E924 –30.

19. Taskinen MR. Diabetic dyslipidemia: from basic research to clinical practice. Diabetologia 2003; 46:733 –49.

20. Albrink MJ, Man EB. Serum triglycerides in coronary artery disease. Arch Intern Med 1959; 103:4 –8.

21. Vogel RA, Corretti MC, Plotnick GD. Effect of a single high fat meal on endothelial function in healthy subjects. Am J Cardiol. 1997; 79:350–354

22. Murphy MC et al. postprandial lipid and hormone responses to meals of varying fat contents: modulatory role of lipoprotein lipase? Eur J Clin Nutr 1995; 49(8): 578-88

23. Hokanson JE, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. J Cardiovasc Risk 1996; 3:213 –9.

24. Groot PH, Van Stiphout WA, Krauss XH, et al. Postprandial lipoprotein metabolism in normolipidemic men with and without coronary artery disease. Arterioscler Thromb 1991; 11:653 –62.

25. Stampfer MJ, Krauss RM, Ma J, et al. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. JAMA 1996; 276:882 –8.

26. Teno S, Uto Y, Nagashima H, et al. Association of postprandial hypertriglyceridemia and carotid intima-media thickness in patients with type 2 diabetes. Diabetes Care 2000; 23:1401 –6.

27. Lewis GF, O ’Meara NM, Soltys PA, et al. Fasting hypertriglyceridemia in noninsulin-dependent diabetes mellitus is an important predictor of postprandial lipid and lipoprotein abnormalities. J Clin Endocrinol Metab 1991; 72:934 –44.

28. Karpe F, Steiner G, Uffelman K, et al. Postprandial lipoproteins and progression of coronary atherosclerosis.Atherosclerosis 1994; 106:83 –97.

29. Karpe F. Postprandial lipoprotein metabolism and atherosclerosis. J Intern Med 1999; 246:341 –55.

30. Mero N, Malmstrom R, Steiner G, et al. Postprandial metabolism of apolipoprotein B-48-and B-100-containing particles in type 2 diabetes mellitus: relations to angiographically verified severity of coronary artery disease. Atherosclerosis 2000; 150:167 –77.

31. Silveira A, Karpe F, Johnsson H, et al. In vivo demonstration in humans that large postprandial triglyceride-rich lipoproteins activate coagulation factor VII through the intrinsic coagulation pathway. Arterioscler Thromb Vasc Biol 1996; 16:1333 –9.

32. Silva KR, Kelly CN, Jones AE, et al. Chylomicron particle size and number, factor VII activation and dietary monounsaturated fatty acids. Atherosclerosis 2003; 166:73 –84.

33. Byrne CD, Wareham NJ, Martensz ND, et al. Increased PAI activity and PAI-1 occuring with an oral fat load: associations with PAI-1 genotype and plasma active TGF-b levels. Atherosclerosis 1998; 140:45 –53.

34. Wasir J, Ram BK, Madhu SV, Kaur J. Postprandial triglyceride metabolism in young non obese diabetics and its relation to insulin sensitivity. JAPI 2001; 49: 204.

35. Madhu SV, Malhotra A, Mehrotra G. Postprandial lipemia and carotid intima media thickness in patients with type 2 diabetes mellitus. Diabetologia 2003; 46: A380.

36. Ryu JE, Howard G, Craven TE, Bond MG, Hagaman AP. Postprandial triglyceridemia and carotid atherosclerosis in middle aged subjects. Stroke 1992; 23: 823-8.

37. Mack WJ, Krauss RM, Hodis HN. Lipoprotein subclasses in the Monitored Atherosclerosis Regression Study (MARS). Treatment effects and relation to coronary angiography progression. Arterioscler Thromb Vasc Biol 1996; 16: 697-704.

38. Huff MW. Dietary cholesterol, cholesterol absorption, postprandial lipemia and atherosclerosis. Can J Clin Pharmacol. 2003; 10 (suppl A): 26A–32A

39. Eaton CB. Can reduced or modified dietary fat prevent cardiovascular disease? Am Fam Physician. 2002; 65: 53–55.

40. D. J. A. Jenkins, C. W. C. Kendall, A. R. Josse, S. Salvatore, F. Brighenti, L. S. A. Augustin, P. R. Ellis, E. Vidgen, and A. V. Rao. Almonds Decrease Postprandial Glycemia, Insulinemia, and Oxidative Damage in Healthy Individuals. J. Nutr, December 1, 2006;136(12):2987-2992.

41. Ceriello A, Taboga C, Tonutti L, et al. Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial dysfunction and oxidative stress generation. Circulation 2002; 106:1211 –8.

42. M E Tushuizen, M Diamant, R J Heine. Postprandial dysmetabolism and cardiovascular disease in type 2 diabetes. Postgrad Med J 2005; 81:1 –6.

43. Tripathy BB et al. observations on clinical patterns of Diabetes mellitus in India. Diabetes 1965; 14:404-12

44. Sahay BK. Profile of lean NIDDM as seen in Hyderabad. In: Kanpur (ed). Proceedings of the second Novo Nordisk diabetes update. Health care communication, Bombay, 1993: 161-4.

45. Mohan V et al. clinical profile of lean NIDDM in South India. Diabetes Res Clin Practice 1997; 38:101-8.

46. Syvänne M, Taskinen MR. Lipids and lipoproteins as coronary risk factors in non-insulin-dependent diabetes mellitus. Lancet 1997; 350 (Suppl 1): SI20–SI23

47. West KM, Ahuja MM, Bennett PH, Czyzyk A, De Acosta OM, Fuller JH, et al. The role of circulating glucose and triglyceride concentrations and their interactions with other "risk factors" as determinants of arterial disease

in nine diabetic population samples from the WHO multinational study. Diabetes Care 1983; 6: 361–369

48. Fontbonne A, Eschwege E, Cambien F, Richard JL, Ducimetiere P, Thibult N, et al. Hypertriglyceridaemia as a risk factor of coronary heart disease mortality in subjects with impaired glucose tolerance or diabetes: Results from the 11-year follow-up of the Paris Prospective Study. Diabetologia 1989; 32: 300–304

49. De Man FH, Cabezas MC, Van Barlingen HH, Erkelens DW, de Bruin TW. Triglyceride-rich lipoproteins in non-insulin-dependent diabetes mellitus: post-prandial metabolism and relation to premature atherosclerosis. Eur J Clin Invest 1996; 26: 89–108

50. Rajmohan L, Deepa R, Mohan A, Mohan V. Association between isolated hypercholesterolemia, isolated hypertriglyceridemia and coronary artery disease in south Indian type 2 diabetic patients. Indian Heart J 2000; 52: 400–406

51. Anderson RA, Evans ML, Ellis GR, Graham J, Morris K, Jackson SK, et al. The relationships between post-prandial lipaemia, endothelial function and oxidative stress in healthy individuals and patients with type 2 diabetes. Atherosclerosis 2001; 154: 475–483

52. Eliasson B, Mero N, Taskinen MR, Smith U. The insulin resistance syndrome and postprandial lipid intolerance in smokers. Atherosclerosis 1997; 129: 79–88

53. Patsch JR, Miesenbock G, Hopferwieser T, Muhlberger V, Knapp E, Dunn JK**,** et al. Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. Arterioscler Thromb 1992; 12: 1336–1345

54. Sharrett AR, Heiss G, Chambless LE, Boerwinkle E, Coady SA, Folsom AR**,** et al. Metabolic and lifestyle determinants of postprandial lipemia differ from those of fasting triglycerides: The Atherosclerosis Risk In Communities (ARIC) study. Arterioscler Thromb Vasc Biol 2001; 21: 275–281

55. Patsch JR. Triglyceride-rich lipoproteins and atherosclerosis. Atherosclerosis 1994; 110 (Suppl): S23–S26

56. Golay A. Are postprandial triglyceride and insulin abnormalities neglected cardiovascular risk factors in type 2 diabetes? Eur J Clin Invest 2000; 30 (Suppl): 12–18

57. Cambien F, Jacqueson A, Richard JL, Warnet JM, Ducimetiere P, Claude JR. Is the level of serum triglyceride a significant predictor of

coronary death in "normocholesterolemic" subjects? The Paris Prospective Study. Am J Epidemiol 1986;124: 624–632

58. Hokanson JE, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. J Cardiovasc Risk 1996; 3: 213–219

59. Assmann G, Schulte H. Relation of high-density lipoprotein cholesterol and triglycerides to incidence of atherosclerotic coronary artery disease (the PROCAM experience). Prospective Cardiovascular Munster Study. Am J Cardiol 1992; 70: 733-737

60. Manninen V, Tenkanen L, Koskinen P, Huttunen JK, Manttari M, Heinonen OP, et al. Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study: implications for treatment. Circulation 1992; 85: 37–45.

PROFORMA

Name: Age: Sex:

Occupation: Address:
Height:
Weight:
BMI:

Diabetes : Yes / No
Type : Type 1/Type 2 DM
Duration :
Treatment:

Hypertension : Yes /No
Coronary Artery Disease : Yes /No
Cerebro Vascular Disease : Yes /No
Peripheral Vascular Disease : Yes /No

Diet History: Vegetarian/Non Vegetarian
Personal History: Smoker/Alcoholic
Family History:

General examination:

Pulse: BP: Temp: RR:
CVS:
RS:
ABDOMEN:
CNS:

INVESTIGATION:

HB: TC: DC: Urine Alb: Sug: Dep:
SUGAR:
Fasting 2hrs PP 4hrs PP
UREA: CREATININE:

LIPID PROFILE- TC LDL HDL VLDL

TRIGLYCERIDES:
Fasting 2hrs PP 4hrs PP

ECG:

ECHO:

FIGURE 1: PATHOPHYSIOLOGY OF TYPE 2 DIABETES MELLITUS

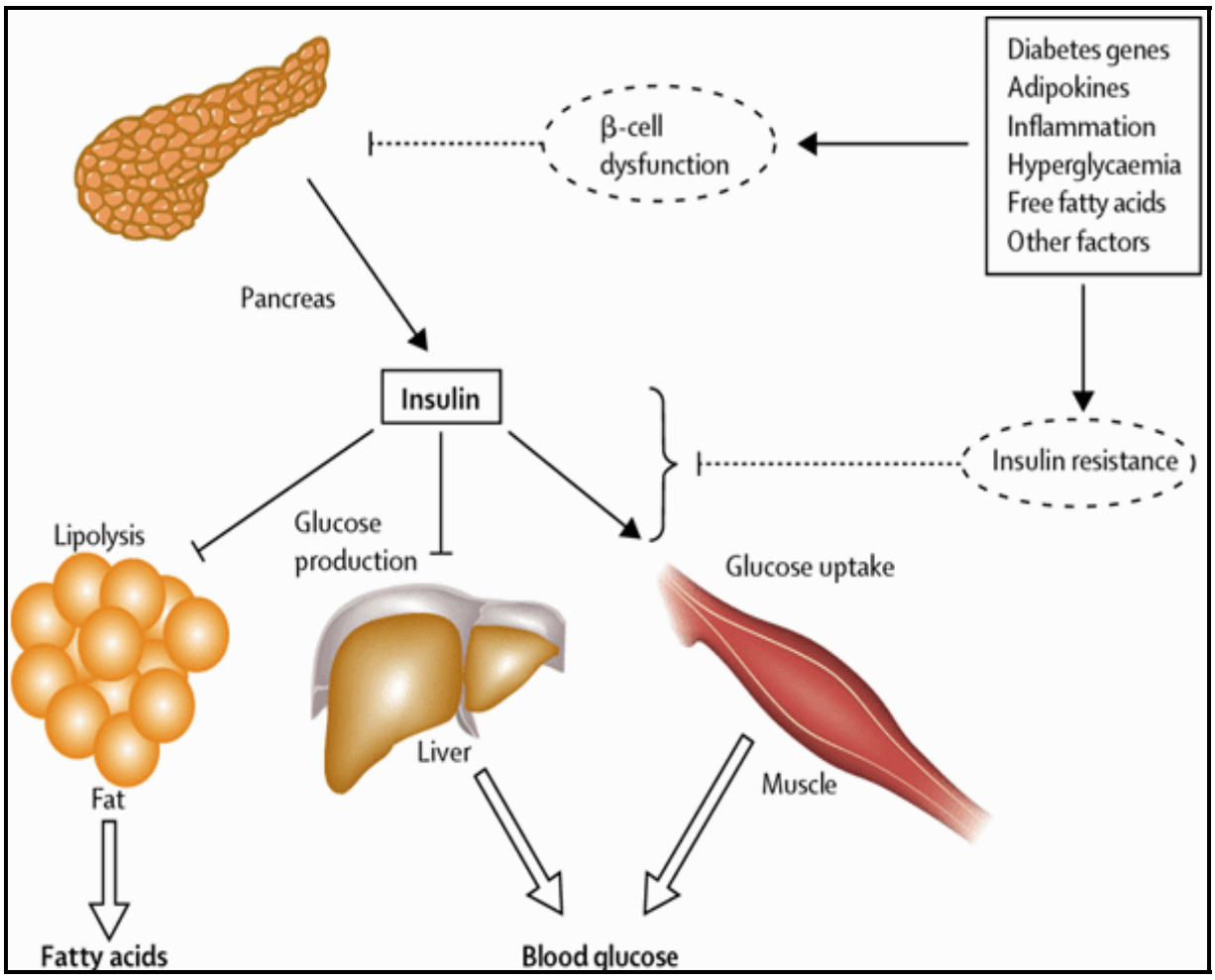

FIGURE 2: OXIDATIVE STRESS AND ENDOTHELIAL DAMAGE

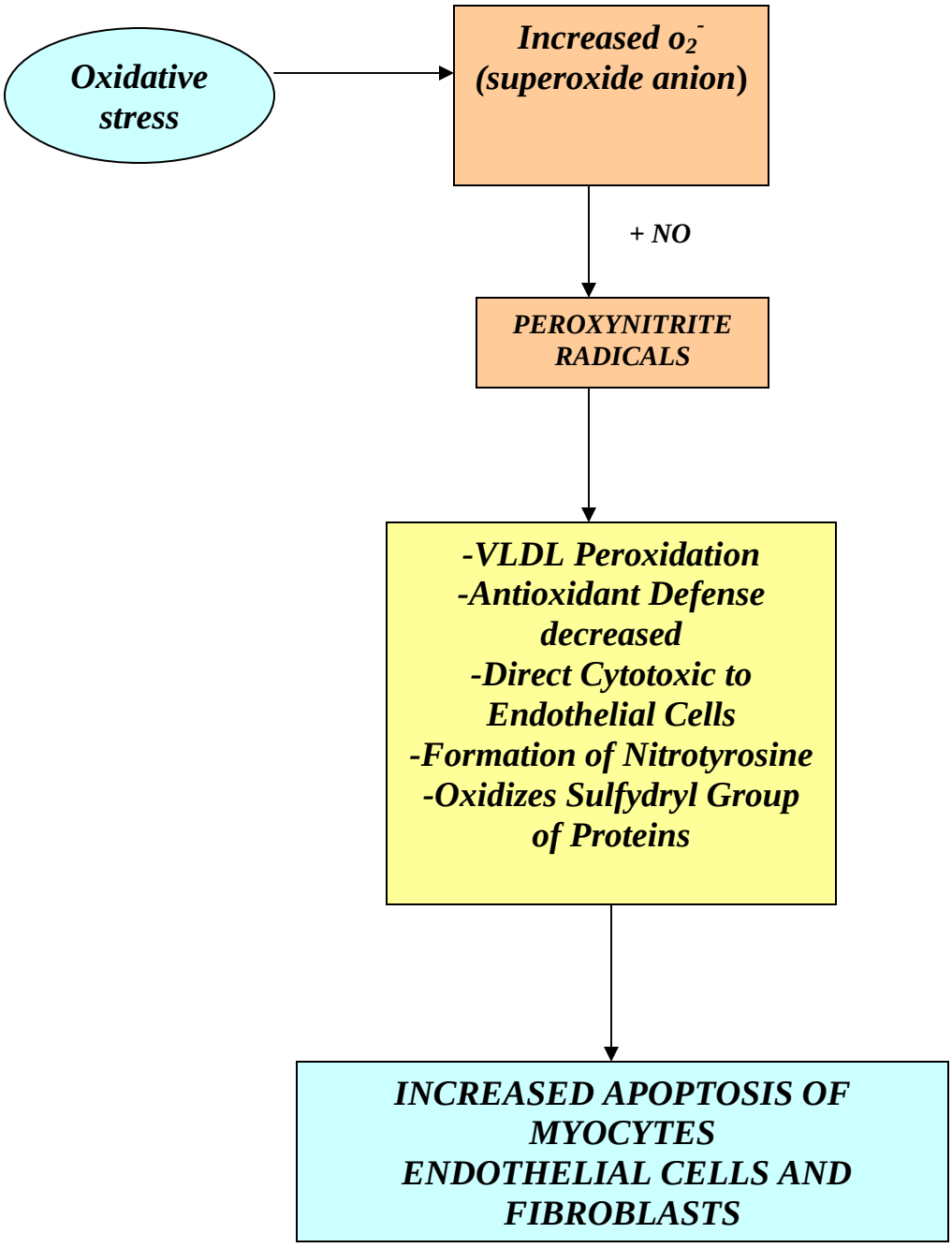

FIGURE 3: AGE DISTRIBUTION OF PATIENTS

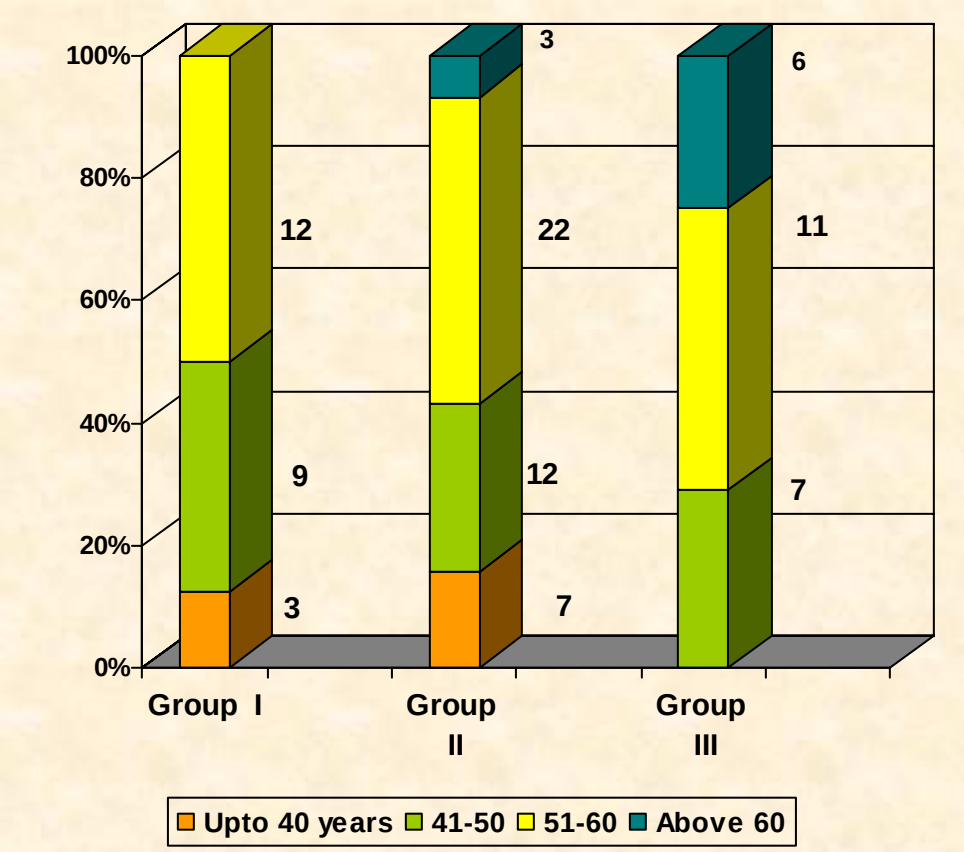

FIGURE 4: MEAN BLOOD SUGAR VALUES

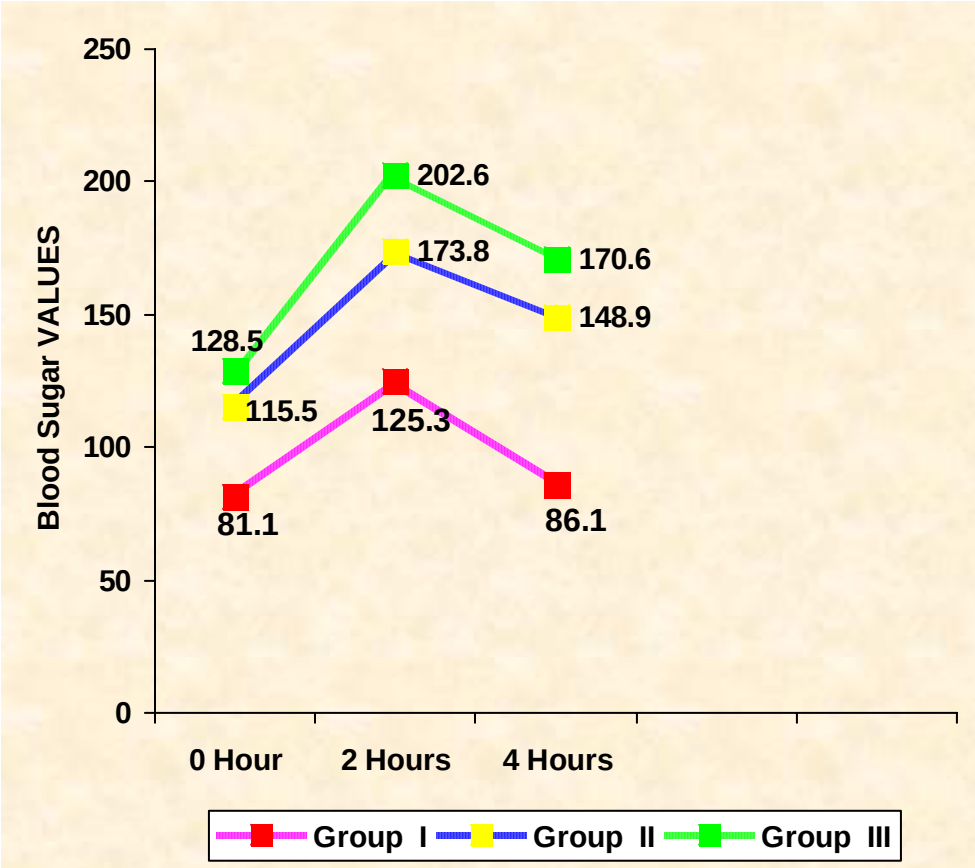

FIGURE 5: MEAN TRIGLYCERIDE LEVELS

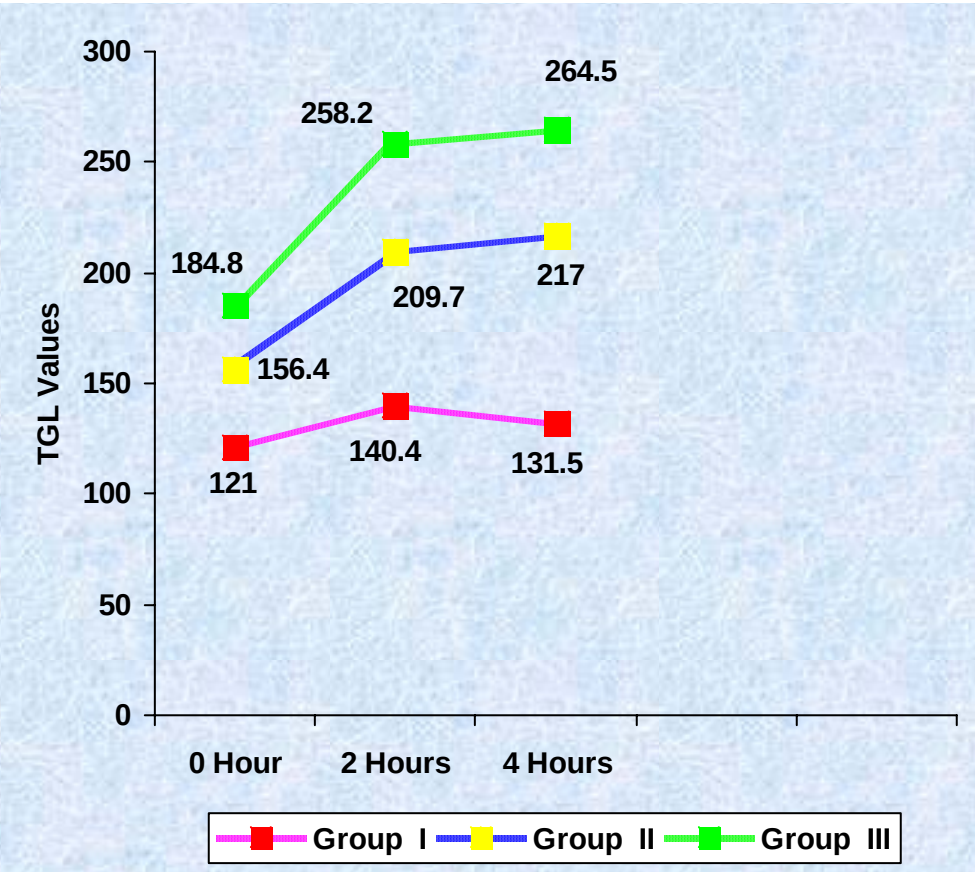

FIGURE 6: 4-HOUR HYPERTRIGLYCERIDEMIA IN VARIOUS GROUPS

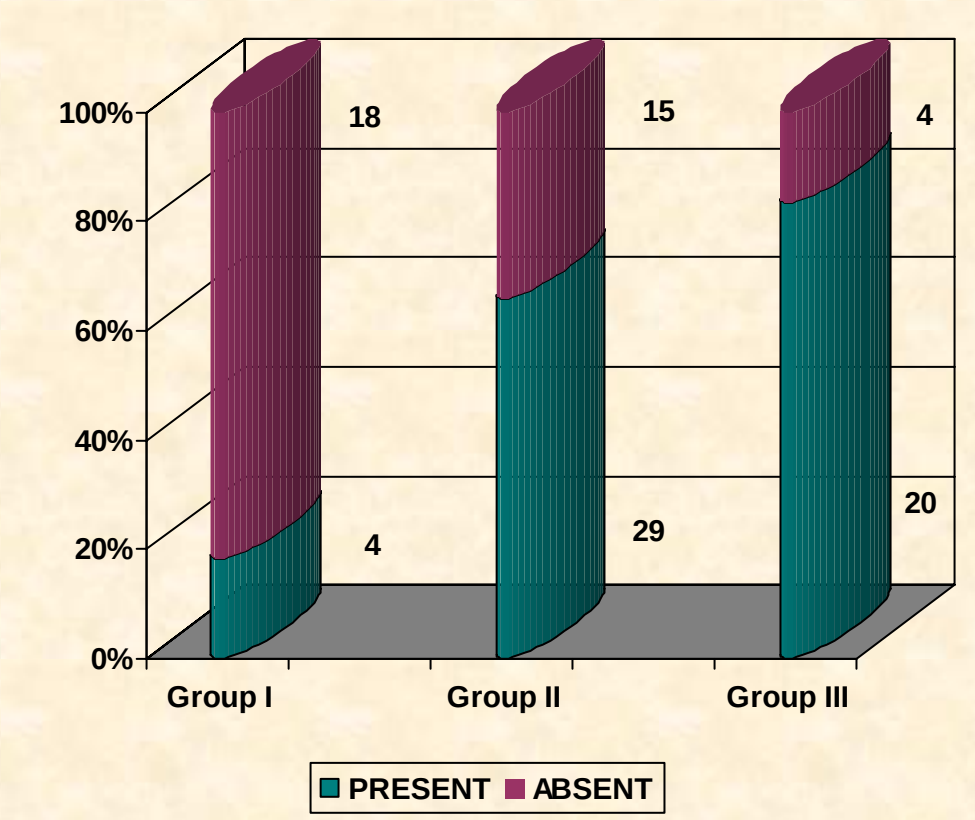

FIGURE 7: CORRELATION BETWEEN HYPERTRIGLYCERIDEMIA AND BLOOD SUGAR VALUES

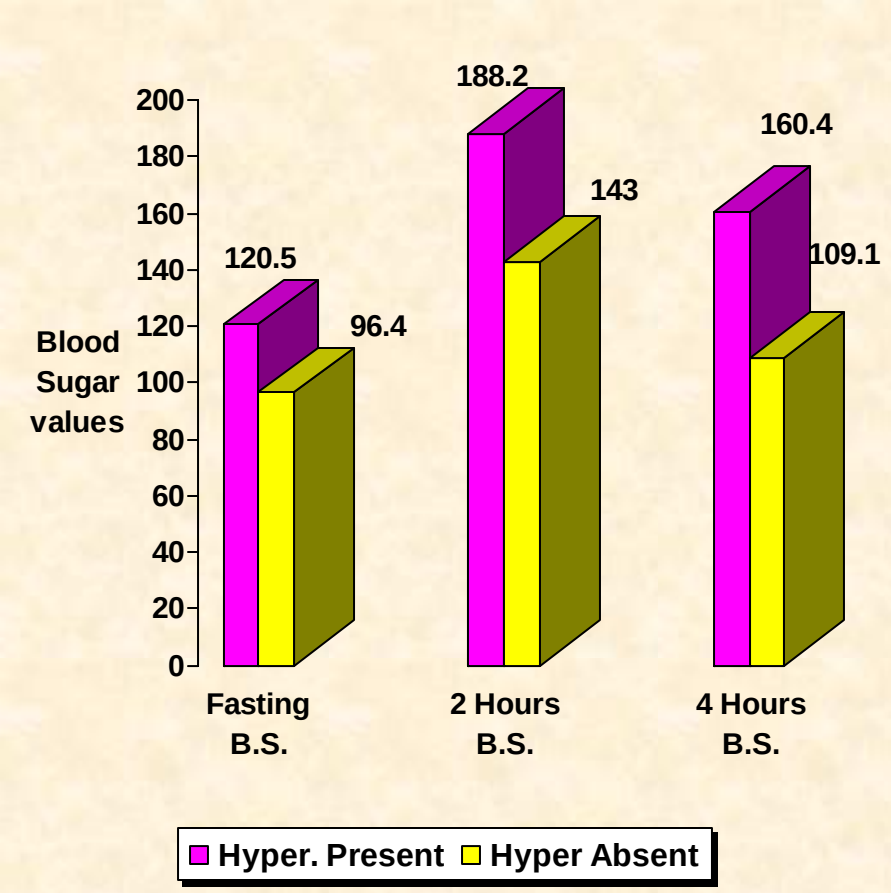

FIGURE 8: HYPERTRIGLYCERIDEMIA VERSUS GENDER

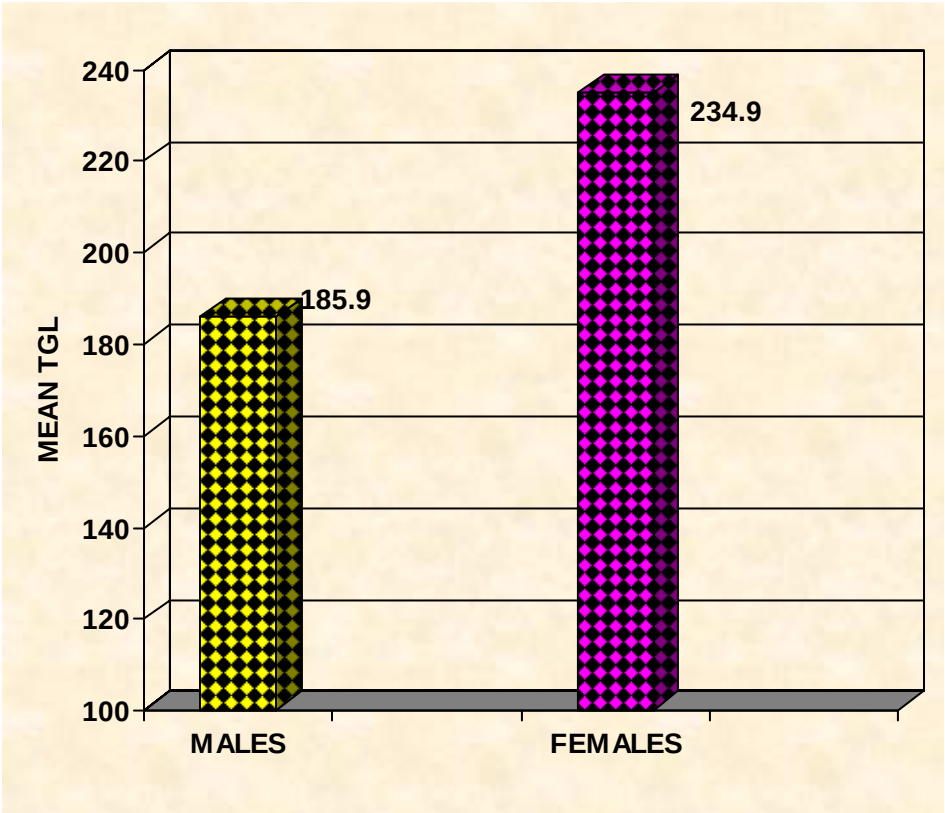

FIGURE 9: HYPERTRIGLYCERIDEMIA VERSUS BMI