DISSERTATION ON

# "SIGNIFICANCE OF C-PEPTIDE LEVELS IN TYPE 2 DIABETES MELLITUS"

Dissertation submitted to

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment of the regulations

for the award of the degree of

M.D. IN GENERAL MEDICINE

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**THANJAVUR MEDICAL COLLEGE**
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CHENNAI – 600 032

APRIL - 2016

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I**, Dr. M. RAJAVEL**, solemnly declare that dissertation titled " **STUDY ON SIGNIFICANCE OF C- PEPTIDE LEVELS IN TYPE 2 DIABETES MELLITUS"** is a bonafide work done by me at Thanjavur Medical College Hospital, Thanjavur during December 2014 to May 2015 under the guidance and supervision of Prof. **Dr. K. NAMASIVAYAM M.D.,** Unit Chief M-3, Department of Internal Medicine, Thanjavur Medical College Hospital, Thanjavur. This dissertation is submitted to **THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI, TAMILNADU** as partial fulfillment for the requirement of M.D. Degree Examination – Branch 1 ( General Medicine ) to be held in April 2016.

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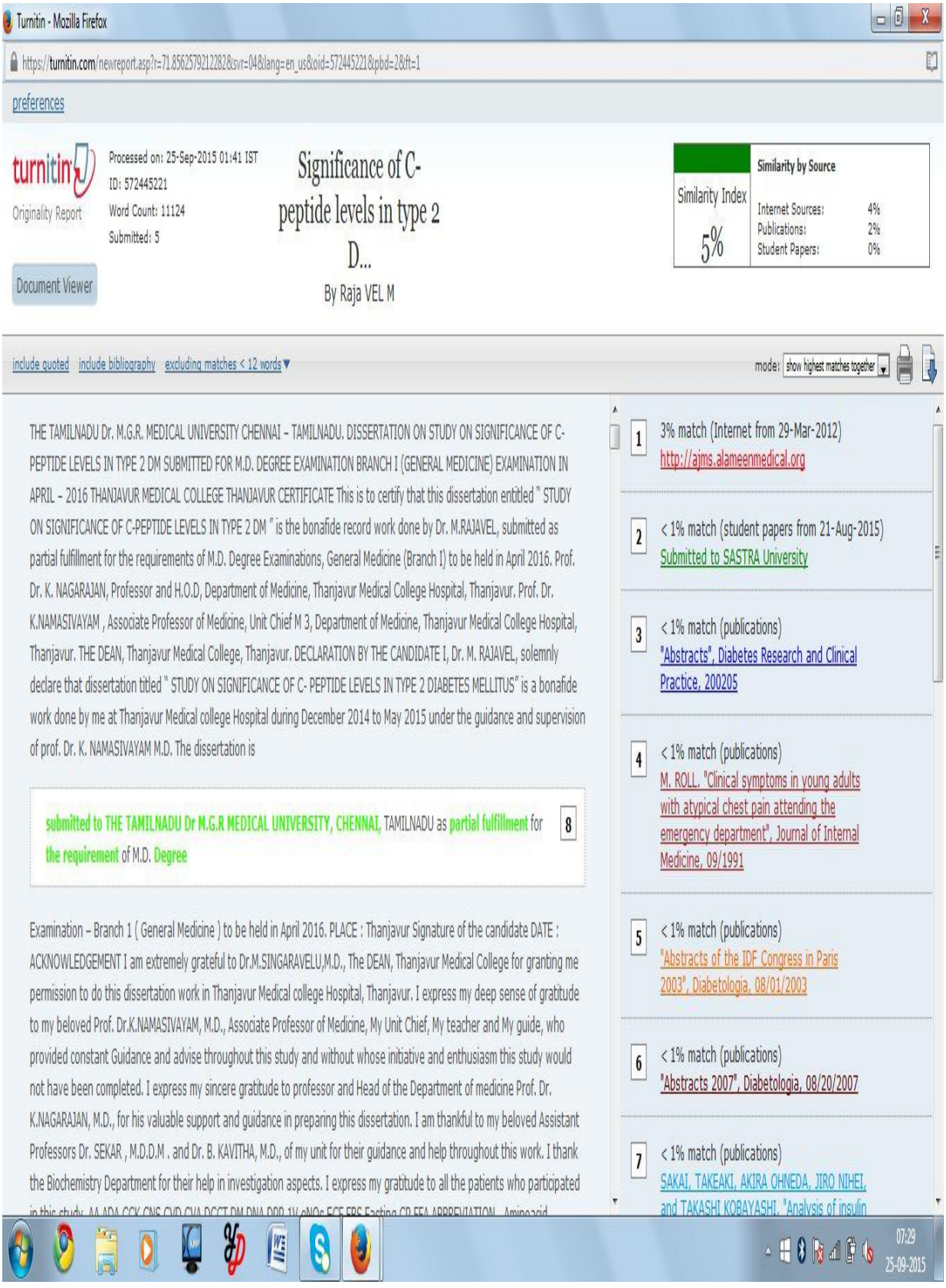
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Examination – Branch 1 ( General Medicine ) to be held in April 2016. PLACE : Thanjavur Signature of the candidate DATE : ACKNOWLEDGEMENT I am extremely grateful to Dr.M.SINGARAVELU,M.D., The DEAN, Thanjavur Medical College for granting me permission to do this dissertation work in Thanjavur Medical college Hospital, Thanjavur. I express my deep sense of gratitude to my beloved Prof. Dr.K.NAMASIVAYAM, M.D., Associate Professor of Medicine, My Unit Chief, My teacher and My guide, who provided constant Guidance and advise throughout this study and without whose initiative and enthusiasm this study would not have been completed. I express my sincere gratitude to professor and Head of the Department of medicine Prof. Dr. K.NAGARAJAN, M.D., for his valuable support and guidance in preparing this dissertation. I am thankful to my beloved Assistant Professors Dr. SEKAR , M.D.D.M . and Dr. B. KAVITHA, M.D., of my unit for their guidance and help throughout this work. I thank the Biochemistry Department for their help in investigation aspects. I express my gratitude to all the patients who participated in this study. AA ADA CCK CNS CVD CVA DCCT DM DNA DPP 1V eNOs FCF FBS Fasting GP FFA ABBREVIATION Aminoacid
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7 < 1% match (publications) SAKAI, TAKEAKI, AKIRA OHNEDA, JIRO NIHEI, and TAKASHI KOBAYASHI. "Analysis of insulin
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# ABBREVIATION

| | |
|---|---|
| AA | - Aminoacid |
| ADA | - American Diabetes Association |
| CCK | - Cholecystokinin |
| CNS | - Central nervous system |
| CVD | - Cardiovascular Disease |
| CVA | - Cerebrovascular accident |
| DCCT | - Diabetes complications and control trial |
| DM | - Diabetes mellitus |
| DNA | - Deoxy Ribonucleic acid |
| DPP 1V | - Dipeptidyl peptidase 4 inhibitor |
| eNOs | - Endothelial Nitric Oxide Synthetase |
| ECF | - Extracellular Fluid |
| FBS | - Fasting blood sugar |
| Fasting CP | - Fasting C-peptide |
| FFA | - Free fatty acids |
| GHB | - Glycated Haemoglobin |

| | |
|---|---|
| GIP | - Gastric inhibitory polypeptide |
| GIT | - Gastrointestinal tract |
| GLP-1 | - Glucagon like peptide 1 |
| HbA1c | - Glycosylated Haemoglobin A1C |
| HDL | - High Density Lipoprotein |
| HTN | - Hypertension |
| Ht | - Height |
| IDDM | - Insulin Dependent Diabetes Mellitus |
| IGT | - Impaired Glucose Tolerance Test |
| IRS | - Insulin Receptor Signalling pathway |
| Kcal | - Kilocalories |
| MW | - Molecular Weight |
| NEFA | - Non esterified fatty acids |
| NIDDM | - Non Insulin Dependent Diabetes Mellitus |
| ng / ml | - nanogram / millilitre |
| NBG | - Normal blood glucose |
| NSAID | - Non steroidal anti- inflammatory drugs |

| | |
|---|---|
| PAS | - Periodic acid Schiff stain |
| PI-3 Kinase | - Phosphatidyl inositol 3 –kinase |
| PPAR | - Peroxisome Proliferator Activated Receptor |
| RNA | - Ribonucleic acid |
| TG | - Triglycerides |
| TNF | - Tumour necrosis factor |
| UKPDS | - United Kingdom Prospective Diabetes Study |
| VLDL | - Very Low Density Lipoprotein |
| Wt | - Weight. |

## ABSTRACT

**BACKGROUND** :

Diabetes mellitus is a group of metabolic disorders characterised by chronic hyperglycaemia associated with disturbance of carbohydrate, fat and protein metabolism due to absolute or relative deficiency in insulin secretion or its action. Treatment should be based on underlying pathophysiology not on symptoms.

**AIM OF THE STUDY :**

1. In type 2 DM patients, estimation of C-peptide levels to assess the level of insulin secretion in the body.
2. Obesity and C-peptide level correlation in patients with type 2 DM.
3. Treatment modification in type 2 DM patients based on C- peptide levels.

**Settings & designs :**

It is a prospective study done in the diabetic outpatient clinic of our hospital.

**Materials & methods :**

From Dec 2014 to May 2015, 50 type 2 diabetic patients were evaluated for fasting plasma glucose level, fasting C-peptide level and BMI taking into account the inclusion and exclusion criterias.

**INCLUSION CRITERIA** :

Patients labelled as type 2 diabetes mellitus.

**EXCLUSION CRITERIA** :

a. Type 1 diabetes mellitus patients.

b. Patients with acute infections, renal failure and pregnancy.

**KEY WORDS** :

Type 2 DM , C- peptide, BMI, duration of diabetes mellitus, insulin resistance, blood sugar, β cell of pancreas, family history of diabetes. Glycosylated haemoglobin.

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# INTRODUCTION

## INTRODUCTION :

Diabetes mellitus is the commonest endocrine disorder in population. This clinical syndrome due to either absolute or relative insulin deficiency. Currently number of diabetics worldwide is 160 million[1]. In India the projected increase is from 20 to 62 million[2,3] .

The term diabetes is derived from Greek words 'Dia' meaning 'through', 'Bainein' meaning 'to go'. This disease causes loss of weight as if the body mass is passed through urine. It is a disease known from ancient times. Charaka in his treatise, elaborated this disorder as 'Madhumeha' (meaning sweet urine) around 400 BC[4]. Type 1 DM is caused by pancreatic β cell destruction by auto antibodies, leading to defect in insulin synthesis and secretion[5]. Type 2 DM results from a combination of impaired β cell function[6] and marked increase in peripheral insulin resistance at receptor/ post receptor levels. Their circulatory levels of insulin may be variable from hyper to normo insulinemic levels in majority of patients. Type 2 DM is a major health problem worldwide[7]. Its development can be prevented in many instances[8]. A family history of diabetes , an increase in BMI and impaired insulin secretion and action are important risk factors[9]. Studies from Urban India suggests that one in four adults over the age of 20 years has IGT or DM[10].

Type 2 DM causes long term damage , dysfunction and failure of various organs especially the eyes, kidneys, nerves, heart and blood vessels. Complications include both micro & macro angiopathy. Though macro angiopathy is the major cause of morbidity and mortality, the microvascular complications are more common. The incidence of type 2 DM is on the rise.

The mortality from cardiovascular disease and the incidence of non- fatal coronary heart disease is 2-4 times higher in patients with NIDDM than in normal subjects and is the major cause of death in these patients[11].

It is a proven fact that diabetes mellitus is an ongoing , chronic progressive disorder. Its progressive nature is not only in terms of worsening hyperglycaemia but also a relentlessly progressive nature of its various complications. Treatment should be based on underlying pathophysiology of diabetes mellitus not on symptoms, taking into account the insulin secretory capacity of pancreatic β cell and peripheral resistance to insulin action occurring primarily in liver, muscle and adipose tissue.

# OBJECTIVES

# OF

# THE STUDY

## OBJECTIVES OF THE STUDY

1. In type 2 DM patients, estimation of C-peptide levels to assess the level of insulin secretion in the body.

2. Obesity and C-peptide level correlation in patients with type 2 DM.

3. Treatment modification in type 2 DM patients based on C- peptide levels.

# REVIEW OF LITERATURE

# REVIEW OF LITERATURE

## HISTORY :

## INSULIN :

Insulin is one of the hormones known from ancient times. In 1869, Langerhans identified alpha and beta cells in islets of pancreas. In 1909, De Meyer named the hypothetical secretion from pancreas as Insulin from the word 'Insula' meaning island (i.e) Islet of Langerhans.

In 1922, Banting and Best extracted the insulin from pancreas. It was the first hormone to be isolated in pure form. Previous workers failed to isolate, as trypsin digested insulin beforehand. Banting and Best circumvented this problem by ligating the pancreatic duct and waiting for 7 weeks. This caused acinar cells producing trypsin to atrophy. For this Banting was awarded Nobel prize in 1923. In 1954 Sanger studied the aminoacid sequence of insulin; first protein with complete aminoacid sequencing done. He was awarded Nobel prize in 1958. Insulin was the first protein to be synthesized in 1964.

## C- PEPTIDE :

In 1967, Donsteiner and his co- workers[12] discovered the pro – insulin. In 1969, Rubenstein et al[13] detected C- peptide in human serum. Between 1973 and 1976, there is a considerable increase of

immunological studies. Chemically synthesized C-peptides, their derivatives and fragments allow exploring the immunological properties of human C-peptide[14]. The enzyme responsible for conversion of pro – insulin to insulin and C- peptide was discovered in 1990/1991[15].

**HAEMOGLOBIN A1C :**

HbA1c was first separated from other forms of haemoglobin by Huisman and Meyering in 1958 using a chromatographic column[16]. It was characterised as a glycoprotein by Bookchin and Gallop in 1968. Its increase in diabetes was first described in 1969 by Samuel Rahbar and his co-workers[17]. The use of HbA1c for monitoring the degree of glucose control in diabetic patients was proposed in 1976 by Anthony Cerami, Ronald Koenig and co-workers.

**INSULIN :**

Insulin has varied effects on every organ system in the body. Hence a brief overview of the structure and physiologic effect of insulin is needed, to understand the manifestations of diabetes.

## FORMATION AND SECRETION OF INSULIN :

### 1. STRUCTURE OF INSULIN :

Insulin is a protein hormone with 2 poly peptide chains; the A chain with 21 amino acids and B chain with 30 amino acids linked together by disulfide bridges. There is another intra chain disulfide bridges between $6^{th}$ and $11^{th}$ residues. There are minor differences in the amino acid composition from species to species. If insulin of one species is injected into another, it induces antibody formation.

### 2. ANATOMY OF THE ISLET CELLS :

The Islets of Langerhans are ovoid collections of cells scattered throughout the pancreas, more in tail than head or body. They constitute around 2% of volume of the gland. There are around 1-2 million islets. Each islet is 0.3mm in diameter surrounded by fenestrated capillaries. It contains 4 different types of cell types namely A, B, D and F. 'A' cells secrete glucagon; 'B' cells secrete insulin; 'D' cells secrete somatostatin and 'F' cells secrete polypeptide. B cells constitute 60% of the islet cells located in the centre of each islet. The B cell granules are packets of insulin with zinc aggregates[18].

## 1. SYNTHESIS OF INSULIN :

Insulin like other polypeptide hormone is synthesized as prepro insulin containing 108 amino acids with molecular weight of 11500 MW . The gene for insulin is located in the short arm of chromosome 11; has two introns and three exons. This has 23 – amino acid signal peptide (leader sequence) which is hydrophobic and is cleaved as it passes through cisternae of endoplasmic reticulum. Pro insulin so formed provides configuration necessary for disulphide bridges. The pro insulin contains 84 amino acids and is transported to golgi apparatus. The amino terminal of pro insulin is the B chain; then a connecting sequence called C-peptide . Pro insulin is cleaved to insulin & C-peptide by enzymes trypsin and carboxy peptidase – B. The site specific cleavages occur between arginine residues at position 31 and 32, as well as lysine and arginine at position 64 and 65 of the pro insulin. Equimolar concentrations of C-peptide with 33 amino acids and insulin with 51 amino acids are thus formed. Then they are packaged into secretory granules in the golgi apparatus. These granules mature as they traverse the cytoplasm towards the plasma membrane. About 95 % of pro insulin is converted into insulin within the secretory granules. Both pro insulin and insulin combine with zinc in the granules to form hexamers. (7)

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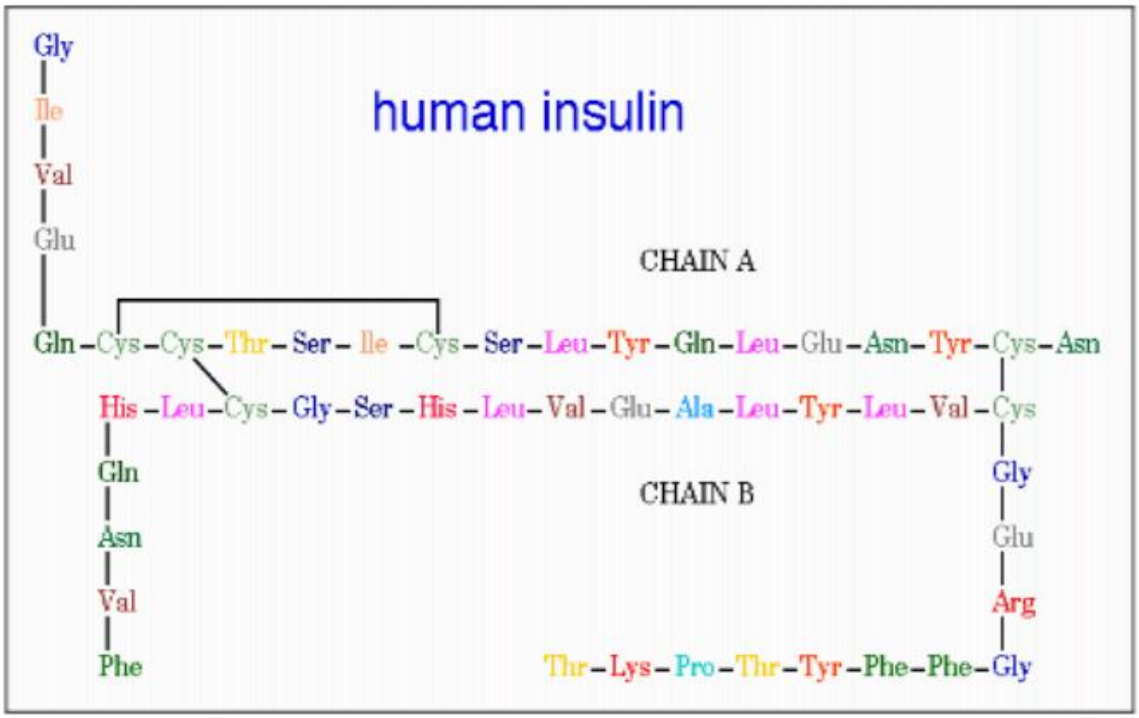
**Figure 1 STRUCTURE OF INSULIN**




**4) INSULIN SECRETION** : Insulin is released from β cells by the process of emiocytosis. Microtubules along with microfilaments ( actin &

myosin ) play an important role in insulin secretion. Glucose, the most important physiological substance involved in regulation of insulin release. Insulin secretion is greater after oral than after intravenous glucose by incretin effect.

Approximately 50 % of the total insulin secreted into the portal system is removed during initial passage through liver ( first pass effect) , which leads to 2.5 – 3 times greater concentration of insulin in the portal vein compared with a peripheral vein[19]. More over the fractional hepatic extraction of insulin is variable.

Insulin clearance also occurs in the kidney, with normal kidney removing nearly 40 % of insulin presented to it[20]. The pro insulin related peptides constitute 20 % of total circulating insulin like immune reactivity. It has been estimated that the biologic potency of pro insulin is only 10 % of insulin, under normal physiologic conditions. The in vivo effects of pro insulin are negligible.

**KINETICS OF INSULIN SECRETION :**

Glucose is the major physiological determinant of insulin secretion. Insulin release is stimulated by transport of glucose into β cells of the pancreas via glucose transporter ( GLUT 2). Glucose

stimulated insulin release is biphasic : Initial response is a transient rapid rise in release which terminates in 5-10 minutes - first phase ( acute insulin response ). This first phase response is important in maintaining normal glucose tolerance, suppresses glucose production in the body and prevents postprandial hyperglycemia. The second phase is the prolonged phase, which persists during stimulus of the high glucose[21].

**STIMULATORS OF INSULIN RELEASE :**

1. **Nutrients :**
    a. Glucose
    b. Amino acids - Arginine is a powerful stimulant. Leucine is also a potent stimulator[22].
    c. Fat regulation : Small increase in insulin secretion occurs following ingestion of medium chain fats.

2. **Hormonal :** Gut hormones like Gastric inhibitory polypeptide & GLP-1 may mediate the incretin effect by stimulating greater insulin release following oral ingestion rather than parenteral administration of glucose.

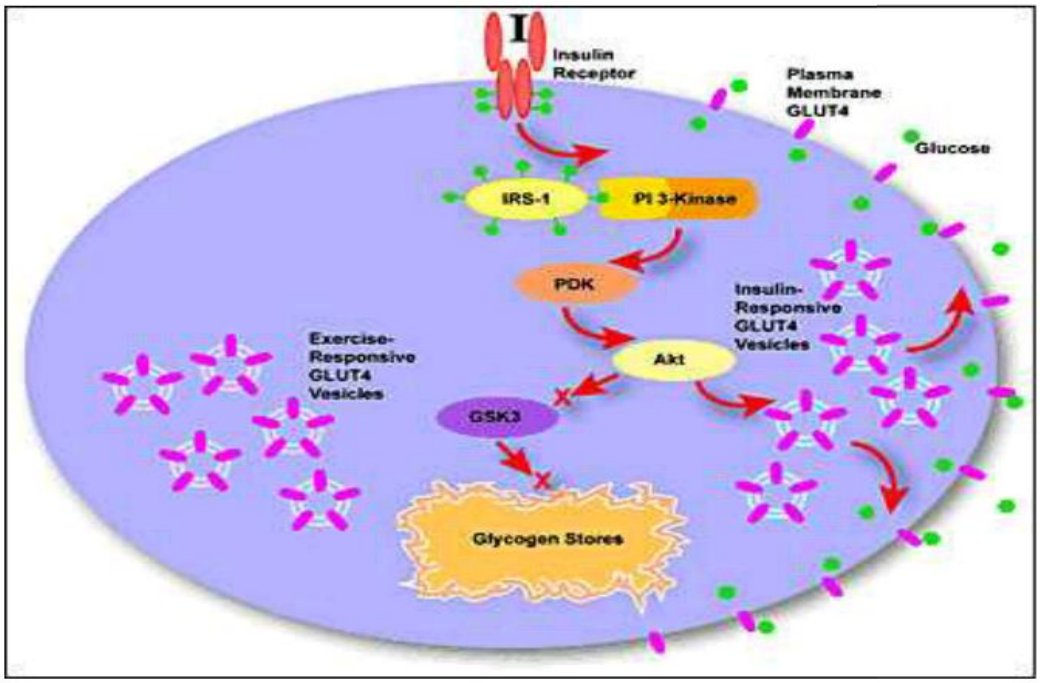
**Neural :** Hypothalamo - entero – insular axis via vagus nerve stimulates insulin release on sight, smell of food. Minimizes early rise of postprandial plasma glucose.

## CELLULAR ACTIONS OF INSULIN :

Insulin mediates its intracellular effect by binding to its receptor which is present on the external surface of cell. The receptor is a glycoprotein with 2α and 2β subunits.

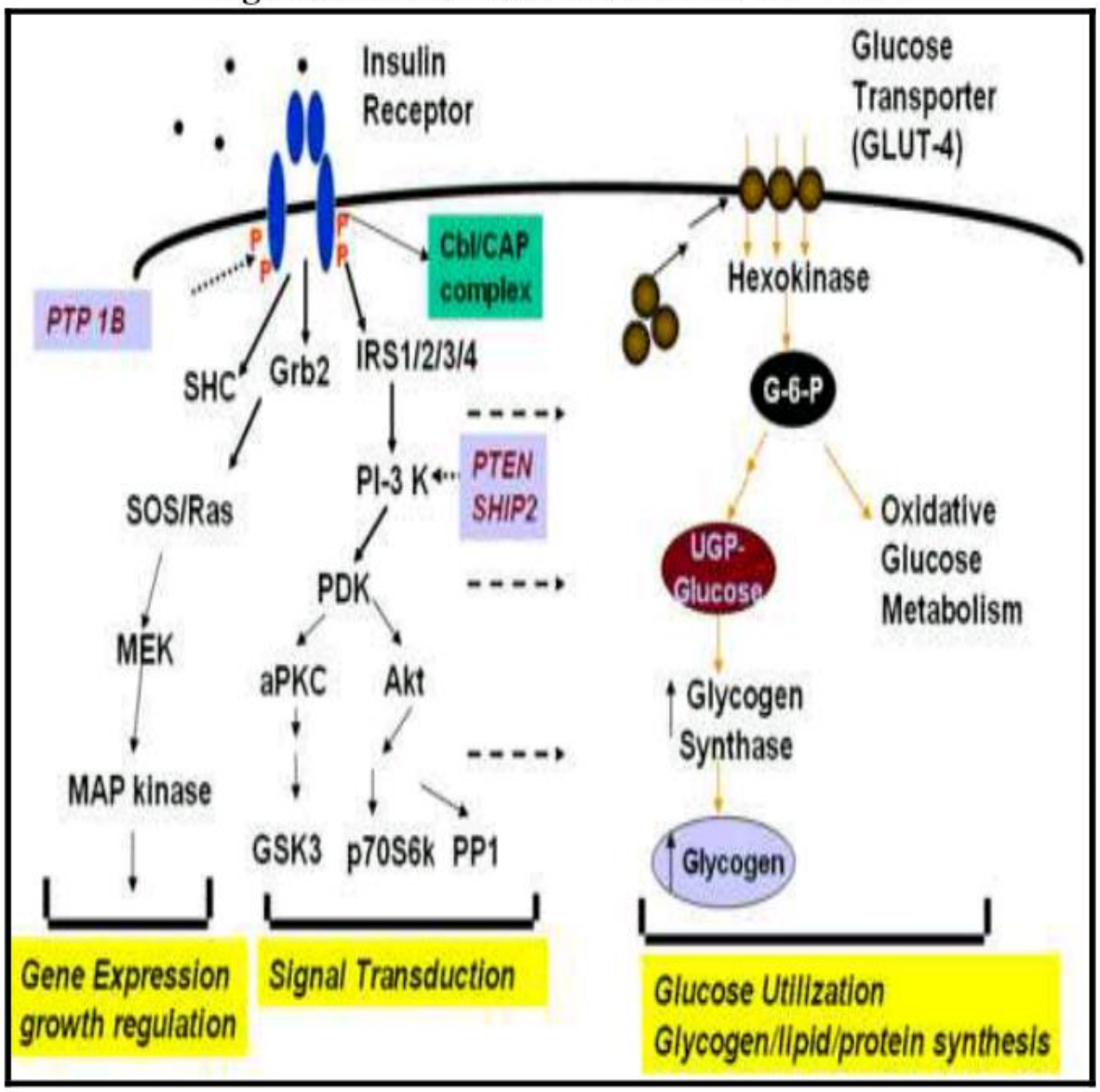
After binding to the receptor site present in α subunit, insulin activates the β subunit's protein kinase activity leading to phosphorylation of several tyrosine residues[24], which mediates multiple intracellular events. As an example, activation of PI-3 kinase pathway stimulates translocation of glucose transporters (GLUT 4) to the cell surface.

**Figure 2 INTRACELLULAR ACTION OF INSULIN**



## DIAGRAM DEPICTING EFFECTS OF INSULIN ACTION

**Figure 3 EFFECTS OF INSULIN ACTION**



# PHYSIOLOGICAL ACTIONS OF INSULIN

## EFFECT ON CARBOHYDRATE METABOLISM :

The entry of glucose into muscle and adipose tissue via Glucose Transporters ( GLUT 4) is mediated by insulin except in nervous tissue. It increases rate of entry of glucose into the muscle 15 fold. In the liver insulin induces glucose uptake. Infact most of the glucose absorbed from GIT is taken up by liver and converted to glycogen. In between meals, this glycogen is converted back to glucose and released into the circulation. Thus glucose level is maintained within a constant range.

Glucose uptake and storage in the liver occurs with the help of insulin by multiple mechanisms :

1. An enzyme phosphorylase pertaining to the liver is inactivated - this prevents glycogenolysis.
2. Increases glucokinase activity, which phosphorylates glucose and hence the phosphorylated glucose cannot diffuse out of the liver.
3. Insulin increases activity of the enzyme glycogen synthetase and hence glycogen synthesis.

**EFFECT ON FAT METABOLISM :**

Insulin has several effects that lead to fat storage in adipose tissue. Insulin increases glucose utilisation by most of the body's tissues, which automatically decreases fat utilisation. So insulin acts as a fat sparer and promotes fatty acid synthesis. This happens when more carbohydrates are consumed than which is needed for energy, thus providing substrate for fat synthesis. This is achieved by conversion to pyruvate, which is then converted to acetyl COA the substrate from which fat can be synthesised. The triglycerides thus formed are incorporated into lipoproteins mainly VLDL and transported to the blood from where they are taken up by other cells, especially the adipose tissue.

Insulin activates lipoprotein lipase, which increases triglyceride uptake into fat cells. Insulin has two more effects pertaining to fat metabolism :

1. It inhibits hormone sensitive lipase.
2. Insulin promotes glucose transport through the cell membrane into fat cells. This forms L – glycerol phosphate which supplies the glycerol that combines with fatty acids to form triglycerides.

## EFFECTS ON PROTEIN METABOLISM :

Insulin causes active transport of many aminoacids into the cells. Thus, it plays an important role in protein anabolism and positive nitrogen balance, by favouring protein synthesis and preventing its degradation.

## SIGNIFICANCE OF C- PEPTIDE

C- peptide is used as a measure of insulin secretion in humans. Because serum insulin level is affected by various factors like exogenous insulin administration and significant amount of secreted insulin undergoes hepatic extraction before it appears in the peripheral blood[32], while secreted C- peptide undergoes only minimal change and its level in the peripheral blood equals to that of in the portal blood.

## CHEMISTRY AND SYNTHESIS :

Human insulin and C- peptide are synthesised as a single polypeptide chain known as proinsulin in the pancreatic islet by the

beta cells. Proinsulin is cleaved proteolytically to form equimolar amounts of mature insulin and C- peptide which are released into the portal vein.

It is called as C- peptide because it connects the A and B chains of insulin. C – peptide is a single peptide chain of 31 aminoacids with molecular weight of 30,200. Half life of C- peptide is about 30 minutes.

**Figure 4 STRUCTURE OF C-PEPTIDE**

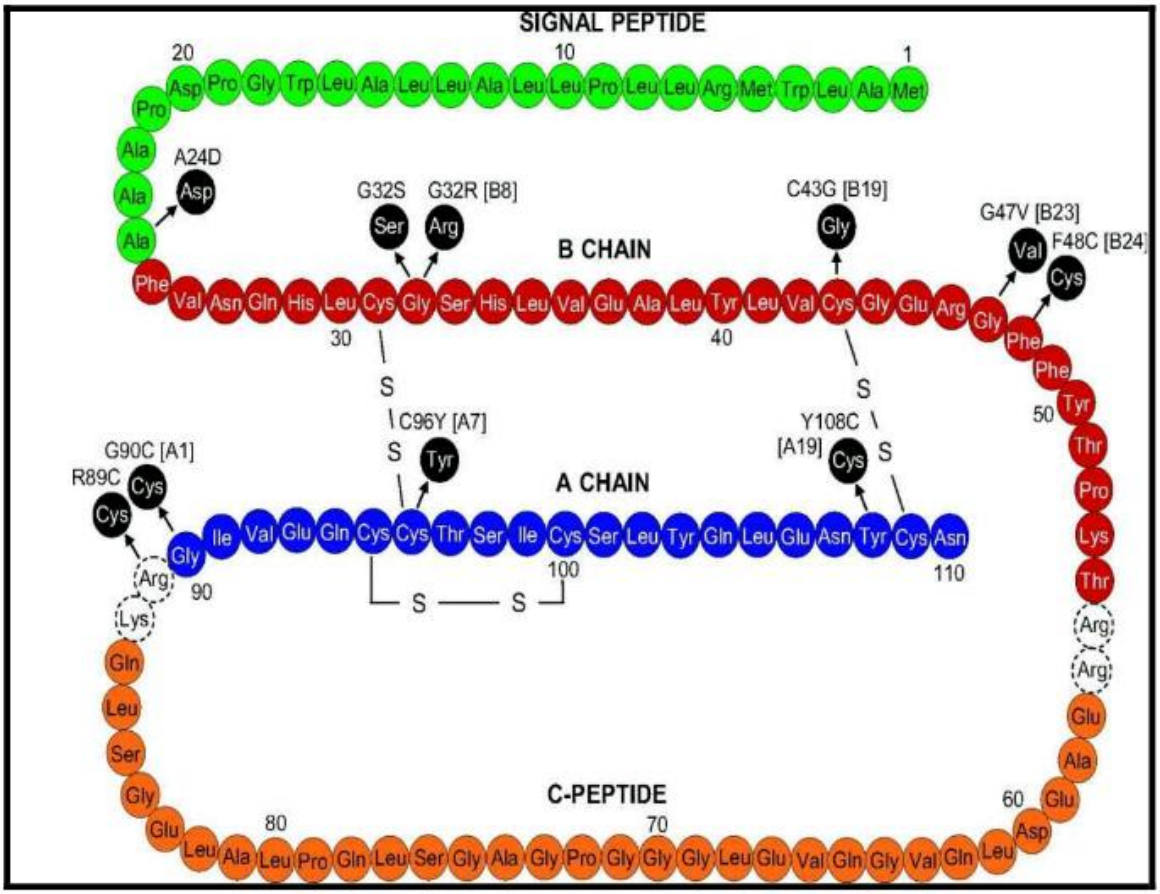
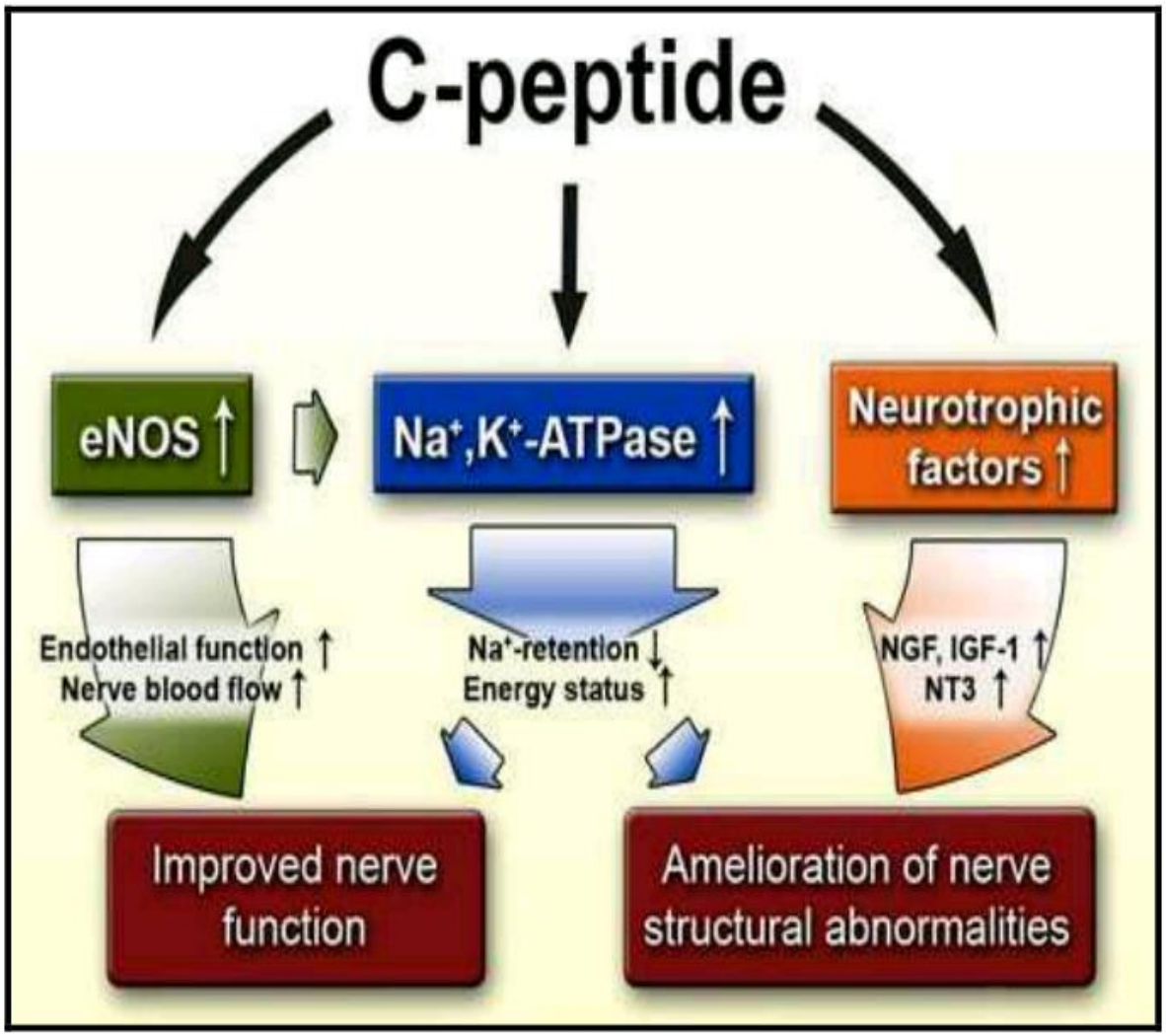


**Figure 5 - PHYSIOLOGICAL EFFECTS OF C – PEPTIDE :**




C – peptide has previously been considered to be without intrinsic physiological effects. However recent studies have suggested that C – peptide in physiological concentrations increases glucose transport and glycogen content in skeletal muscle[33]. (18)

In IDDM patients, short term 2 hour infusion of C – peptide to a steady state plasma level of 0.8 nmol/l (2.45 ng/ml ) , significantly increases the whole body glucose uptake by about 25 %. A further uptake of 15 % of glucose was observed when the plasma C – peptide level was increased to 2.1 nmol/l (6.34 ng/ml ). Glomerular hyperfiltration was reduced by 7 %[34]. Whether the effect on glucose metabolism reflects increased glucose utilisation or suppression of endogenous hepatic glucose production is unknown, but enhanced peripheral glucose utilisation seems to be one possible explanation, since glucose uptake increases in the forearm during C- peptide infusion[35]. This suggest that C – peptide is of physiological importance for the maintenance of normal blood flow and capillary function and that C – peptide may have a role in the regulation of carbohydrate metabolism.

C- peptide is not biologically inert as previously believed. The intracellular signal transduction involves the activation of calcium dependent signalling pathways, with subsequent stimulation of Na+ - K+ ATPase and Enos activities[36]. Both of these systems show attenuated activities particularly in renal and nervous tissue. There is now evidence to indicate that replacement of C – peptide in type 1 diabetes is accompanied by improved renal function, as evidenced by correction of glomerular hyperfiltration and amelioration of nerve dysfunction[37]. (19)

**KINETICS AND THE VALIDITY OF C – PEPTIDE AS A MARKER OF INSULIN SECRETION :**

Plasma C – peptide concentrations can be used to determine the insulin secretion rates. The validity of C- peptide measurements as an indicator of beta cell secretion depends on following assumptions :

A) C – peptide and insulin are secreted in equimolar quantities , the C-peptide : insulin ratio approximates 1.0[38]

B) Hepatic extraction of C – peptide is negligible under physiologic conditions [39]. In humans, portal to peripheral C – peptide ratio is 1.4 in the fasting state.

The overall kinetics of C – peptide is known under the condition studied, and the behaviour of C- peptide in the systemic circulation is such that, its rate of appearance can be predicted from its plasma concentrations[40]. The plasma C – peptide has a half life of approximately 30 minutes.

**URINARY C- PEPTIDE AS A MEASURE OF INSULIN SECRETION**

It has been proposed as an indicator of endogenous insulin secretion, because in both lean and obese non – diabetic subjects and in most studies of patients with NIDDM,

urinary C- peptide has been found to correlate with plasma C- peptide levels. Further more in children with IDDM urinary excretion may be the parameter of choice as it can be measured by non – invasive procedures [41]. Several studies also indicate that urinary C – peptide estimation can be a more sensitive indicator of residual beta cell function in patients with IDDM of long duration.

The relatively high urinary clearance of C- peptide and the absence of significant hepatic uptake, suggest that urinary C- peptide might be a reliable indicator of the amount of insulin secreted over specific time intervals. Small amount (5 % ) of secreted C- peptide appears in the urine and so decrease in GFR results in decline in urinary C- peptide clearance leads to a large change in the quantity of urinary C- peptide.

**CLNICAL APPLICATION OF C- PEPTIDE :**

C- peptide has been the most important research tool in the study of natural course of beta cell destruction and of therapeutic interventions to arrest or delay this process[42]. Most IDDM patients have residual beta cell function at the onset of their disease and all continue to secrete insulin during the first 2 years.

Thereafter the prevalence of residual beta cell function declines to about 10 – 15 % after approximately 5 years and remains at this level in patients with upto 40 years duration.

The age at onset of the disease is the most important variable in predicting the duration and magnitude of residual beta cell function[43]. Thus, the younger the subjects are, at disease onset, the shorter is the beta cell survival. During the first 2 years after diagnosis, a faster reduction in residual beta cell function has been demonstrated in patients having islet cell antibodies positive and in males[44] as opposed to females. After initiation of insulin therapy and subsequent metabolic stabilisation, most diabetic subjects experience an improvement in beta cell function. On average, beta cell function doubles in magnitude after 7 – 14 days of conventional insulin treatment. In most patients, maximal beta cell function is observed after 1 – 6 months of insulin treatment. The patients with highest concentration at onset will display the best degree of beta cell function during the first 1- 2 years after diagnosis[45]. The clinical remission is a result of both an improvement in endogenous insulin secretion and an enhancement in insulin sensitivity.

On the other hand, remaining beta cell function has been found to protect the blood retinal - barrier function more effectively (22)

than good metabolic control[46]. A significant inverse relationship between residual beta cell function and the degree of retinopathy has been observed.

**USE OF C- PEPTIDE IN CLASSIFICATION AND PREDICTION OF INSULIN REQUIREMENT :**

It is important, both for scientific and for therapeutic purposes to monitor the beta cell function in diabetic patients. The classification of newly diagnosed patients with DM is normally without problems in young patients with ketoacidosis. There are however patients with less straight forward classification, usually middle aged or elderly patients. Even when these patients present with hyperglycaemia and ketoacidosis, some can be managed without insulin after treatment of the initial metabolic decompensation[47]. Other patients may progress towards insulin dependency, but cannot initially be distinguished from other NIDDM patients.

A group of NIDDM patients may need insulin to control symptomatic hyperglycaemia. In other patients starting insulin treatment during periods of intercurrent disease, insulin is often continued unnecessarily. This heterogeneity makes it difficult to classify patients according to clinical criteria.

Measurement of C- peptide after beta cell stimulation appears to be a useful aid for decisions regarding DM classification and clinical management.

**OTHER USES OF C- PEPTIDE :**

**a) IN DIFFERENTIAL DIAGNOSIS OF HYPOGLYCAEMIA** :

Values will be low if a person has taken an overdose of insulin but not suppressed if hypoglycaemia is due to an insulinoma or sulphonylureas.

**b) IN POLYCYSTIC OVARIAN SYNDROME** : To determine the degree of insulin resistance .

**c)** To monitor insulin production after the removal of a tumor of the pancreas ( insulinoma ) .

## GLYCATED HAEMOGLOBIN : HbA1c

Glycated haemoglobin comprise HbA1 and other haemoglobin- glucose adducts, whereas HbA1 is made up of HbA1a, HbA1b and HbA1c. HbA1c is the major component of HbA1, (24)

accounting for 80 %. Glycated proteins are formed post translationally from the slow, non- enzymatic reaction between glucose and amino groups on proteins.

Table showing HbA1c values indicating the various stages of glycaemia :

| HbA1c targets | % |
|---|---|
| **Normoglycaemia** | 4 – 6.0 |
| **At high risk of diabetes** | 5.7 – 6.4 |
| **Diabetes** | 6.5 |
| **Diabetes with good glycaemic control** | 7.0 |
| **Diabetes at higher risk of hypoglycaemia** | 7.5 |

Recently the ADA, the European Association for study of Diabetes and the WHO have recommended the use of HbA1c levels $\geq 6.5\%$ for diagnosing diabetes mellitus. Levels between 5.7 – 6.4 % indicate increased risk for diabetes.

ADVANTAGES of using HbA1c :

- Random sampling of whole blood. (25)

- High biological stability.
- Unaffected by acute factors such as stress, physical activity.
- Index of long term status of glycaemia[48]
- Predicts development of vascular complications of diabetes[49]
- Helps to guide treatment.

**DISADVANTAGES :**

Affected by various factors such as factors influencing RBC life span (hemolysis), erythropoiesis (iron, vitamin B12 deficincy), chronic liver or renal problems, splenectomy.

**GOAL** in type 2 DM patients on therapy, aims to keep HbA1c < 7% and in patients having HbA1c > 8% , treatment regimen should be modified.

**MONITORING OF HbA1c :** Testing should be done at least two times a year in patients meeting treatment goals and more frequently in patients whose therapy has changed or who are not meeting glycaemic goals[50].

Hence it is best to continue using HbA1c as a monitor for effectiveness of glycaemic therapy and indicator for when therapy needs to be modified[51].

**INSULIN RESISTANCE :**

Condition in which body's cells become resistant to the effects of insulin. That is the normal response to a given amount of insulin is reduced. As a result, high levels of insulin are needed in order to have its proper effects[52]. So the pancreas compensates by trying to produce more and more insulin until the pancreas can no longer produce sufficient insulin towards the body's demands, then blood sugar rises[53].

**CONTRIBUTION OF INSULIN RESISTANCE IN SKELETAL MUSCLE:**

In a normal person, 75 % of glucose in carbohydrate meal is taken up by the muscle and stored as glycogen. In insulin resistant patients given supraphysiologic insulin infusion, glycogen synthesis in skeletal muscle is decreased more than oxidative metabolism. When physiologic insulin levels are maintained, both muscle glycogen storage and glucose oxidation are impaired[54.]The insulin receptor levels and tyrosine kinase activity in skeletal muscle are reduced, but these alterations are secondary to hyperinsulinemia and are not a primary defect.

**ABNORMAL INHIBITORS :** Insulin resistance in NIDDM may be the result of inhibition of insulin action. Inhibition of insulin receptor (tyrosine kinase), by a membrane glycoprotein called PC – I[55], was increased in fibroblasts of patients with typical NIDDM.

**INSULIN RESISTANCE IN ADIPOCYTES :**

Insulin resistance in adipose tissue may play a role in the obese diabetic patients. The cellular content of GLUT 4, the facilitative glucose transporter of adipocytes and skeletal muscle is reduced by 40 % in obese patients without NIDDM and by 85 % in obese patients with NIDDM. The increased fat cell mass of obesity in addition to being intrinsically insulin resistant may export insulin resistance to muscle both by releasing FFA into general circulation and by infiltrating muscle with adipocytes ( marbleization of muscle ). The concentration of glycerol in intrinsic fibres of muscle are 42 times higher than in plasma[56], making it likely that marbleized muscle of obese individuals is exposed to high levels of substrate released from adipocytes such as FFA and TNF – alpha.

**FREE FATTY ACIDS :**

Elevated plasma FFA in obesity can cause insulin resistance[57], probably by inhibiting muscle glucose metabolism and

inducing hyper insulinemia through upregulation of low km glucose metabolism in islets[58]. Fat cells store fuel in the form of TG's at times of metabolic abundance and release FFA and glycerol when food is not available. FFA release conserves the dwindling supply of glucose, the essential fuel for CNS, by substituting for glucose as fuel and thereby limiting glucose utilisation in tissues for which it is not essential (i.e. skeletal muscle )[59]. Because glucose is spared in times of glucose need, the increase in fatty acyl CoA in tissues prolongs the survival time of starving organism.

**TNF alpha :**

It has important effects on whole body lipid and glucose metabolism. TNF alpha can cause many changes seen in insulin resistant state[60]. It downregulates GLUT 4 mRNA levels, inhibits glucose transport, impairs the tyrosine kinase activity of insulin receptors, by lowering its autophosphorylation and decreases the phosphorylation of IRS – 1 through activation of phosphotyrosine phosphatase[61].

So, insulin resistance associated with obesity accounted for increased levels of FFA and TNF alpha. However insulin resistance in lean glucose tolerant relative of NIDDM patient cannot be explained on this basis and probably involves defect in skeletal muscle.

## OBESITY AND INSULIN RESISTANCE

Obesity induced hyperinsulinemia results from both an expansion of beta cell mass and from an increase in low Km gene metabolism in enlarged islets. A hyperinsulinemic obese patient becomes overtly diabetic, only if the beta cells do not maintain the hyperinsulinemia at a level sufficient to counteract insulin resistance.

## RELATION BETWEEN BETA CELL DYSFUNCTION AND PERIPHERAL INSULIN RESISTANCE IN TYPE 2 DM :

Islet dysfunction and peripheral insulin resistance coexist in overt NIDDM as noted previously. First possibility is that insulin resistance is the primary lesion and causes a secondary defect in beta cell function through exhaustion[62]. Second possibility is hyperinsulinemia could be the initial lesion and insulin resistance, a secondary defence against hyperinsulinemia. A third possibility is that both the insulin resistance and beta cell changes could be secondary to a common abnormality such as high levels of FFA.

The co- existence of high FFA and hyperinsulinemia is a evidence of resistance in adipocytes to the antilipolytic action of insulin, suggesting that a primary antilipolytic insulin resistance in adipocytes causes a secondary glucoregulatory insulin resistance in muscle.

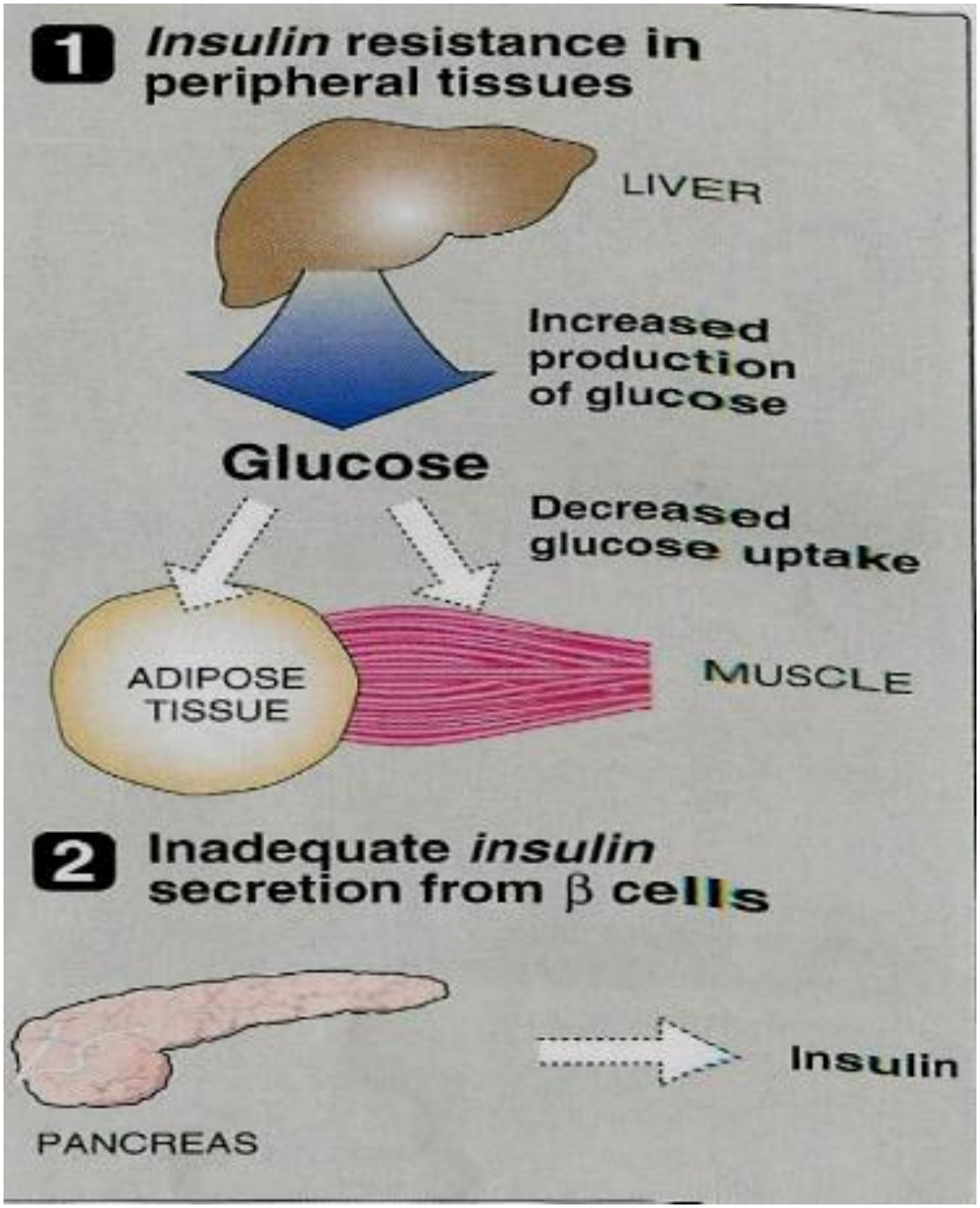
## PATHOPHYSIOLOGY

The cardinal manifestation of DM is hyperglycaemia and results from :

1. Inadequate insulin secretion from β cells of pancreas.
2. Insulin resistance in peripheral tissues

Leads to increased glucose production from liver and

decreased glucose uptake in cells particularly muscle and adipose tissue.



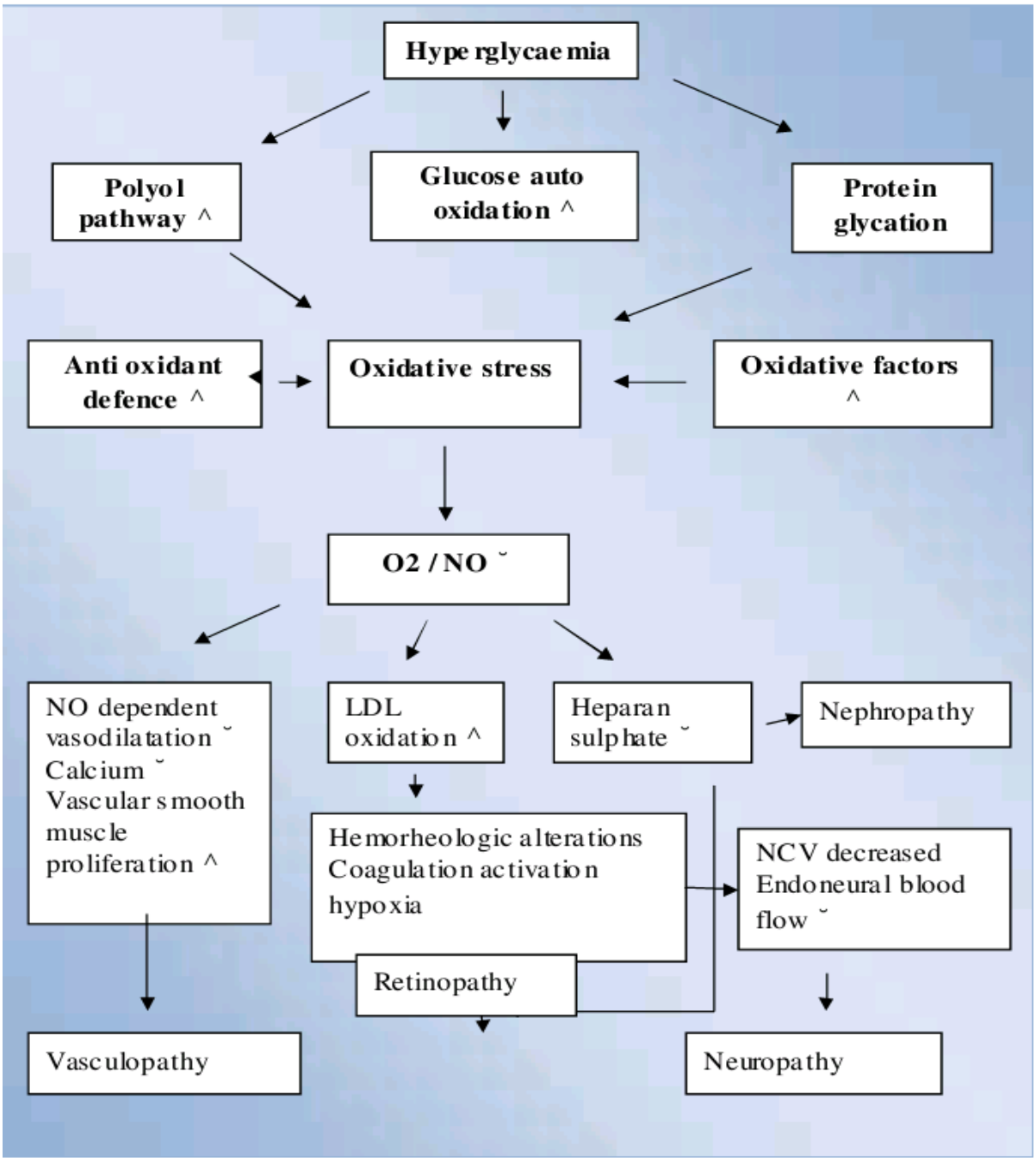

Polyuria, polydipsia and weight loss occurs. Insulin deficiency leads to high levels of plasma glucose. After a certain level ( > 180 mg/dl ) the maximum level of renal tubular reabsorption of glucose is exceeded and sugar is excreted in urine. The urine volume is increased owing to osmotic diuresis and coincident

obligatory water loss. This inturn leads to dehydration, thirst and excessive drinking. Glycosuria causes substantial loss of calories (i.e.) 4.1 K cal/gm of glucose excreted, thus resulting in weight loss.

In the absence of insulin, catabolic activity of protein and lipids predominates. When the liver's ability to oxidise FA to carbondioxide is exceeded, beta hydroxy butyric acid and acetoacetic acid accumulate. Initial compensation occurs by increasing respiratory loss of carbon dioxide, but if unchecked leads to diabetic ketoacidosis.

**PATHOGENESIS OF DIABETIC COMPLICATIONS :**

The links between hyperglycaemia induced oxidative stress and diabetic complications[64] are as follows :




**PATHOGENESIS OF DIABETIC COMPLICATIONS**

## ROLE OF POLYOL PATHWAY - DM and atherosclerosis :

Both are associated with high incidence in females. MI, CVA and peripheral vascular occlusive disease can occur[65]. (34)

**DIABETIC MICROANGIOPATHY :**

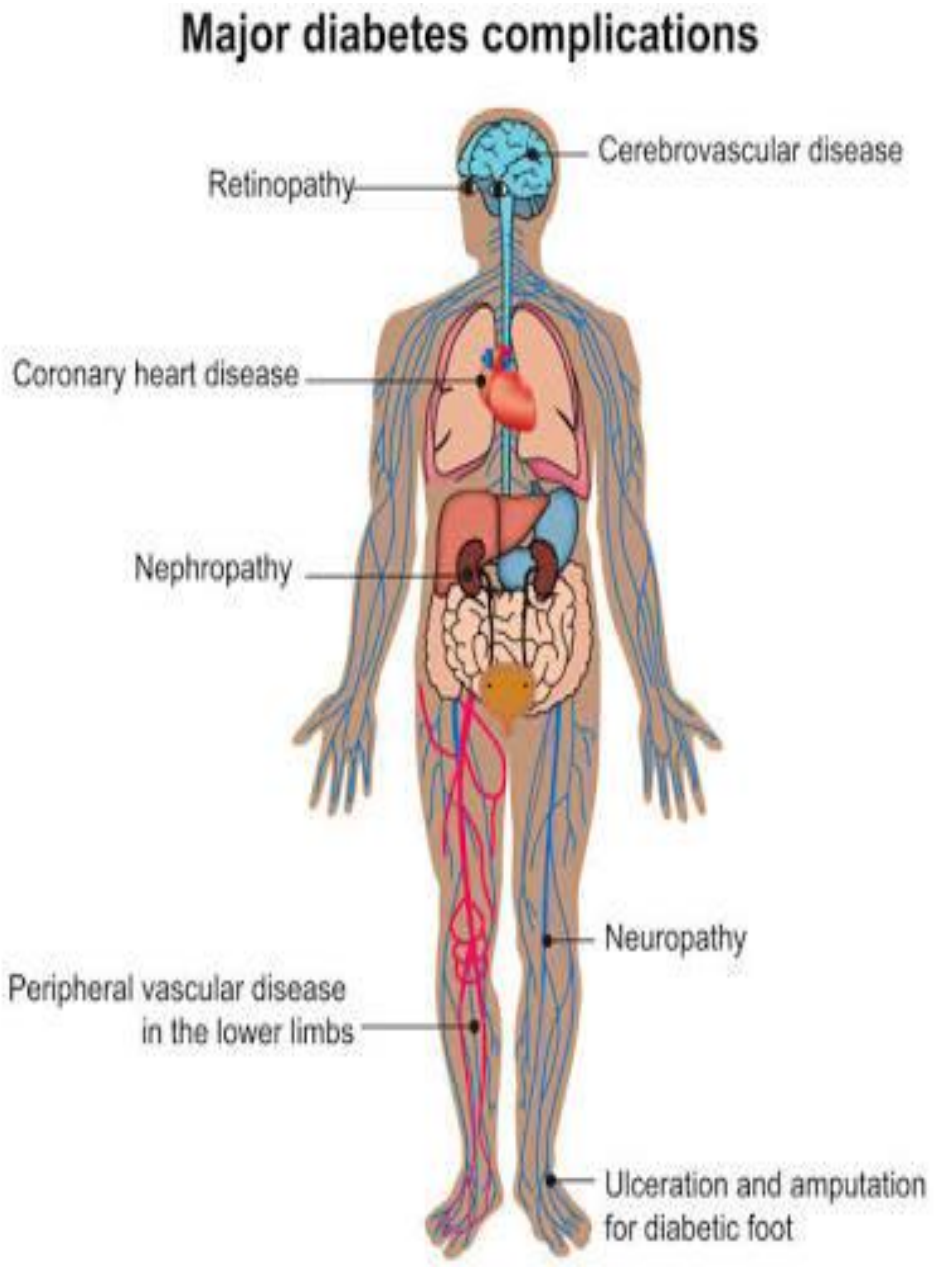
It affects small vessels, especially of kidneys and eyes. It affects capillaries, occasionally arterioles and venules[66]. Thickening of capillary walls by deposition of PAS positive material beneath endothelial cells. It is seen in all tissues except adipose.

**DIABETIC NEPHROPATHY :** It is the commonest manifestation of microangiopathy. 30 – 40 % of type 1 and 15 – 20 % of type 2 patients develop nephropathy. Typical lesion is glomerulosclerosis ( diffuse or nodular ). Nodular glomerulosclerosis present as Kimmelsteil Wilson lesion [67] due to hyaline deposition in mesangium.

**DIABETIC RETINOPATHY :** Due to selective degeneration of pericytes from retinal capillaries[68].

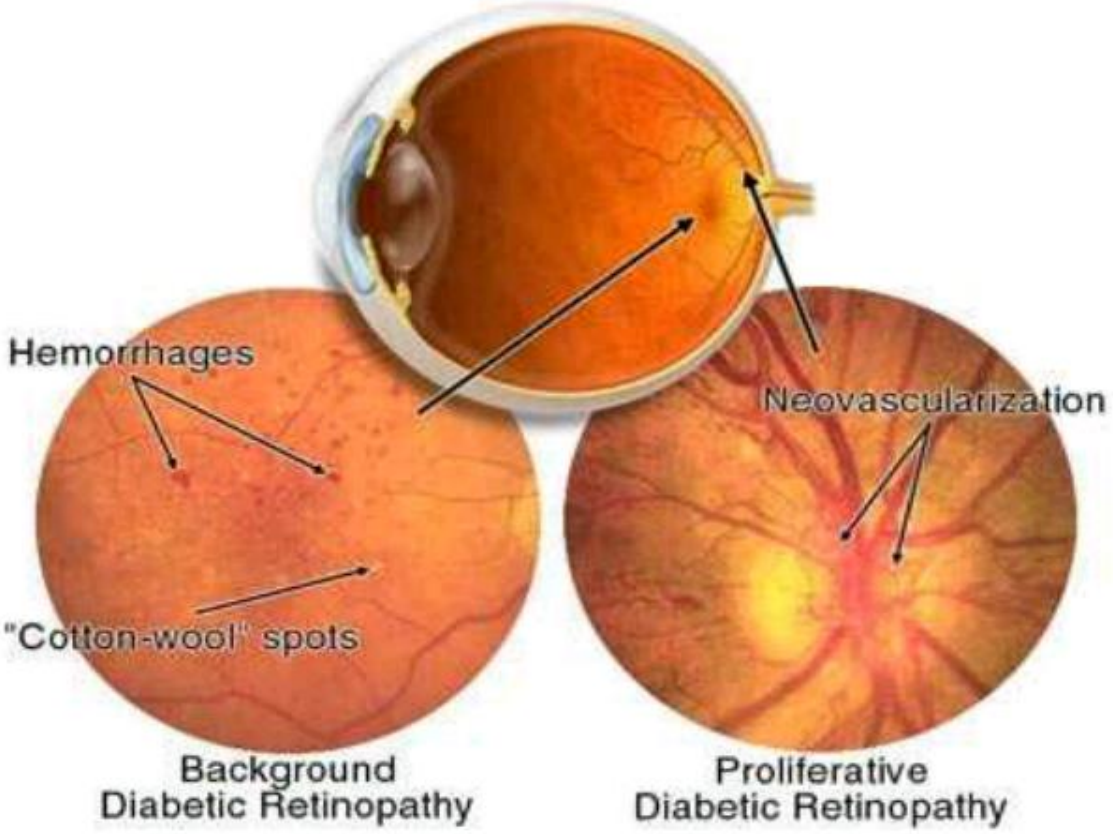
**DIABETIC NEUROPATHY :** Peripheral nerves are involved due to occlusion of vasanervosum. There is symmetric distal polyneuropathy due to Schwann cell injury causing myelin degeneration.

Figure 6




The above diagram showing complications of diabetic mellitus : Neuropathy, nephropathy, retinopathy, stroke, peripheral vascular disease and coronary artery disease.

**Figure 7 DIABETIC RETINOPATHY**




The above diagram depicting features of diabetic retinopathy ( proliferative & non- proliferative retinopathy ) like hemorrhages, cotton wool spots and Neovascularisation.

## DIAGNOSIS OF DIABETES MELLITUS :

### Criteria for the diagnosis :

1) Symptoms of diabetes plus random blood sugar concentration ≥ 200 mg/dl or
2) Fasting plasma glucose ≥ 126 mg/dl or
3) Two hour plasma glucose ≥ 200 mg/dl during an OGT.

## IMPAIRED GLUCOSE TOLERANCE :

1) Fasting plasma glucose : 100 – < 126 mg/dl
2) Two hour plasma glucose : 140 – <200 mg/dl during an OGT.

## MANAGEMENT OF TYPE 2 DM

The goals of treatment in type 2 DM is to control symptoms, achievement of fasting blood glucose < 126 mg/dl, HbA1c < 7 % and management of complications due to long standing DM. The UKPDS demonstrated that intensive blood glucose control using oral hypoglycaemic agents and insulin can substantially decrease the risk of the micro vascular complications like retinopathy, (38)

neuropathy and nephropathy in adults with type 2 DM.

However the UKPDS did not reveal any significant decrease in macro-vascular complications through the control of blood glucose alone.

**GENERAL PRINCIPLES :**

**DIET AND EXERCISE :**

Lifestyle modifications are important components of the treatment of type 2 DM. Weight loss improves insulin sensitivity in obese non-diabetic subjects[69]; This has also been shown to ameliorate insulin resistance, but may not restore beta cell dysfunction[70]. UKPDS study showed that the effect of diet therapy was transient, reducing plasma glucose levels and TG's, increasing HDL cholesterol[71].

**PHARMACOLOGICAL THERAPY :**

1. Insulin,
2. Insulin secretagogues,
3. Insulin sensitizers,
4. α - glucosidase inhibitors[72] ,
5. Bile acid sequestrants and
6. **Newer drugs :** Amylin analogue, GLP-1 analogue, DPP 4 inhibitors and SGLT-2 inhibitors.

**INSULIN :**

**1.Rapid acting** - Lispro, Aspart and glulisine.

**2.Short acting** - Regular insulin. Given 30 minutes before a meal.

**3.Intermediate acting** - Isophane insulin ( NPH ). They are given twice a day.

**4.Long acting - Glargine and Detemir** ; They are peakless insulin and provide a basal level of insulin for 24 hours[73].

**INSULIN SECRETAGOGUES :**

Two types, sulfonylureas and non- sulfonylureas.

**SULFONYLUREAS :**

**1st generation** : Tolbutamide and chlorpropamide.

**2nd generation** : Glyburide, glipizide, glimepiride[74] ,

glycazide and glibenclamide.

Their principal mechanism of action is the stimulation of insulin secretion from the pancreatic beta cells in response to glucose[75]. Hypoglycaemia is the important side effect.

**NON- SULFONYLUREAS - Repaglinide and nateglinide[76]**

These agents are taken 30 minutes before food. Hypoglycaemia and weight gain are side effects .

**INSULIN SENSITISING AGENTS :**

Biguanides and thiazolidinediones.

**Biguanides :** Metformin.

Acts by inhibiting hepatic glycogenolysis[77] , thus decrease glucose production from the liver. May be used as monotherapy at diagnosis of type 2 DM. Best taken with meals to minimize GI upset .

**Thiazolidinediones :** Rosiglitazone and Pioglitazone.

Acts by binding to PPAR, thus improving sensitivity of liver & tissues to insulin[78]. They decrease FFA and lower TG concentration[79] . They cause weight gain. Liver disease is under scrutiny and peripheral edema may occur.

**GLUCOSE ABSORPTION INHIBITORS :**

They competitively inhibit the alpha glucosidase in the brush border of enterocytes of the GIT, preventing the breakdown of oligo and disaccharides into monosaccharides[80].

DRUGS : **Acarbose, miglitol and voglibose.**

Adverse effects are bloating, abdominal discomfort, diarrhoea and flatulence[81].

**NEWER DRUGS :**

**AMYLIN ANALOGUES :** They are non- insulin injectables.

**Pramilintide** is a synthetic analogue co secreted with insulin.

**Mechanism of action :**

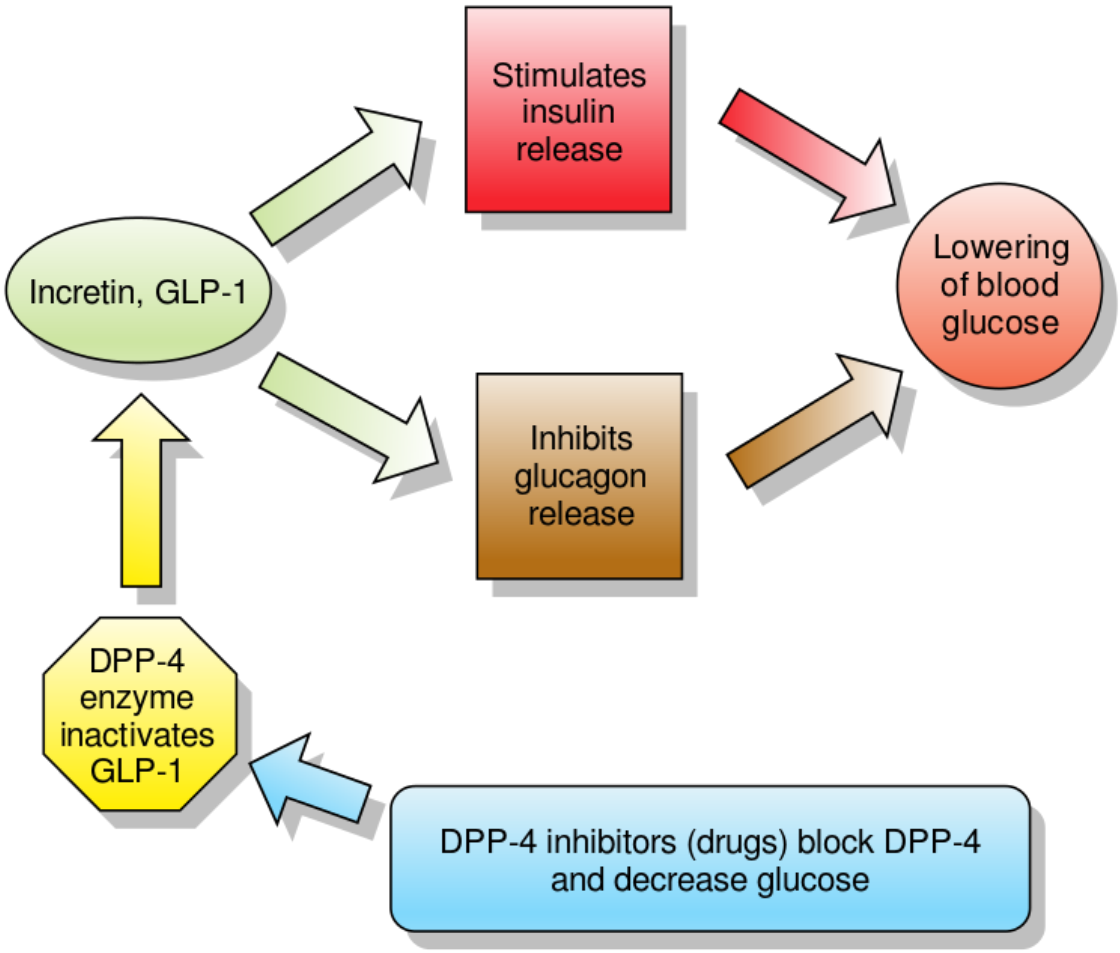
1. Gastric emptying rate is slowed, thus reducing postprandial rise in blood sugar.
2. Reduce glucagon levels post prandially
3. Inhibits Ghrelin, leads to early satiety[83]

**Adverse effects :** Nausea, vomiting, loss of appetite and hypoglycaemia.

**INCRETIN MIMETICS :**

Incretins are insulin secretagogues. They are Glucagon like peptide – 1(GLP-1) and gastric inhibitory peptide (GIP). Both are rapidly inactivated by the enzyme DPP- 4 [84].

Figure 8 - ACTION OF INCRETIN




**GLP –1 ANALOGUE :** Bind to a membrane GLP – 1 receptor. As a result, it inhibits glucagon secretion and stimulates insulin release from pancreatic beta cells. These analogues are not degraded by DPP- 4. Analogues are **Exenatide, Liraglutide and Taspoglutide.**

**DPP – 4 INHIBITORS :** Increase blood concentration of the incretin, GLP- 1 by inhibiting its degradation by the

enzyme DPP- 4.

**Drugs used are :** sitagliptin, saxagliptin, vildagliptin, linagliptin.

**BILE ACID SEQUESTRANTS :** Colesevelam

Used as an add on therapy for type 2 DM.

Initially approved to lower LDL cholesterol; then approved for use in type 2 DM in 2008.

**Dose :** 1875 mg orally twice a day with meals.

**SGLT- 2 ( Sodium** glucose transporters – 2 ) inhibitors :

Block the re-uptake of glucose in the renal tubules, promoting loss of glucose in the urine. This causes both mild weight loss and a mild reduction in blood sugar levels with little risk of hypoglycaemia.

**Side effect :** Urinary tract infection.

DRUGS : **Canagliflozin and Dapagliflozin**.

# METHODOLOGY

**SELECTION OF CASES :**

50 type 2 DM patients who attended the diabetic outpatient clinic of our hospital were randomly selected for the study, then methods, procedures of the study were adequately explained to the patient and consent obtained. The study subjects were asked to come in the fasting state, after cessation of taking oral medications for type 2 DM for 3 days. Then sample for FBS & C- peptide were taken in the fasting state and its level estimated.

**INCLUSION CRITERIA :**

Type 2 diabetes mellitus patients.

**EXCLUSION CRITERIA** :

A. Type 1 diabetes mellitus patients.

B. Patients with acute infections, renal failure and pregnancy.

## ESTIMATION OF C –PEPTIDE

For estimation of C- peptide two methods are available :

1) **ELISA** ( Enzyme linked immuno sorbent assay )
2) **CLIA** ( Chemi luminescent immunoassay ) - For serum C- peptide level estimation in quantities.

**PROCEDURE OF CLIA :**

It is based on a solid phase ELISA. In this test, microtiter well coated with anti C – peptide antibody and horseradish peroxidase enzyme labelled with anti-C-Peptide antibody is used. To the anti-C-Peptide antibody coated microtiter wells, the standards and test specimen are added. Then the enzyme labelled anti-C-Peptide antibody is added to the well. If C- peptide is present in the serum then it forms a sandwich between the solid phase and enzyme-linked antibodies. Then chemi luminescent substrate is added and read in a Luminometers.

Amount of C-peptide present in the sample is estimated by the intensity of emitting light measured by Luminometers.

**MATERIALS REQUIRED :**

Anti-C-Peptide Antibody Coated Microtiter Wells - 96 wells

Enzyme conjugate reagent - 12 ml

Reference Standard : 0, 0.5, 1.0, 2.0, 5.0, and 10.0 ng/ml, liquid, ready for use - 1 set

50x Wash Buffer - 15 ml

Chemiluminescence Reagent A - 6.0 ml

Chemiluminescence Reagent B - 6.0 ml

**PRECAUTIONS :**

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface antigen, HIV 1&2 and HCV antibodies .

**LIMITATIONS :**

1. Heterophile antibodies in human serum, interfere with in vitro immunoassays.
2. Lipemeic, hemolysed samples should not be used.

**MEASUREMENT OF BMI :**

Also called **Quetelet** index, is defined as the body mass divided by the square of the body height and is expressed in units of $kg/m^2$

$$\textbf{BMI} = \frac{\text{Weight in kilogram}}{\text{Height in metre square}}$$

# RESULTS

## 1. DISTRIBUTION OF THE PATIENTS BASED ON AGE

### TABLE - 1

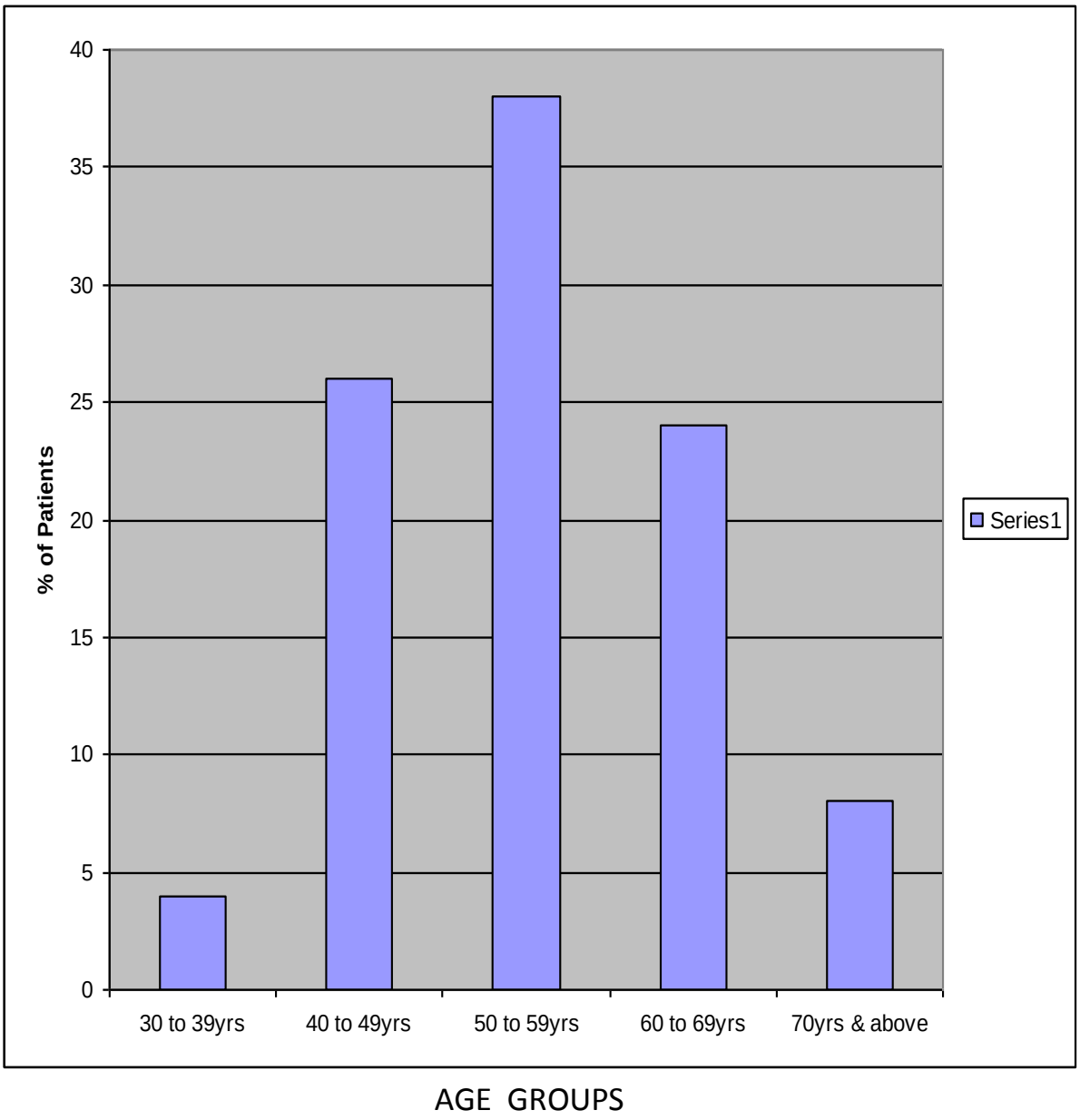
| Age groups | NO. OF CASES (n=50) | 100% |
|---|---|---|
| **30 to 39yrs** | 2 | 4.0 |
| **40 to 49yrs** | 13 | 26.0 |
| **50 to 59yrs** | 19 | 38.0 |
| **60 to 69yrs** | 12 | 24.0 |
| **70yrs & above** | 4 | 8.0 |
| | 50 | 100 |

From the above table it was inferred that out of 50 cases with type 2 DM, a maximum of 19 (38 %) cases were between the 50 – 59 years, 13 (26 %) cases were between the 40-49 years.

Mean age group = 54.40 ± 10.319.

## AGE WISE DISTRIBUTION

**Figure 1**



## 2. DISTRIBUTION OF THE PATIENTS BASED ON SEX

### TABLE - 2

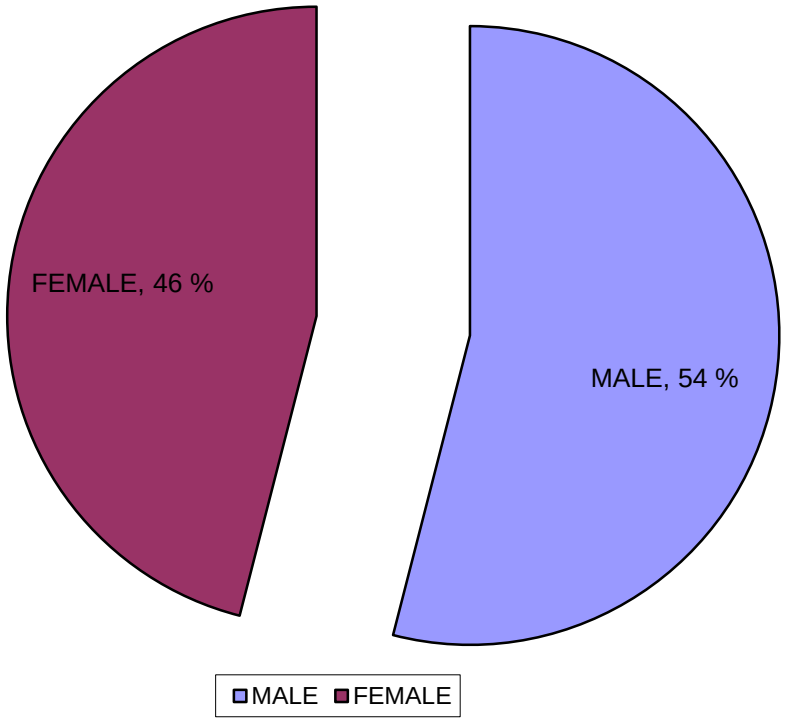
| SEX | No. of cases (n=50) | 100% |
|---|---|---|
| **MALE** | 27 | 54.0 |
| **FEMALE** | 23 | 46.0 |
| | 50 | 100 |

From the above table it was inferred that out of 50 cases of type 2 DM, males constitute 27 (54 %) and females constitute 23 (46 % ).

Male : Female = **1.17 : 1**

## DISTRIBUTION OF THE PATIENTS BASED ON SEX

**Figure - 2**



## 3. DISTRIBUTION OF THE PATIENTS BASED ON DM DURATION :

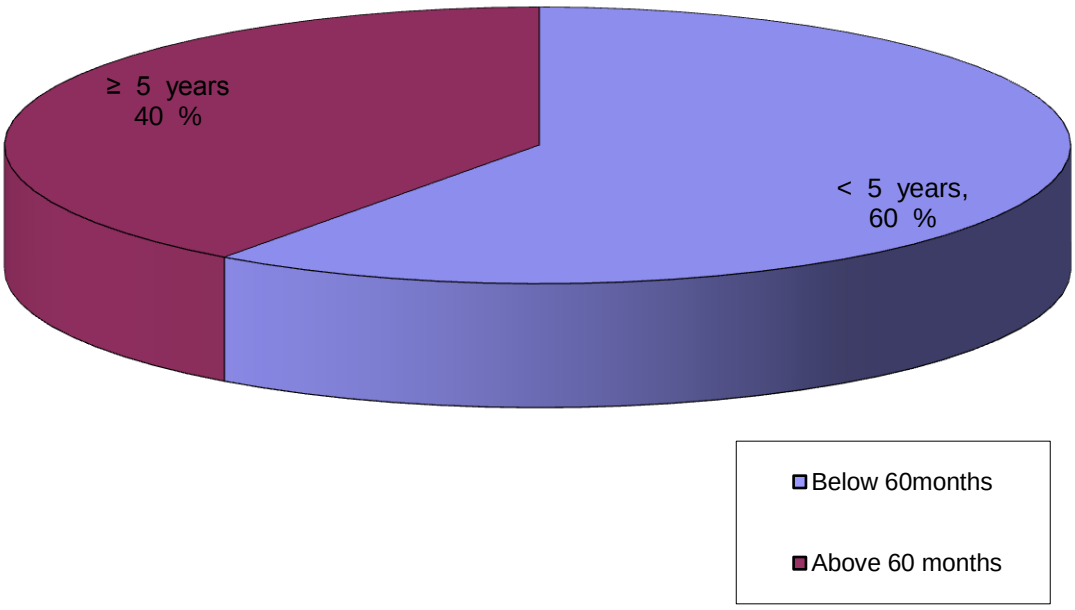
**TABLE 3**

| DURATION | No. of cases (n=50) | 100% |
|---|---|---|
| < 5 Years | 30 | 60.0 |
| ≥ 5 Years | 20 | 40.0 |
| | 50 | 100 |

From the above table it was inferred that out of 50 cases of type 2 DM, 30 (60 %) patients had DM with duration of < 5 years and the remaining 20 (40 %) had DM with duration of ≥ 5 years.

## DISTRIBUTION OF THE PATIENTS BASED ON DM DURATION

**Figure - 3**



## 4. DISTRIBUTION OF THE PATIENTS BASED ON THE PRESENCE OR ABSENCE OF FAMILY HISTORY :

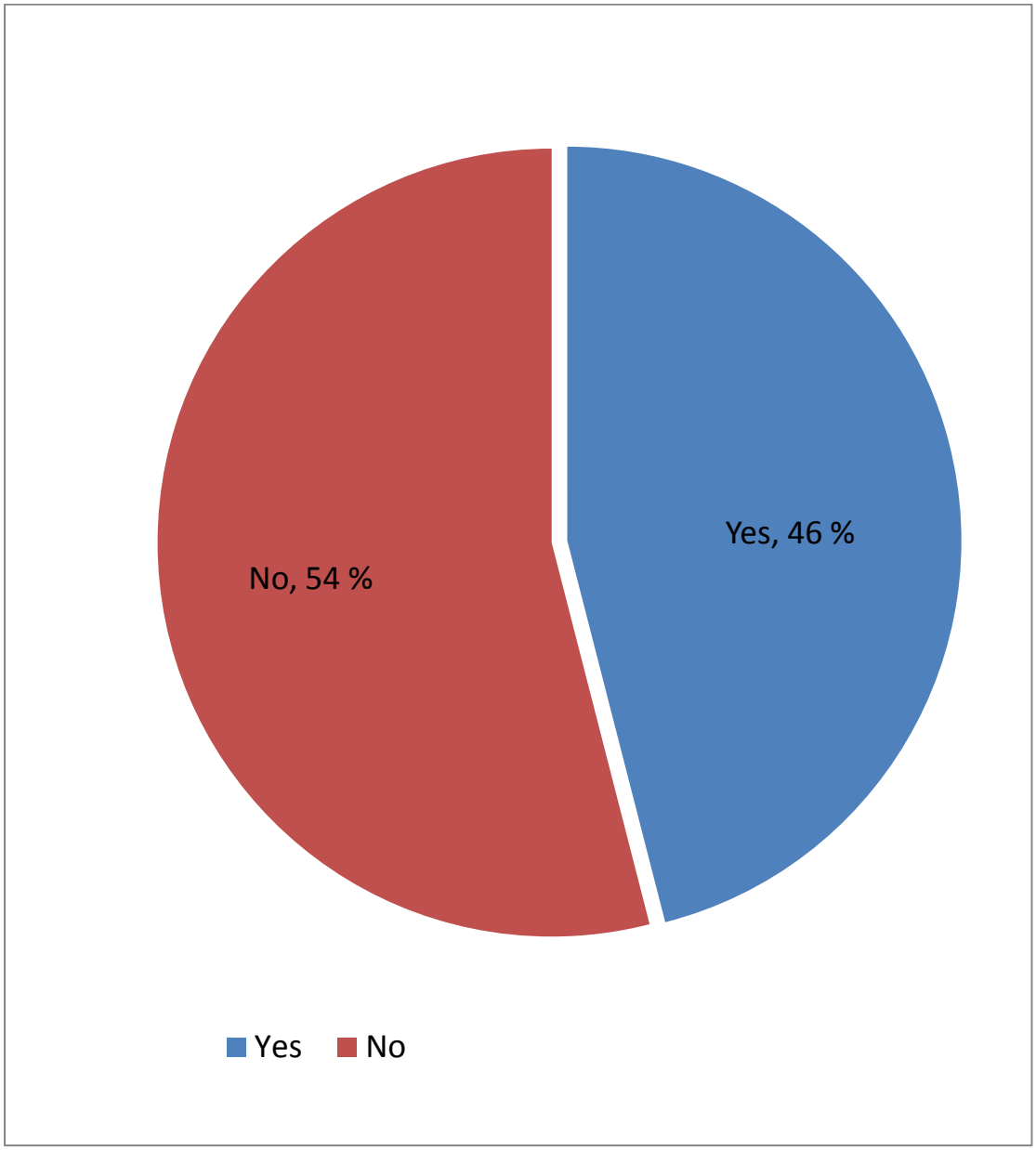
**TABLE 4**

| FAMILY H/O | NO. OF CASES (n=50) | 100% |
|---|---|---|
| **Yes** | 23 | 46.0 |
| **No** | 27 | 54.0 |

From the above table it was inferred that out of 50 cases of type 2 DM, 23 (46 %) patients has family history of DM and the remaining 27 (54 %) had no family history.

**DISTRIBUTION OF THE PATIENTS BASED ON PRESENCE OR ABSENCE OF FAMILY HISTORY :**

**Figure 4**



## 5. DISTRIBUTION OF THE PATIENTS ON THE BASIS OF BMI

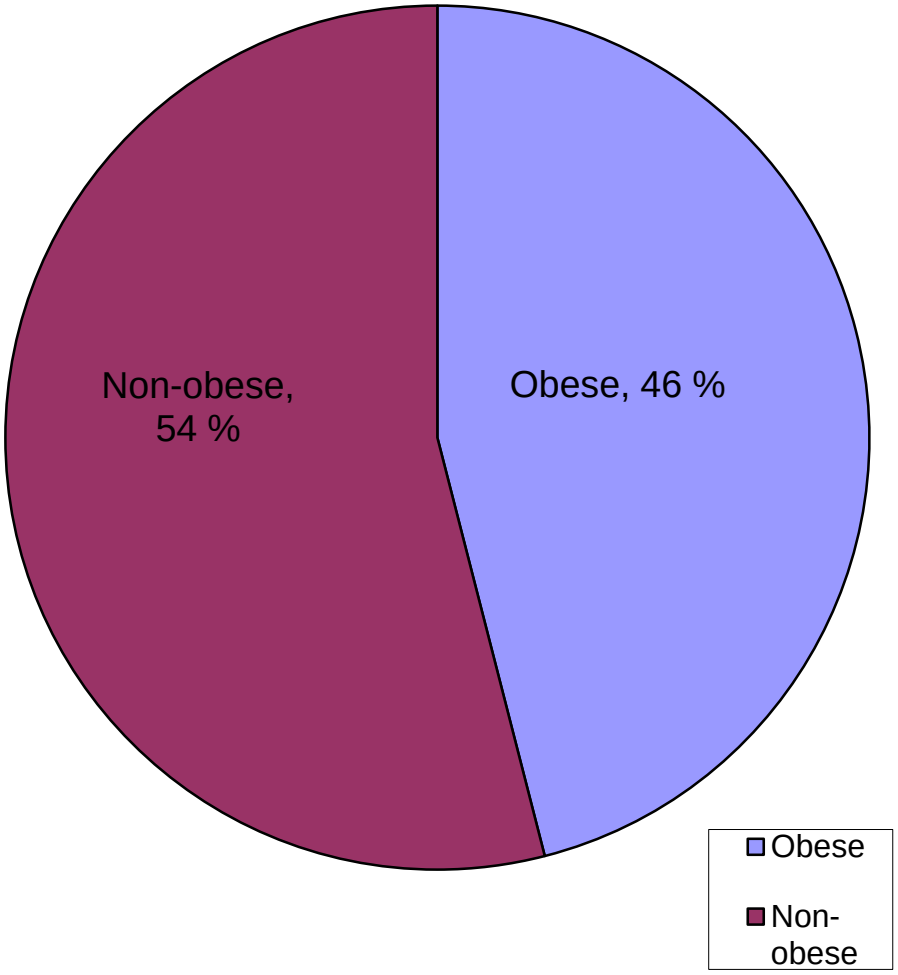
**TABLE 5**

| BMI | NO. OF CASES (n=50) | 100% |
|---|---|---|
| **Obese ( ≥ 25)** | 23 | 46.0 |
| **Non-obese (< 25 )** | 27 | 54.0 |

From the above table it was inferred that out of 50 cases of type 2 DM, 23 ( 46 % ) patients had BMI ≥ 25 and the remaining 27 ( 54 % ) patients had BMI < 25 .

## DISTRIBUTION BASED ON BMI

**Figure 5**



## 6. DISTRIBUTION OF THE PATIENTS ON THE BASIS OF FASTING C- PEPTIDE LEVELS

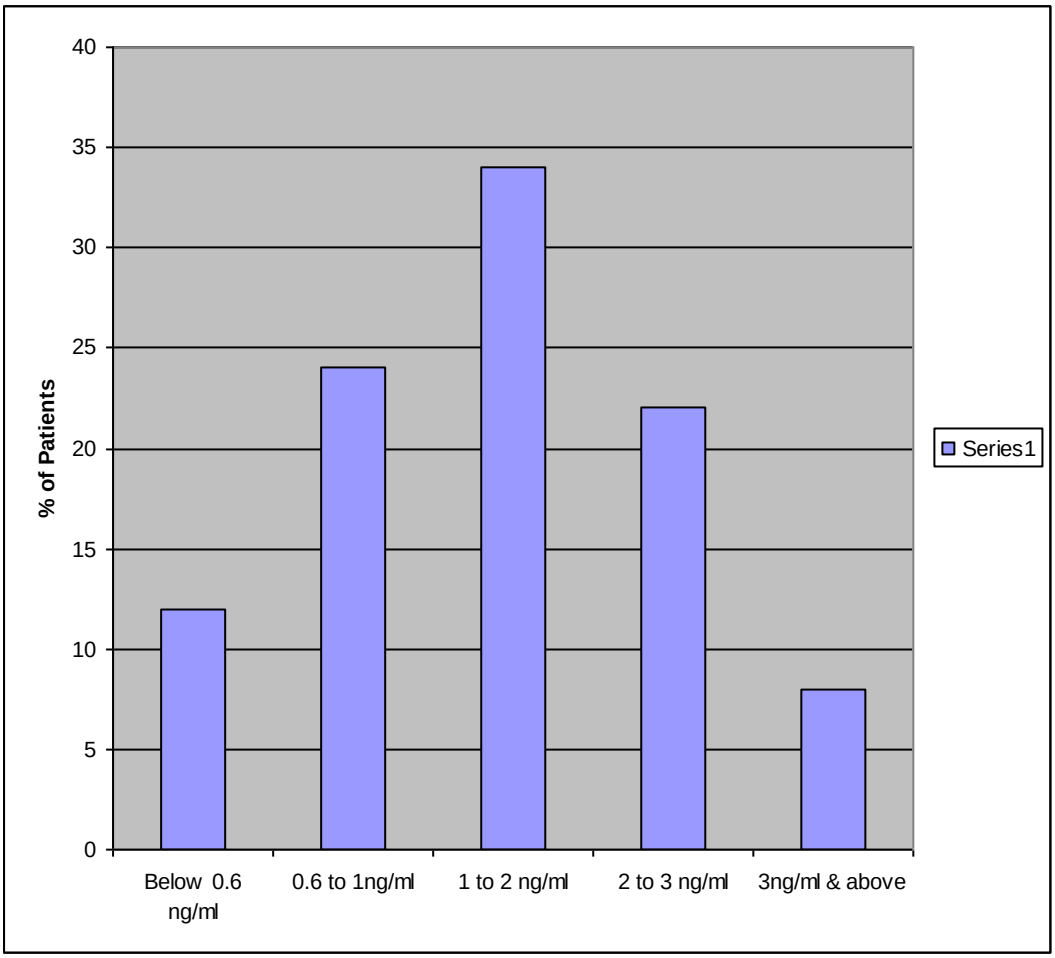
**TABLE 6**

| C-PEPTIDE LEVELS | NO. OF CASES (n=50) | 100% |
|---|---|---|
| **< 0.6 ng/ml** | 6 | 12.0 |
| **0.6 to 1ng/ml** | 12 | 24.0 |
| **1 to 2 ng/ml** | 17 | 34.0 |
| **2 to 3 ng/ml** | 11 | 22.0 |
| **≥ 3ng/ml** | 4 | 8.0 |

From the above table it was inferred that out of 50 cases of type 2 DM, fasting C- peptide levels of < 0.6 ng/ml was found in 6 (12 %) patients , 0.6 – 1 ng/ml was found in 12 (24 %) patients, 1-2 ng/ml in 17 (34 %) patients and > 2 ng/ml in 15 ( 30 %) of patients.

**DISTRIBUTION OF THE PATIENTS ON THE BASIS OF FASTING C- PEPTIDE LEVELS**

**Figure 6**




**C- peptide levels**

## 7. FASTING BLOOD SUGAR LEVELS IN TYPE 2 DM

### TABLE 7

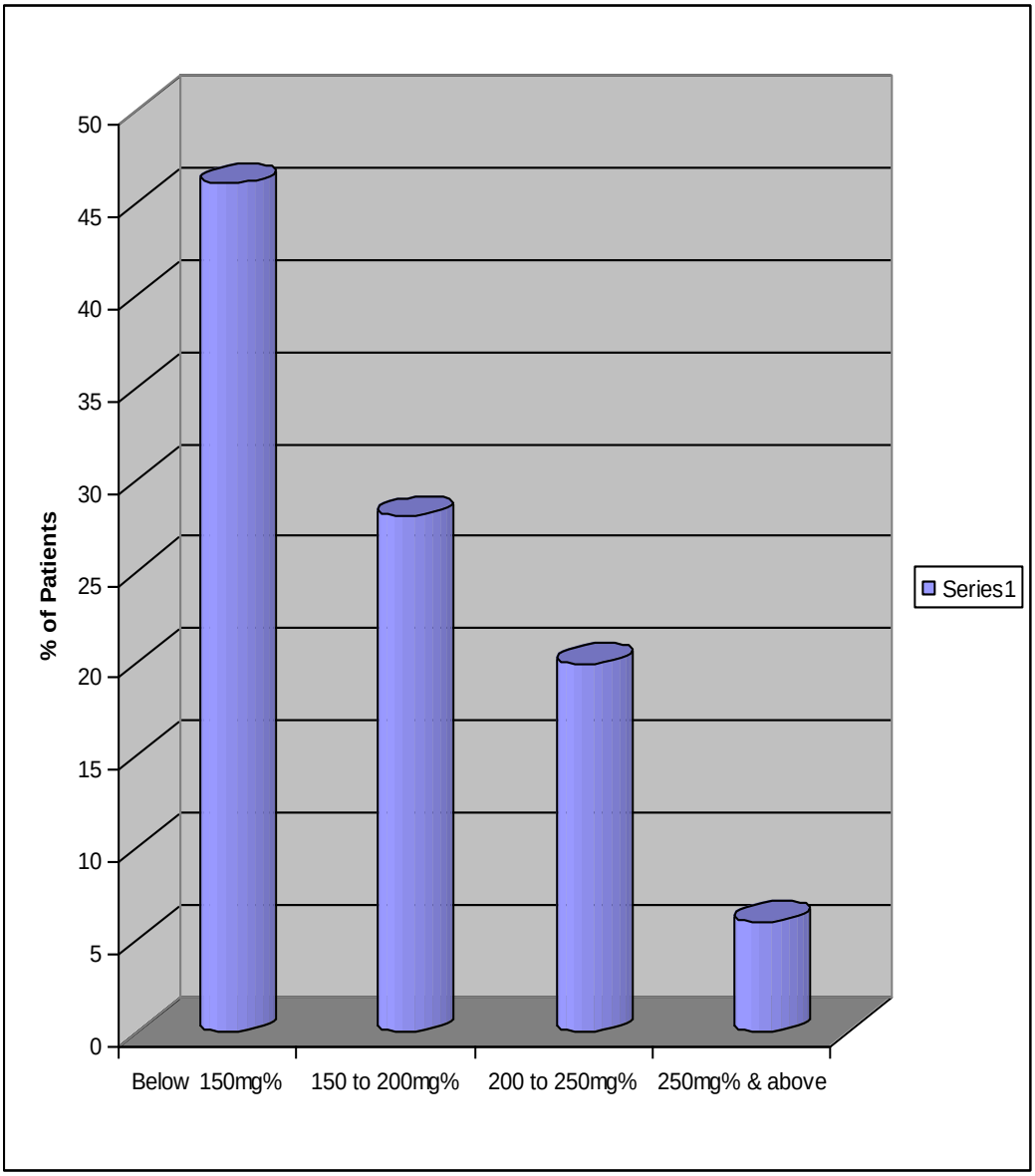
| FBS | No.of Patients (n=50) | Percentage (100%) |
|---|---|---|
| **< 150 mg%** | 23 | 46.0 |
| **150 to 200 mg%** | 14 | 28.0 |
| **200 to 250 mg%** | 10 | 20.0 |
| **> 250 mg%** | 3 | 6.0 |

From the above table it was inferred that out of 50 cases of type 2 DM, 23 (46%) patients had FBS level below 150 mg/dl, 14 (28%) patients had FBS level between 150 – 200 mg/dl, 13 (26%) patients had FBS level above 200 mg/dl.

**Mean FBS = 166.32 ± 44.215.**

## DISTRIBUTION BASED ON FBS LEVELS

**Figure 7**




**FBS levels**

## DESCRIPTIVE STATISTICS

| Parameters | Min | Max | Mean | S.D |
|---|---|---|---|---|
| **1. Age(yrs)** | 36 | 83 | 54.40 | 10.319 |
| **2. Duration of Diabetes in Years** | 1 | 240 | 67.02 | 66.478 |
| **3. Height ( cms )** | 139 | 171 | 156.82 | 8.337 |
| **4. Weight (kg)** | 38 | 81 | 59.24 | 9.642 |
| **5. BMI** | 16 | 35 | 24.15 | 3.907 |
| **6. Fasting C-peptide (ng/ml)** | 0.37 | 4.93 | 1.5900 | 0.99284 |
| **7. Fasting blood sugar (mg/dl)** | 86 | 260 | 166.32 | 44.215 |

## 8. C- PEPTIDE LEVEL AND BMI CORRELATION IN TYPE 2 DM

### TABLE 8

| FASTING C-PEPTIDE (ng/ml) | BMI | | | Statistical inference |
|---|---|---|---|---|
| | Obese (n=23) | Non-obese (n=27) | Total (n=50) | |
| **< 0.6 ng/ml** | 2 (8.7%) | 4 (14.8%) | 6 (12%) | $X^2$= 24.405. **P = 0.010 < 0.05 Significant**. |
| **0.6 to 1 ng/ml** | 4 (17.4%) | 8 (29.6%) | 12 (24%) | |
| **1 to 2 ng/ml** | 8 (34.8%) | 9 (33.3%) | 17 (34%) | |
| **2 to 3 ng/ml** | 7 (30.4%) | 4 (14.8%) | 11 (22%) | |
| **≥ 3 ng/ml** | 2 (8.7%) | 2 (7.4%) | 4 (8%) | |

From the above table it was inferred that out of 50 patients, 23 (46%) patients were obese, among the obese patients : 17 patients had adequate or higher C- peptide levels and low C- peptide level of < 0.6 ng/ml was found only in 2 (8.7%) patients.

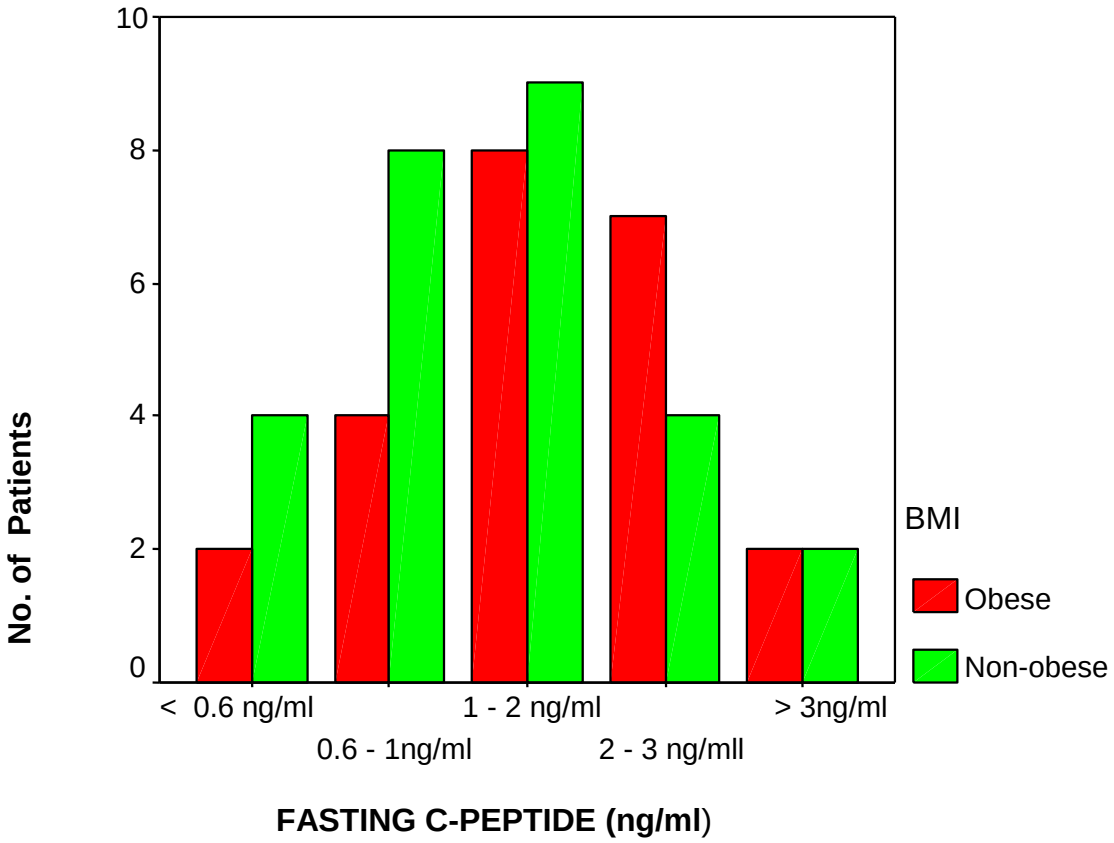
Non- obese patients constitute 27 (54%) of the 50 cases. Among them, 8 (29.6%) patients had C- peptide level in the range of 0.6-1 ng/ml, 9 (33.3%) patients had 1-2 ng/ml, 6 (22.1%) patients had > 2 ng/ml and 4 (14.8%) patients had < 0.6 ng/ml.

In this study, it was found that there is positive correlation between BMI & C- peptide levels as indicated by P value (64)

which is significant (P= 0.010) . So, the C- peptide level increases with increase in BMI indicating the resistance to insulin action.

**FASTING C-PEPTIDE AND BMI CORRELATION**

**Figure 8**



## 9. ASSOCIATION OF BMI AND FBS LEVEL

### TABLE - 9

### Chi- square test

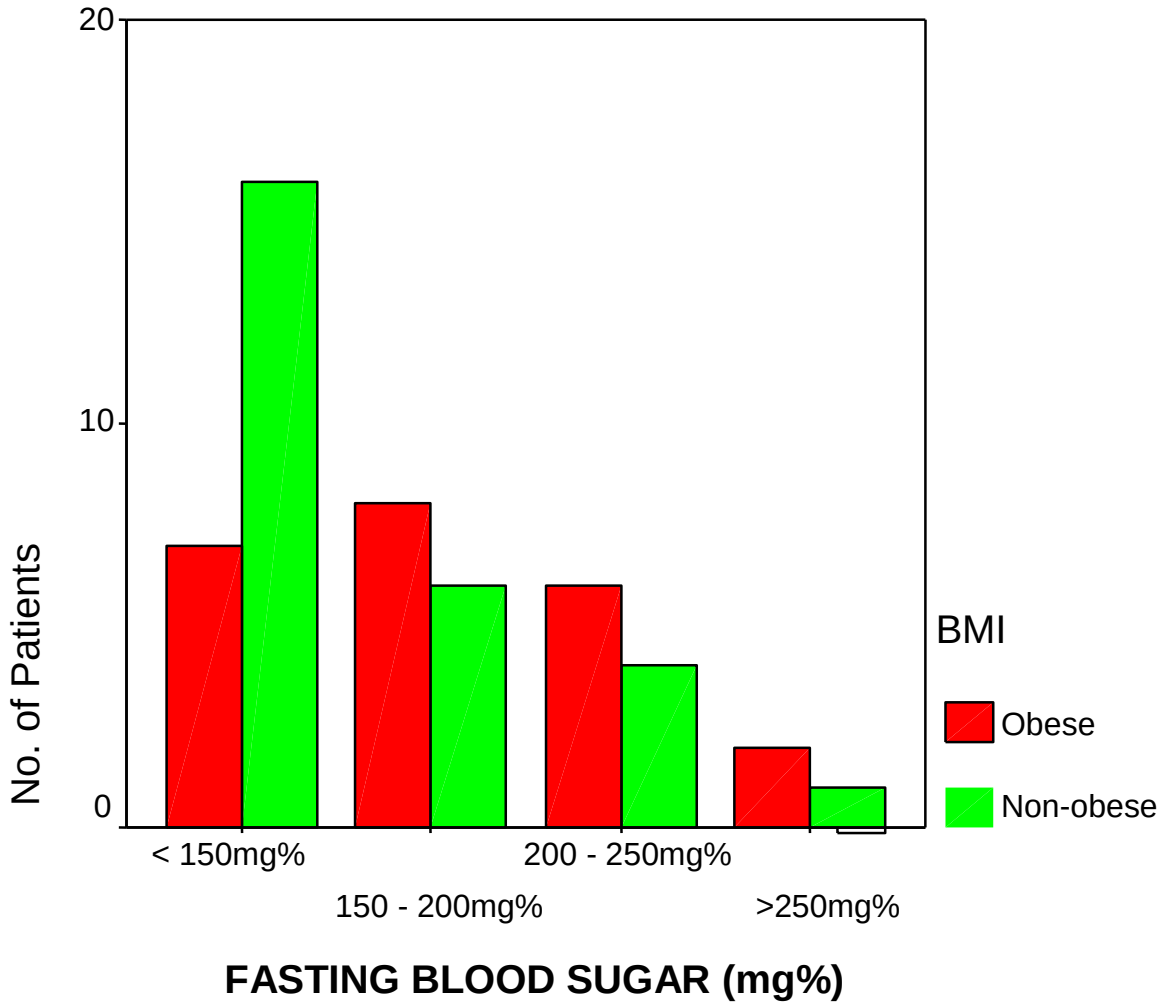
| FASTING BLOOD SUGAR ( mg%) | BMI | | | Statistical inference |
|---|---|---|---|---|
| | Obese (n=23) | Non-obese (n=27) | Total (n=50) | |
| **< 150 mg%** | 7 (30.4%) | 16 (59.3%) | 23 (46%) | **$X^2$ = 20.248 P = 0.036 < 0.05 Significant** |
| **150 to 200 mg%** | 8 (34.8%) | 6 (22.2%) | 14 (28%) | |
| **200 to 250 mg%** | 6 (26.1%) | 4 (14.8%) | 10 (20%) | |
| **≥ 250 mg%** | 2 (8.7%) | 1 (3.7%) | 3 (6%) | |

From the above mentioned table it was inferred that out of 50 patients, in the obese group of 23(46%) patients , FBS level was ≥ 150 mg/dl in 16 (69.6%) patients, <150 mg/dl in only 7 (30.4%) patients.

In the non- obese group of 27 patients, 16 (59.3%) patients had FBS level below 150 mg/dl and 11 (40.7%) patients had level above 150 mg/dl.

The above observation suggests that the association between FBS levels and BMI was significant (p= 0.036) and positively correlated, indicating that in obese patients inspite of elevated C-peptide levels, FBS levels increased due to insulin resistance.

**Figure 9 : ASSOCIATION OF BMI AND FBS LEVELS**



## 10. ASSOCIATION OF FASTING C- PEPTIDE AND FBS LEVEL

### TABLE 10

### Chi- square test

| FASTING C-PEPTIDE (ng/ml) | FASTING BLOOD SUGAR (mg%) | | | | | Statistical inference |
|---|---|---|---|---|---|---|
| | Below 150 mg% (n=23) | 150 to 200 mg% (n=14) | 200 to 250mg% (n=10) | 250 mg% & above (n=3) | Total (n=50) | |
| **< 0.6** | 0 | 2(14.3%) | 3 (30%) | 1 (33.3%) | 6 (12%) | **$X^2$ = 16.119 Df=12 P = 0.186 (> 0.05) Not Significant** |
| **0.6 to 1** | 5 (21.7%) | 4 (28.6%) | 2 (20%) | 1 (33.3%) | 12(24%) | |
| **1 to 2** | 11(47.8%) | 3 (21.4%) | 3 (30%) | 0 | 17(34%) | |
| **2 to 3** | 5 (21.7%) | 5 (35.7%) | 1 (10%) | 0 | 11(22%) | |
| **≥ 3** | 2 (8.7%) | 0 | 1 (10%) | 1 (33.3%) | 4(8%) | |

From the above mentioned table it was inferred that out of 50 patients, those with C-peptide level < 0.6 ng/ml, fasting plasma glucose level between 150-200 mg/dl was found in 2 patients & fasting plasma glucose level above 200 mg/dl was found in 4 patients .

Among 29 patients with C-peptide level between 0.6 - 2 ng/ml, fasting plasma glucose level between 150-200 mg/dl was

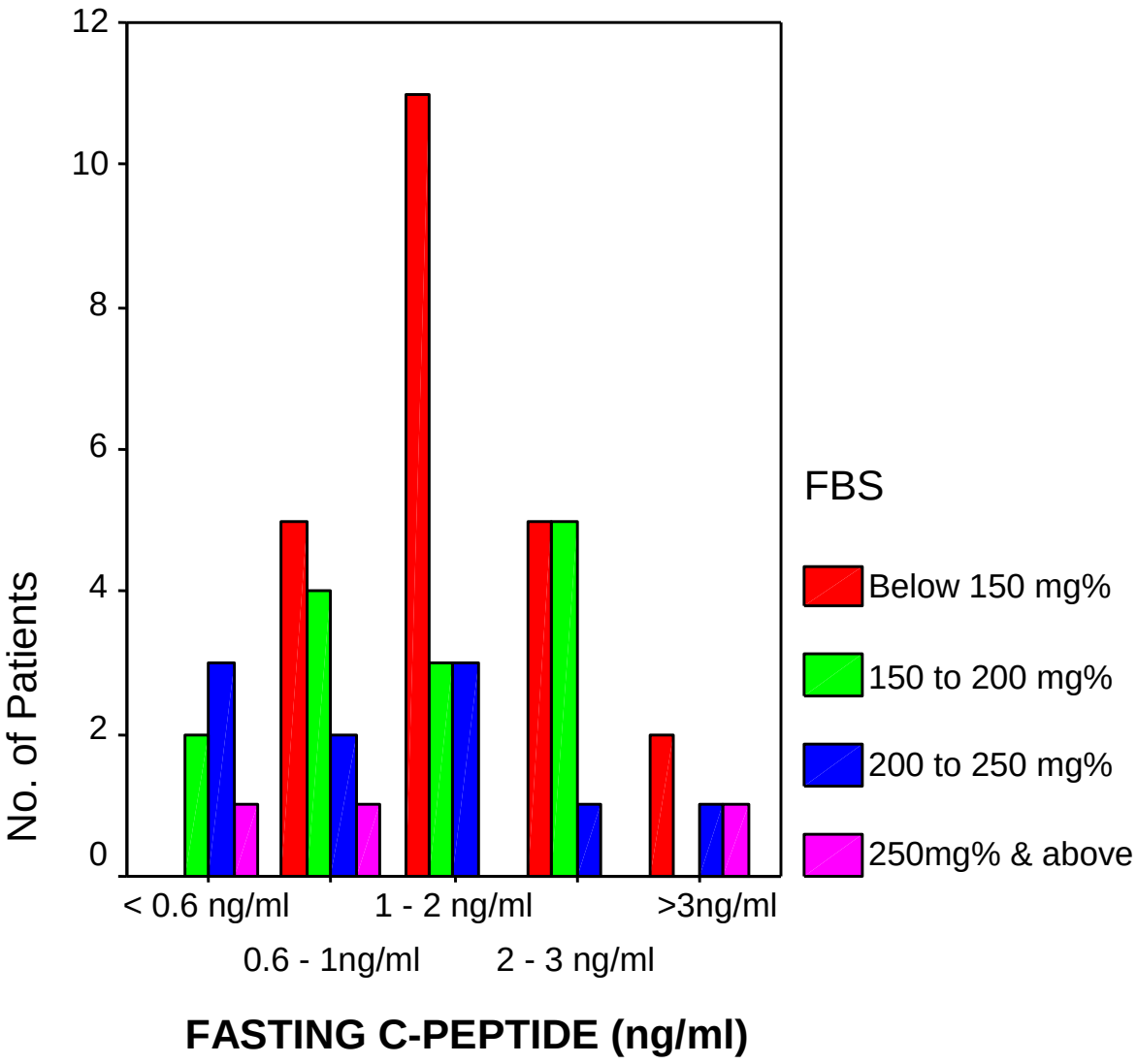
found in 7 patients , above 250 mg/dl was found in 6 patients and 16 patients had level below 150 mg/dl.

Among 15 patients with C-peptide level > 2 ng/ml, FBS < 150 mg/dl was found in 7 patients and > 150 mg/dl was found in 8 patients.

From the above mentioned data it was inferred that , the fasting C-peptide and fasting blood sugar level associations were not significant as indicated by the P value (P = 0.186).

## 10. ASSOCIATION OF FASTING C- PEPTIDE AND FBS LEVELS

**Figure 10**



## 11. ASSOCIATION OF DURATION OF DIABETES AND BMI

### TABLE 11

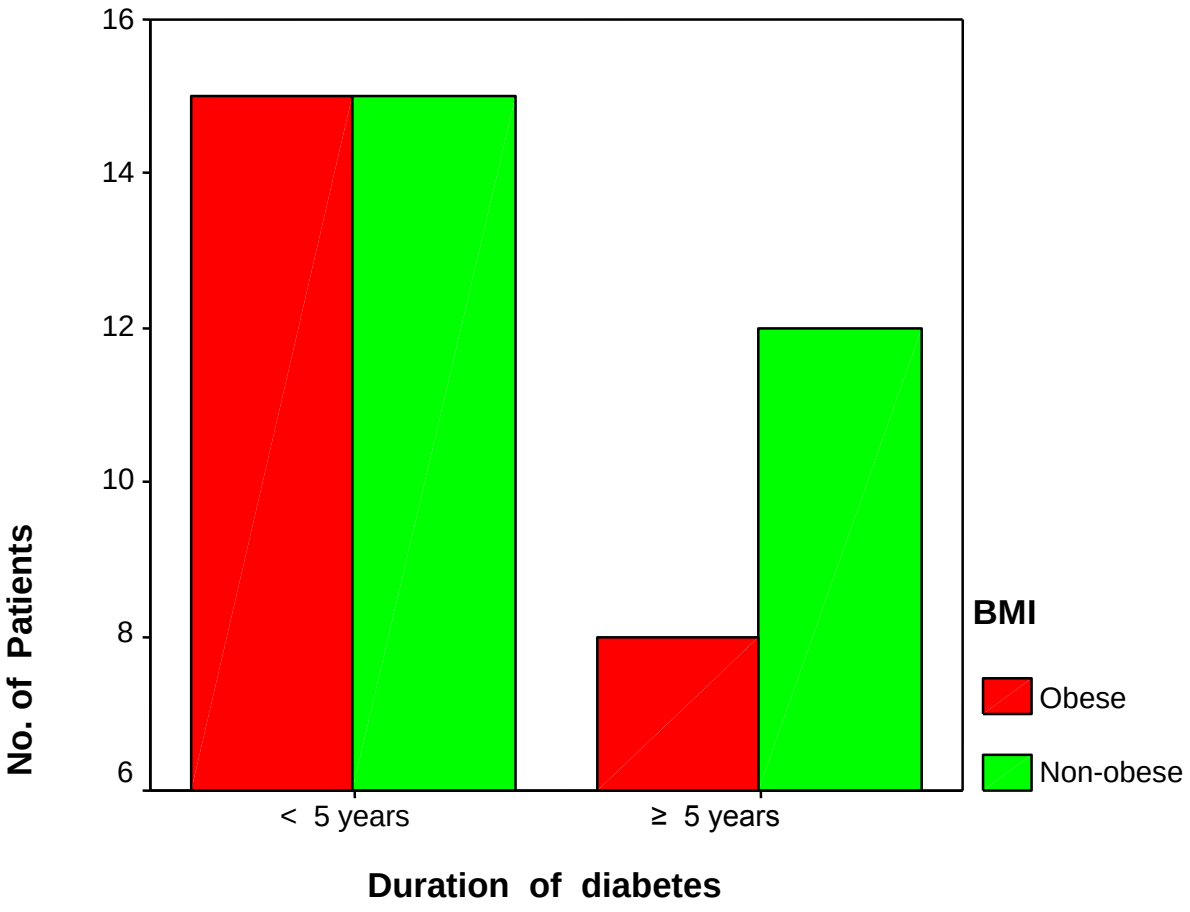
### Chi- square test

| DURATION OF DIABETES | BMI | | | Statistical inference |
|---|---|---|---|---|
| | Obese (n=23) | Non-obese (n=27) | Total (n=50) | |
| < 5 years | 15 (65.2%) | 15 (55.6%) | 30 (60%) | $X^2$= 0.483; Df =1 P = 0.487 (>0.05) **Not Significant** |
| ≥ 5 years | 8 (34.8%) | 12 (44.4%) | 20 (40%) | |

The above table shows that in this study, among 23 obese patients, duration of diabetes was < 5 years in 15 patients and ≥ 5 years in 8 patients. Among 27 non- obese patients, duration of diabetes was < 5 years in 15 patients and ≥ 5 years in 12 patients. The association between BMI and duration of diabetes were not significant (P = 0.487).

## ASSOCIATION OF DURATION OF DIABETES AND BMI

**Figure 11**



## 12. ASSOCIATION OF DURATION OF DIABETES AND FASTING C- PEPTIDE LEVELS

### TABLE 12 a

| FASTING C-PEPTIDE (ng/ml) | DURATION OF DIABETES | | | Statistical inference |
|---|---|---|---|---|
| | < 5 YEARS (n=30) | ≥ 5 YEARS (n=20) | Total (n=50) | |
| **< 0.6** | 1 (3.3%) | 5 (25%) | 6 (12%) | $X^2$=12.593 Df=4<br>**P = 0.013 (<0.05)**<br>**Significant** |
| **0.6 to 1** | 6 (20%) | 6 (30%) | 12 (24%) | |
| **1 to 2** | 9 (30%) | 8 (40%) | 17 (34%) | |
| **2 to 3** | 10 (33.3%) | 1 (5%) | 11 (22%) | |
| **> 3** | 4 (13.3%) | 0 | 4 (8%) | |

From the above mentioned table it was inferred that out of 50 patients, DM duration < 5 years was found in 30 patients, among which C-peptide level of 1-2 ng/ml was found in 9 patients, > 2 ng/ml in 14 patients and < 0.6 ng/ml in 1 patient.

DM duration ≥ 5 years was found in 20 patients, among which C-peptide level of < 0.6 ng/ml was found in 5 patients, 0.6-1 ng/ml in 6 patients and > 2 ng/ml in 1 patient.

From the above observed data it was inferred that there was negative correlation between DM duration and C- peptide level in this study, as indicated by significant P value (P = 0.013). So, the C-peptide level decreases with increase in DM duration.

**DM DURATION AND MEAN C- PEPTIDE LEVEL CORRELATION**

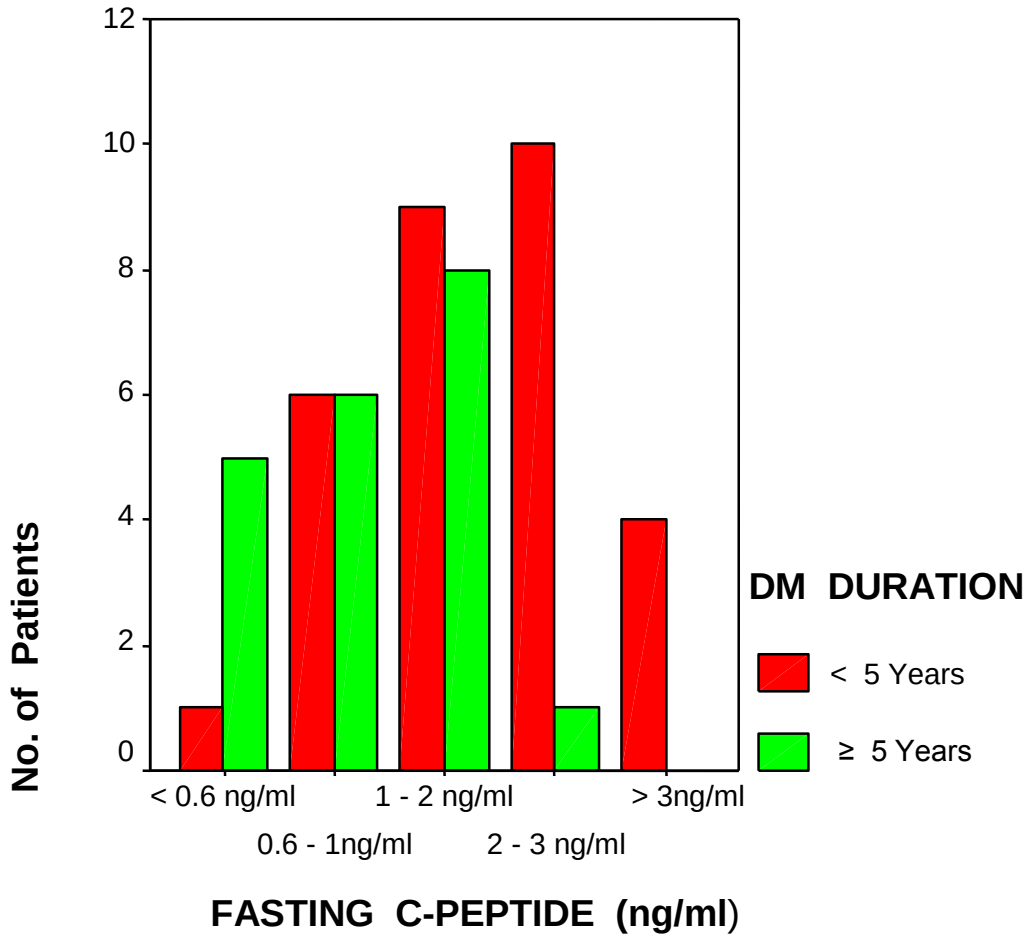
**TABLE 12 b**

| **DM duration** | **NO. of cases** | **C- peptide Mean ± SD** | **Test applied ( T – test )** |
|---|---|---|---|
| **< 5 years** | 30 | 1.953 ± 1.031 | **T = 3.518** |
| **≥ 5 years** | 20 | 1.045 ± 0.629 | **P = 0.001(< 0.05) Significant** |

From the above table it was inferred that , by comparing the mean ± SD of C- peptide with patients of DM duration < 5 years and ≥ 5 years by T- test , it was found that the association were significant as denoted by T value (3.518) and P value (P = 0.001), thus the C- peptide level decreases with increase in DM duration

## ASSOCIATION OF DURATION OF DIABETES AND FASTING C- PEPTIDE LEVELS

**Figure 12**



## ASSOCIATION OF DURATION OF DM AND FBS LEVEL

## TABLE 13

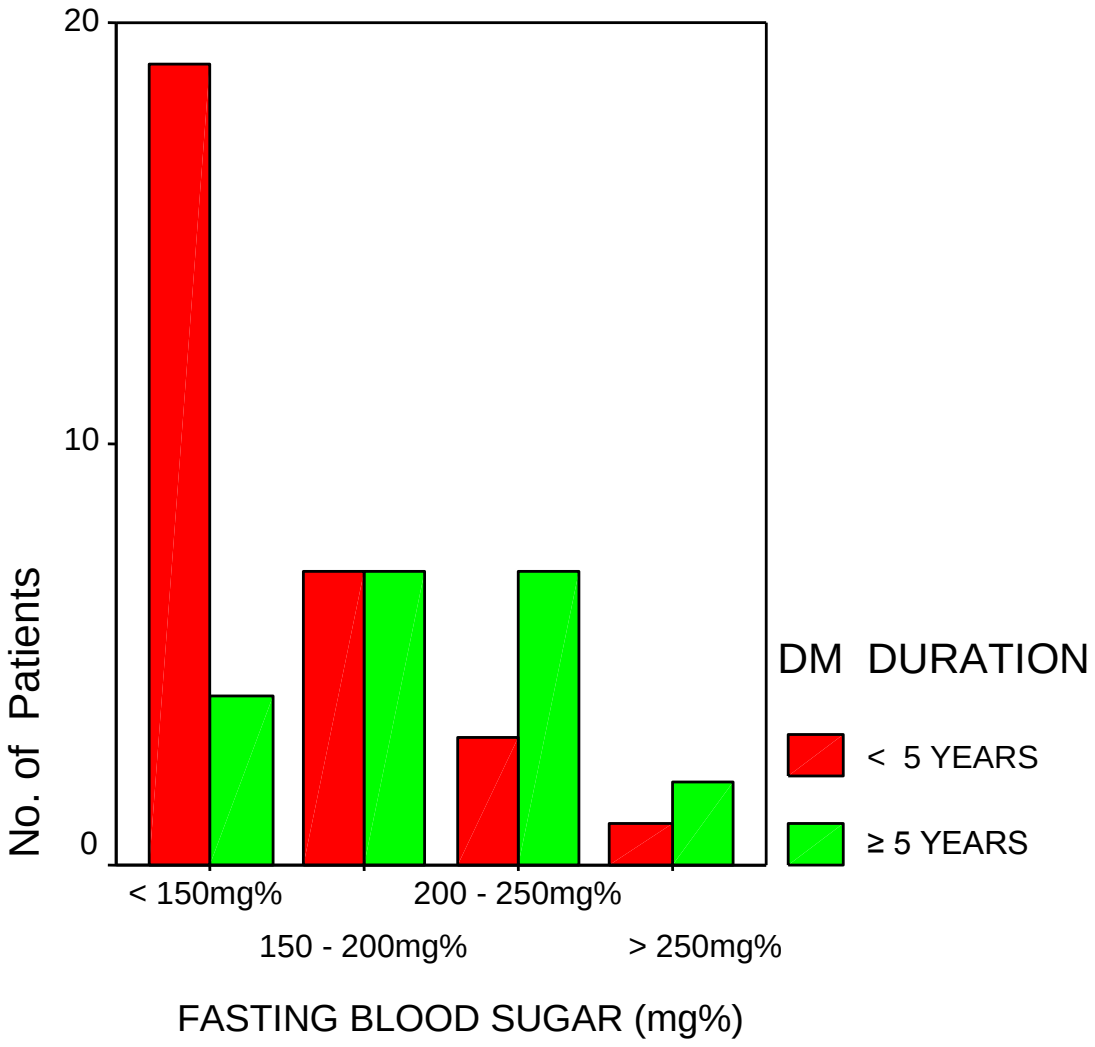
| FASTING BLOOD SUGAR (mg%) | DURATION OF DIABETES | | | Statistical inference |
|---|---|---|---|---|
| | < 5 YEARS (n=30) | ≥ 5 YEARS (n=20) | Total (n=50) | |
| **< 150** | 19 (63.3%) | 4 (20%) | 23 (46%) | $X^2$=10.121 Df=3<br>**P = 0.018 (<0.05) Significant** |
| **150 to 200** | 7 (23.3%) | 7 (35%) | 14 (28%) | |
| **200 to 250** | 3 (10%) | 7 (35%) | 10 (20%) | |
| **≥ 250** | 1 (3.3%) | 2 (10%) | 3 (6%) | |

The above table shows that, among 30 patients with duration of DM < 5 years, 19 patients had FBS level < 150 mg/dl and only 4 patients had FBS level above 200 mg/dl. Among 20 patients with duration of DM ≥ 5 years, 9 patients had FBS level > 200 mg/dl and only 4 patients had FBS level < 150 mg/dl.

This observation suggests that there is an association between duration of DM and FBS levels as indicated by P value which was found to be significant (P = 0.018). This association had moderately positive correlation, as indicated by increase in fasting plasma glucose values with increase in DM duration. (76)

## ASSOCIATION OF DIABETES DURATION AND FBS LEVEL

**Figure 13**



**COMPARISON OF AGE WITH MEAN FASTING C-PEPTIDE LEVEL IN PATIENTS WITH BMI < 25 (Non- obese).**

**TABLE 14 a**

| Particular | No.of patients (n=27) | Percentage (100%) |
|---|---|---|
| Non-obese | 27 | 100.0 |

**Oneway ANOVA ( Non- obese)**

| FASTING C-PEPTIDE (ng/ml) | Mean | S.D | SS | DF | MS | Statistical inference |
|---|---|---|---|---|---|---|
| **Between Groups** | | | 0.691 | 3 | 0.230 | F=78<br>P = 0.841 (>0.05)<br>**Not Significant** |
| ***40 to 49yrs (n=5)*** | 1.3780 | 0.71054 | | | | |
| ***50 to 59yrs (n=10)*** | 1.3970 | 1.09673 | | | | |
| ***60 to 69yrs (n=9)*** | 1.5989 | 0.82442 | | | | |
| ***70yrs & above (n=3)*** | 1.0600 | 0.62746 | | | | |
| **Within Groups** | | | 19.070 | 23 | 0.829 | |

The above table shows that there is no association between fasting C-peptide level and age , in patients with BMI < 25, as the P value is not significant (P = 0.841).

**COMPARISON OF AGE WITH MEAN FASTING C-PEPTIDE LEVEL IN PATIENTS WITH BMI ≥ 25 (obese).**

**TABLE 14 b**

| Particular | No.of Patients (n=23) | Percentage (100%) |
|---|---|---|
| Obese | 23 | 100.0 |

**Oneway ANOVA (Obese )**

| FASTING C-PEPTIDE (ng/ml) | Mean | S.D | SS | DF | MS | Statistical inference |
|---|---|---|---|---|---|---|
| **Between Groups** | | | 12.505 | 4 | 3.126 | F=3.907 P = 0.219 (> 0.05) **Not Significant** |
| ***30 to 39yrs (n=2)*** | 0.9200 | 0.19799 | | | | |
| ***40 to 49yrs (n=8)*** | 1.4438 | 0.70737 | | | | |
| ***50 to 59yrs (n=9)*** | 2.4233 | 1.15302 | | | | |
| ***60 to 69yrs (n=3)*** | 0.7600 | 0.33719 | | | | |
| ***70yrs & above (n=1)*** | 3.5900 | 0.000 | | | | |
| **Within Groups** | | | 14.405 | 18 | 0.800 | |

The above table shows that there is no association between fasting C-peptide level and age , in patients with BMI ≥ 25, as the P value is not significant (P = 0.219). (79)

# DISCUSSION

## DISCUSSION

In this study, there are totally 50 patients labelled as type 2 DM, of which female patients constitute 23 (46%) and males constitute 27 (54%). Age group of study patients is in the range of 30-80 years. The age group in the range of 50-60 years, constitute maximum number of study patients . **Mean age** was found to be **54.40 ± 10.319.**

**Khatib et al in**[86] his study also showed that the 50- 60 years age group constitute many type 2 DM cases.

In this study, 60 % patients had DM duration of < 5 years, while 40 % had duration of ≥ 5 years.

As per **ADA 2002** recommendation, patients with BMI ≥ 25 is considered obese. In this study, 23 (46%) patients were considered obese and 27(54%) patients were considered non- obese as measured by BMI. So, it is inferred from the study that obesity plays an important role in the development of insulin resistance and subsequent type 2 DM.

**INSULIN SECRETORY FUNCTION :**

By measuring C-peptide levels in the fasting state, insulin secretion from the β cell of pancreas was measured in this study. Because C- peptide is secreted from β cell of pancreas along with insulin in equal amounts and it undergoes only negligible liver extraction

and its level in the peripheral blood equals to that of in the portal blood, C- peptide is used as a measure of insulin secretion in the body.

In this study, C- peptide level was found to be adequate or high in 44 patients ( indicating good insulin reserve ) and low in 6 patients

( indicating poor insulin reserve).

**Mean C-peptide** level in this study was **1.590 ± 0.992.**

**OBESITY AND C- PEPTIDE LEVEL :**

In this study, obese patients had mean C- peptide level of 1.82 ng/ml while non- obese patients had 1.30 ng/ml, indicating that obese patients had higher mean C- peptide levels compared to non- obese group, this association shows positive correlation as measured by P value which is significant ( $x^2 = 24.405$, P = 0.010 ). In obese patients resistance to insulin action occurs resulting in elevated insulin levels as evidenced by increased C- peptide levels.

Study conducted by **Snehalatha, A. Ramachandran et al**[88] also showed that the insulin secretion was lesser in non- obese compared to obese individuals. **Andrea Tura et al** [89] in his study measured insulin and C- peptide levels during a 3 hour oral glucose tolerance tests. (81)

**S.W. Park et al** [90] in his study noticed that there is an association between BMI and serum C-peptide levels which were positively correlated. .

**Banerjee et al** in his study showed that Asian Indians have high body fat relative to muscle mass and BMI; they have increased resistance to insulin action at cellular level resulting in elevated serum insulin levels.

**ASSOCIATION OF FASTING PLASMA GLUCOSE AND C- PEPTIDE LEVELS :**

In this study, association between fasting C-peptide level and FBS levels were not significant as the P value is 0.186.

**Clare, Jones O et al** [92] in their study noticed that the obese patients had elevated serum insulin, C- peptide and blood glucose levels than non-obese patients.

**Relimpio F et al** [93] studied C-peptide/ blood sugar level association in type 2 DM patients treated with oral hypoglycaemic drugs.

**ASSOCIATION OF BMI AND FBS LEVELS:**

In this study it is noticed that there is an association between BMI & fasting plasma glucose levels. They were positively correlated as indicated by the P value which was found to be significant (P = 0.036).

So, it is inferred that FBS level increases with increase in BMI, indicating the increased resistance to insulin action in obese individuals.

**ASSOCIATION OF INSULIN SECRETION AND DM DURATION :**

In this study , patients with DM duration < 5 years had mean C- peptide level of 1.953 ± 1.031, while patients with duration of diabetes ≥ 5 years had mean C- peptide level of 1.045 ± 0.629.

Negative correlation was found between insulin secretion and duration of DM as indicated by significant T value ( 3.518) & P value (0.001), indicating that insulin secretion from β cell of the pancreas decreases with increase in DM duration.

**ASSOCIATION OF BMI AND DURATION OF DM :**

In this study it was found that the association between BMI and DM duration was not significant as measured by P value .

**DURATION OF DM and FBS level :**

The previous table shows that, among 30 patients with duration of DM < 5 years, 19 patients had FBS level < 150 mg/dl and only 4 patients had FBS level above 200 mg/dl. Among 20 patients with duration of DM ≥ 5 years, 9 patients had FBS level > 200 mg/dl and only 4 patients had FBS level < 150 mg/dl.

This observation suggests that there is association between duration of DM and FBS levels as P value is significant (P = 0.018). The association was found to be moderately positively correlated, indicating that as the duration of diabetes increases, FBS levels increases.

**FAMILY HISTORY :**

In this study, it was found that 46 % patients had family history of DM , while the remaining 54 % had no family history of DM.

Study conducted by **Shobha Malini et al** [95] noticed 58 % patients had family history of DM.

## LIMITATIONS OF THIS STUDY :

1. The sample size of patients with type 2 diabetes mellitus taken for this study were small.
2. Obese patients with type 2 diabetes mellitus constitute less than 50 % of the total sample studied.
3. Glycosylated haemoglobin level estimation for the study subjects were not done in this study.

# CONCLUSION

**CONCLUSION :**

1. Nearly 88 % patients had adequate insulin reserve of > 0.6 ng/ml. The obese patients had higher C- peptide levels compared to the non- obese patients indicating insulin resistance.
2. The insulin reserve decreased with the increase in duration of diabetes as seen by the decrease in fasting C-peptide levels. This indicates that the β cell dysfunction increases with the duration of diabetes.
3. Minority of the patients had very low C- peptide levels and require insulin therapy. The obese patients with adequate insulin reserve, require dietary and exercise management along with oral hypoglycaemic drugs to improve the level of insulin sensitivity to receptors.

**In Conclusion,** this study suggests :

* Early screening of subjects with family history of diabetes,
* C-peptide testing should be done in patients with poor blood glucose control to decide treatment modalities and
* To create awareness about lifestyle modifications and education to prevent obesity.

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# PROFORMA

**PROFORMA**

**NAME :**

**AGE :** **SEX :** **HOSPITAL NO :**

**SYMPTOMS** :

| | | | |
|---|---|---|---|
| POLYURIA | | VOMITING & DIARRHOEA | |
| POLYDYPSIA | | NUMBNESS & PARESTHESIA | |
| LOSS OF WT. | | PRURITIS | |
| POLYPHAGIA | | DIMNESS OF VISION | |
| TIREDNESS | | OTHERS | |
| WT. GAIN | | | |

**PAST HISTORY** : Hypertension, ischemic heart disease, Chronic Kidney disease, others.

**DRUG HISTORY** :

**PERSONAL HISTORY** : Smoking, alcohol, tobacco / betel nut.

**FAMILY HISTORY** : Diabetes mellitus - Yes / No (100)

**EXAMINATION :**

**HEIGHT :**

**WEIGHT :**

**BODY MASS INDEX :**

**GENERAL EXAMINATION :**

Anemia, clubbing, pedal edema, ingrowing toe nail, ulcer, Dermopathy, icterus, Jugular venous pulse.

**VITALS :**

**PULSE RATE :**

**BLOOD PRESSURE :**

**SYSTEMIC EXAMINATION :**

**CARDIOVASCULAR SYSTEM :**

**RESPIRATORY SYSTEM :**

**ABDOMINAL SYSTEM** :

**CENTRAL NERVOUS SYSTEM :**

**EYE :** Cataract :

Fundus :

**FOOT EXAMINATION :**

**INVESTIGATIONS :**

1. Complete blood count :
2. Serum Urea :
3. Serum Creatinine :
4. ECG :
5. Chest X- ray PA view :
6. Fasting blood sugar :
7. Fasting C- peptide :
8. URINE - Albumin

Sugar

# MASTER

# CHART

# *MASTER CHART*

| S.NO | NAME | AGE (YRS) | SEX | DURATION OF DIABETES | FAMILY H/O | HEIGHT (Cm) | WEIGHT (Kg) | BMI | FASTING C-PEPTIDE (ng/ml) | FASTING BLOOD SUGAR(mg%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. | ABDUL MUTHULEAF | 71 | M | 2 YRS | YES | 165 | 55 | 20.2 | 0.77 | 140 |
| 2. | AROCKIA SAMY | 50 | M | 2 Months | NO | 152 | 63 | 27.3 | 1.35 | 139 |
| 3. | BALAGURU | 40 | M | 6 YRS | NO | 163 | 70 | 26.3 | 0.52 | 160 |
| 4. | BARATHI | 50 | F | 3 YRS | NO | 151 | 53 | 23.2 | 3.22 | 124 |
| 5. | BANUMATHI | 55 | F | 6 YRS | NO | 154 | 63 | 26.6 | 0.93 | 260 |
| 6. | BASKAR | 40 | M | 3 YRS | YES | 167 | 60 | 21.5 | 1.00 | 110 |
| 7. | GUNASEKARAN | 50 | M | 2 YRS | NO | 168 | 65 | 23 | 0.88 | 139 |
| 8. | JAHEER USEN | 40 | M | 7 YRS | YES | 165 | 75 | 27.5 | 1.80 | 172 |
| 9. | JEYALAXMI | 62 | F | 8 YRS | YES | 150 | 57 | 25.3 | 0.84 | 130 |
| 10. | JEYARAMAN | 48 | M | 3 YRS | YES | 170 | 65 | 22.5 | 1.88 | 148 |
| 11. | JOSEPH | 63 | M | 5 YRS | NO | 167 | 60 | 21.5 | 2.42 | 140 |
| 12. | KALYANI | 54 | F | 1 Month | YES | 139 | 51 | 26.4 | 2.87 | 180 |
| 13. | KARUNANIDHI | 44 | M | 5 Months | NO | 168 | 72 | 25.5 | 0.70 | 124 |
| 14. | MANGALAM | 65 | F | 1 YR | NO | 150 | 51 | 22.7 | 2.13 | 114 |
| 15. | MANI | 60 | M | 15 YRS | YES | 159 | 60 | 23.7 | 2.81 | 167 |
| 16. | MANICKAM | 62 | M | 15 YRS | NO | 166 | 59 | 21.4 | 0.62 | 236 |
| 17. | NAGARAJAN | 55 | M | 11 YRS | NO | 163 | 55 | 20.7 | 1.29 | 180 |
| 18. | NARAYANAN | 60 | M | 20 YRS | YES | 158 | 65 | 25.4 | 0.39 | 210 |
| 19. | NOORJAHAN | 55 | F | 6 Months | NO | 150 | 79 | 35.1 | 2.94 | 168 |
| 20. | PADMAVATHI | 38 | F | 2 Months | YES | 148 | 55 | 25.1 | 1.06 | 110 |
| 21. | PANDIAMMAL | 52 | F | 5 YRS | YES | 153 | 67 | 28.6 | 2.01 | 141 |
| 22. | PONMANI | 60 | F | 13 YRS | YES | 153 | 51 | 21.8 | 0.71 | 155 |
| 23. | POUNAMBAL | 40 | F | 3 Months | NO | 158 | 57 | 22.8 | 2.15 | 128 |
| 24. | PREMA | 52 | F | 5 Months | YES | 159 | 54 | 21.4 | 3.47 | 130 |
| 25. | RAJA | 58 | M | 3 YRS | YES | 148 | 55 | 25.1 | 1.86 | 140 |

## *MASTER CHART- CONTINUED*

| S.NO | NAME | AGE (YRS) | SEX | DURATION OF DIABETES | FAMILY H/O | HEIGHT (Cm) | WEIGHT (Kg) | BMI | FASTING C-PEPTIDE (ng/ml) | FASTING BLOOD SUGAR (mg%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 26. | RANI | 40 | F | 9 YRS | NO | 146 | 57 | 26.7 | 1.21 | 210 |
| 27. | SAMIPILLAI | 77 | M | 13 YRS | NO | 163 | 60 | 22.6 | 0.63 | 168 |
| 28. | SAROJA | 40 | F | 2 YRS | YES | 149 | 53 | 23.9 | 1.49 | 112 |
| 29. | SARAVANAN | 6O | M | 4 YRS | NO | 168 | 60 | 22 | 1 .40 | 120 |
| 30. | SEKAR | 55 | M | 2 YRS | YES | 160 | 65 | 25.4 | 2.38 | 196 |
| 31. | SELVAM | 45 | M | 1 YR | NO | 164 | 70 | 26 | 2.55 | 120 |
| 32. | SELVARAJ | 44 | M | 4 YRS | YES | 159 | 40 | 16 | 0.37 | 208 |
| 33. | SHANTHI | 48 | F | 3 YRS | YES | 153 | 75 | 32 | 1.47 | 178 |
| 34. | SUBRAMANY | 80 | M | 20 YRS | NO | 155 | 50 | 20.8 | 1.78 | 146 |
| 35. | SUSAI MARY | 57 | F | 9 YRS | NO | 153 | 38 | 16.2 | 0.42 | 198 |
| 36. | SUSEELA | 59 | F | 14 YRS | NO | 144 | 47 | 22.7 | 1.35 | 141 |
| 37. | THAMBAIAH | 69 | M | 2 YRS | YES | 171 | 64 | 21.9 | 1.87 | 132 |
| 38. | THANGARAJ | 64 | M | 8 YRS | NO | 156 | 43 | 17.7 | 0.56 | 210 |
| 39. | THANGARASU | 65 | M | 15 YRS | YES | 151 | 53 | 23.2 | 1.87 | 108 |
| 40. | THIAGARAJAN | 52 | M | 2 YRS | YES | 164 | 70 | 26 | 2.54 | 200 |
| 41. | VASANTHA PRIYA | 49 | F | 6 YRS | YES | 140 | 50 | 25.5 | 1.10 | 221 |
| 42. | VASANTHA | 55 | F | 1 YR | NO | 153 | 66 | 28.2 | 4.93 | 256 |
| 43. | VEERALAXMI | 48 | F | 2 YRS | NO | 146 | 57 | 26.7 | 2.20 | 210 |
| 44. | VEERAYAN | 71 | M | 2 YRS | NO | 159 | 68 | 26.9 | 3.59 | 204 |
| 45. | VELLAISAMY | 56 | M | 7 YRS | YES | 164 | 58 | 21.6 | 0.62 | 241 |
| 46. | VIJAYA | 50 | F | 6 Months | NO | 146 | 52 | 24.4 | 0.80 | 158 |
| 47. | VIJAYALAXMI | 36 | F | 1 MONTH | YES | 156 | 81 | 33.3 | 0.78 | 180 |
| 48. | VIOLET MARY | 60 | F | 15 YRS | NO | 148 | 64 | 29.2 | 1.05 | 212 |
| 49. | VISWANATHAN | 55 | M | 8 Months | NO | 171 | 64 | 21.9 | 1.52 | 86 |
| 50. | YUVARANI | 58 | F | 7 YRS | NO | 158 | 40 | 16 | 0.40 | 256 |