



## “The Evaluation of Serum Amylase and Serum Lipase for Assessment of Pancreatic Function in Type II Diabetes Mellitus Patients”

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### I. INTRODUCTION:

The prevalence of diabetes mellitus (DM) is developing rapidly and expected to double globally from 171 million in 2000 to 366 million in 2030 with a maximum rise in India. It is expected that DM may affect up to 79.4 million individuals in India by 2030<sup>(1,2)</sup>. At present India is the capital of DM disease in the world<sup>(1,4)</sup>.

Diabetes mellitus is a condition in which there is impaired metabolism of carbohydrate; lipid and proteins caused by either lack of insulin secretion or decreased sensitivity of the body tissues to insulin. It is characterized by hyperglycaemia resulting from the body's inability to utilize glucose for energy. In type 1 diabetes, the pancreas cannot produce insulin and glucose in the blood cannot get entry inside the cell to be metabolized to provide energy. In type 2 diabetes, either the pancreas cannot produce enough insulin or the tissues of the body are insensitive to insulin. The complications of diabetes involving the eyes, kidneys, nerves, blood vessels of heart, brain and extremities are common to both types diabetes. The successful treatment of diabetes mellitus depends on keeping blood glucose level as close as possible to normal level to minimize the complications of diabetes mellitus.

The pancreas is a mixed exocrine–endocrine gland, with the exocrine portion of the gland making up the greatest volume (84%). Ductal cells and blood vessels make up around 4% of the volume, while the endocrine part makes up 2% of the volume. The other part (10%) is occupied by an extracellular matrix. The acinar tissue in the pancreas is in the close vicinity of the islets. Because of this close morphological relationship, functional interactions are likely to occur between the exocrine and endocrine pancreas in any disease, which affect this organ<sup>(9,10)</sup>. Defect in islet cells observed in diabetes may disturb the neighbouring acinar cells of the pancreas and may affect the synthesis and release of enzymes amylase and lipase from exocrine pancreas.

Recently some studies showed low serum amylase and lipase values in metabolic syndrome and Diabetes. In contrast there are some studies which showed an increased levels of serum amylase and lipase in Diabetes Mellitus.

Hence the study is designed to estimate the levels of serum amylase and lipase in both type II diabetes mellitus patients and healthy controls and to find out the exocrine pancreatic involvement in type II Diabetes Mellitus.

### II. AIM AND OBJECTIVES

#### III. AIM:

To estimate the levels of serum Amylase and serum Lipase in patients with type II diabetes mellitus and to compare them with controls.

#### OBJECTIVES:

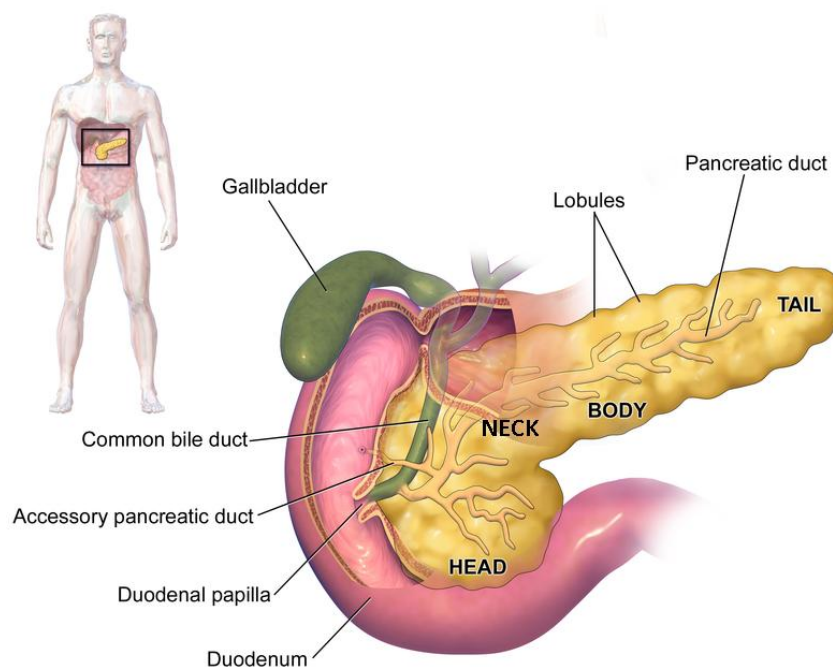
1. To estimate serum amylase in patients with type II diabetes mellitus.
2. To estimate serum lipase in patients with type II diabetes mellitus.
3. To estimate serum amylase and lipase in healthy controls.

4. To compare serum amylase & lipase values between type II diabetes mellitus patients and healthy controls.

#### IV. REVIEW OF LITERATURE

##### ANATOMY OF PANCREAS

The pancreas is one of the largest digestive glands. The major part of the gland is exocrine, secreting enzymes involved in the digestion of lipids, carbohydrates and proteins. It has an additional endocrine function derived from clusters of cells scattered throughout the substance of the gland, which take part in glucose homeostasis and the control of upper gastrointestinal motility and function<sup>(18)</sup>



##### STRUCTURE

The healthy pancreas is creamy pink in colour, with a soft to firm consistency and lobulated surface. The bowl of retort represents its head and the stem of retort represents its neck, body, and tail.<sup>(18)</sup> The pancreas lies more or less horizontally on the posterior abdominal wall in the epigastric and left hypochondriac regions. It crosses the posterior abdominal wall obliquely from concavity of the duodenum to the hilum of spleen. Opposite the level of T12–L3 vertebrae.<sup>(19)</sup>

##### SIZE AND SHAPE

The pancreas is “J”-shaped or retort shaped being set obliquely.<sup>(19)</sup>

Its Measurements are:

Length: 12–15 cm.<sup>(18,19)</sup>

Width: 3–4 cm.

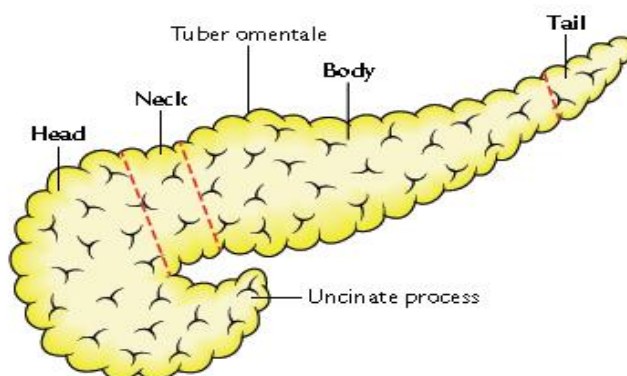
Thickness: 1.5–2 cm.

Weight: 80–90 g.

##### PARTS (SUBDIVISIONS) AND RELATIONS<sup>(19)</sup>

The pancreas is divided (from right to left) into the

1. Head
2. Neck
3. Body
4. Tail
5. Uncinate process.



**Fig. 9.13** Parts of the pancreas.

### **HEAD OF THE PANCREAS <sup>(10)</sup>**

The head of the pancreas lies to the right of the midline, anterior and to the right of the vertebral column, within the curve of the duodenum. It is the thickest and broadest part of the pancreas but is still flattened in the anteroposterior plane. Superiorly, it lies adjacent to the first part of the duodenum; close to the pylorus, however, where the duodenum is on a short mesentery, it overlaps the upper part of the head anteriorly. The duodenal border of the head is flattened, slightly concave and adherent to the second part of the duodenum, particularly around the duodenal papillae. The superior and inferior pancreaticoduodenal arteries lie adjacent to this region. The inferior border of the pancreatic head lies superior to the third part of the duodenum and is continuous with the uncinate process.

The anterior surface of the head is covered by peritoneum and related to the origin of the transverse mesocolon. Posteriorly, the common bile duct is partially embedded within the head of the gland just proximal to where it joins the pancreatic duct near the major duodenal papilla. The posterior surface of the pancreatic head is also related to the inferior vena cava, the right crus of the diaphragm and the termination of the right gonadal vein. Near the midline, the head of the pancreas becomes continuous with the neck.

### **NECK OF THE PANCREAS**

The neck of the pancreas is approximately 2 cm wide and links the head and body. It is often the most anterior part of the gland and may be defined as that part of the pancreas lying anterior to the formation of the portal vein (usually the union of the superior mesenteric and splenic veins) in the transpyloric plane. This is a crucial relationship when evaluating pancreatic cancer because malignant involvement of these vessels may make resection impossible. The superior mesenteric vein and portal vein groove the posterior aspect of the neck. The inferior mesenteric vein joins the confluence of the superior mesenteric vein and splenic vein in one-third of individuals. The anterior surface of the pancreatic neck is covered by peritoneum and lies adjacent to the pylorus. The anterior superior pancreaticoduodenal branch of the gastroduodenal artery descends in front of the gland at the junction of the head and neck.

### **BODY OF THE PANCREAS**

The body of the pancreas is the longest part of the gland and runs from the neck to the tail, becoming progressively thinner. It is slightly triangular in cross-section, and has anterior and posterior surfaces and superior and inferior borders.

#### **Anterior surface**

The anterior surface is covered by peritoneum, which is reflected anteroinferiorly from the surface of the gland to be continuous with the posterior layer of the greater omentum and the transverse mesocolon. The two layers of the transverse mesocolon diverge along this surface. Above the attachment of the transverse mesocolon, the anterior surface of the pancreas is separated from the stomach by the lesser sac. Inferiorly, it lies within the infracolic compartment, and its anterior relations include the fourth part of the duodenum, the duodenojejunal flexure and coils of jejunum.

#### **Posterior surface**

The posterior surface of the pancreas is devoid of peritoneum. It lies on fascia (the fusion fascia of Toldt) anterior to the aorta and the origin of the superior mesenteric artery, the left crus of the diaphragm, left suprarenal gland, the upper pole of the left kidney surrounded by perirenal fascia, and left renal vein. The

splenic vein runs from left to right directly on this surface of the gland and indents the parenchyma to a variable extent, ranging from a shallow groove to almost a tunnel.

### **Superior border**

To the right, the superior border of the body of the pancreas is blunt but it becomes narrower and sharper to the left. An omental tuberosity usually projects from the right end of the superior border above the level of the lesser curvature of the stomach. The superior border is related to the coeliac artery and its branches; the common hepatic artery runs to the right just above the gland, and the splenic artery passes to the left in a tortuous manner, projecting above the superior border at several points.

### **Inferior border**

At the medial end of the inferior border, adjacent to the neck of the pancreas, the superior mesenteric vessels emerge from behind the gland. Laterally, the inferior mesenteric vein runs behind the inferior border to join the splenic vein or the confluence of the splenic and superior mesenteric veins. This is a useful site for identification of the inferior mesenteric vein on computed tomographic (CT) imaging and during left-sided colonic resections.

### **TAIL OF THE PANCREAS**

The tail of the pancreas is the narrowest, most lateral portion of the gland and is continuous medially with the body. It is between 1.5 and 3.5 cm long in adults and lies between the layers of the splenorenal ligament. It may terminate at the base of the splenorenal ligament or extend up to the splenic hilum, when it is at risk of injury at splenectomy during ligation or stapling of the splenic vessels. The splenic artery and its branches, and the splenic vein and its tributaries, lie posterior to the tail.

### **UNCINATE PROCESS OF THE PANCREAS**

The uncinata process is a hook-shaped continuation of the infero medial part of the head of the gland. Embryologically, it is separate from the rest of the gland. The superior mesenteric vein and, occasionally, the superior mesenteric artery descend on its anterior surface before running forwards into the root of the mesentery of the small intestine.

The uncinata process extends medially anterior to the abdominal aorta above the third part of the duodenum, which may be compressed by a pancreatic tumour at this site. On sagittal cross-sectional imaging, the left renal vein, uncinata process, and third part of duodenum can be seen lying between the superior mesenteric artery anteriorly and the abdominal aorta posteriorly.

In calibre within the body of the gland as it receives further lobular ducts that join it almost at right angles to its axis, forming a ‘herringbone pattern’. In adults, the duct can often be demonstrated on ultrasound, measuring approximately 3 mm in diameter in the head, 2 mm in the body and 1 mm in the tail; the calibre of the duct increases from about the fifth decade onwards. As it reaches the neck of the gland, it turns inferiorly and posteriorly towards the bile duct, which lies on its right side. The two ducts unite to form a short common channel, which enters the wall of the descending part of the duodenum obliquely; it may contain a dilation known as the hepato pancreatic ampulla (of Vater). The terminal part of the main pancreatic duct contains a few mucosal folds that impede reflux of pancreatic juice. The length of the common pancreaticobiliary channel is variable and measures up to 5–7 mm in normal individuals.<sup>(18)</sup>

The accessory (dorsal) pancreatic duct (of Santorini) drains the upper part of the anterior portion of the pancreatic head. Much smaller in calibre than the main duct, it is formed within the substance of the head from several lobular ducts and usually communicates with the main pancreatic duct near the neck of the gland or near its first inferior branch. The accessory duct usually opens on to a small, rounded minor duodenal papilla, which lies about 2 cm proximal to the major papilla. If the duodenal end of the accessory duct fails to develop, the lobular ducts drain via small channel(s) into the main duct.<sup>(18)</sup>

### **ARTERIAL SUPPLY**

1. **Splenic artery, a branch of coeliac trunk:** The splenic artery is the branch of coeliac trunk and it is the main source of blood supply to the pancreas. Its branches supply the body and tail of pancreas. Two branches are named. One large branch which arises near the tail and runs toward the neck is called arteria pancreatica magna. Another relatively small branch, which runs toward the tip of the tail, is termed arteria caudae pancreatica.
2. **Superior pancreaticoduodenal artery:** The superior pancreaticoduodenal artery is a branch of gastroduodenal artery.
3. **Inferior pancreaticoduodenal artery:** The inferior pancreaticoduodenal artery is a branch of superior mesenteric artery.

Both the superior and inferior pancreaticoduodenal arteries divide into anterior and posterior branches, which run between the concavity of the duodenum and the head of pancreas. The anastomoses between anterior and posterior branches form anterior and posterior pancreaticoduodenal arterial arcades. <sup>(19)</sup>

### **VENOUS DRAINAGE <sup>(19)</sup>**

The veins of the pancreas drain into

- (a) Portal vein,
- (b) Superior mesenteric vein, and
- (c) Splenic vein

### **LYMPHATIC DRAINAGE <sup>(19)</sup>**

The lymphatics from the pancreas follow the arteries and drain mainly into the following groups of lymph nodes

1. Pancreaticosplenic nodes (main group).
2. Coeliac nodes.
3. Superior mesenteric nodes.
4. Pyloric nodes. <sup>(19)</sup>

### **NERVE SUPPLY**

The sympathetic and parasympathetic nerve fibres reach the gland along its arteries from coeliac and superior mesenteric plexuses.

The sympathetic supply is vasomotor whereas the parasympathetic supply controls the pancreatic secretion. <sup>(19,20)</sup>

### **FUNCTIONS OF PANCREAS**

A healthy pancreas produces the correct chemicals in the proper quantities, at the right times, to digest the foods we eat. The pancreas is a mixed exocrine–endocrine gland, with the exocrine portion of the gland making up the greatest volume (84%). Ductal cells and blood vessels make up around 4% of the volume, while the endocrine part makes up 2% of the volume. The other part (10%) is occupied by an extracellular matrix. The acinar tissue in the pancreas is in the close vicinity of the islets. Because of this close morphological relationship, functional interactions are likely to occur between the exocrine and endocrine pancreas in any disease, which affect this organ. <sup>(21)</sup>

### **THE EXOCRINE PANCREAS:**

The exocrine function purpose is to help in the digestion and further breakdown of carbohydrates, proteins and fat ingested. The pancreas daily secretes approximately 1,5 liters of pancreatic juice composed by 97% of water and electrolytes with abundant enzymes, mainly proteases, amylase, lipase and nuclease that later on is drained into the duodenum in order to neutralize acid gastric secretions to fulfill digestion <sup>(14)</sup> The stomach slowly releases partially digested food into the duodenum as a thick, acidic liquid called chyme. The acini of the pancreas secrete pancreatic juice to complete the digestion of chyme in the duodenum. Pancreatic juice is a mixture of water, salts, bicarbonate, and many different digestive enzymes. <sup>(21)</sup> The bicarbonate ions present in pancreatic juice neutralize the acid in chyme to protect the intestinal wall and to create the proper environment for the functioning of pancreatic enzymes. The pancreatic enzymes each specialize in digesting specific compounds found in chyme <sup>(21)</sup>

- Pancreatic amylase breaks large polysaccharides like starches and glycogen into smaller sugars such as maltose, maltotriose, and glucose. Maltase secreted by the small intestine then breaks maltose into the monosaccharide glucose, which the intestines can directly absorb.

- Trypsin, chymotrypsin, and carboxypeptidase are protein-digesting enzymes that break proteins down into their amino acid subunits. These amino acids can then be absorbed by the intestines.

- Pancreatic lipase is a lipid-digesting enzyme that breaks large triglyceride molecules into fatty acids and monoglycerides. Bile released by the gallbladder emulsifies fats to increase the surface area of triglycerides that pancreatic lipase can react with. The fatty acids and monoglycerides produced by pancreatic lipase can be absorbed by the intestines <sup>(21)</sup>

Endocrinal derangement observed in diabetes may interfere with the exocrine function of the pancreas.

Recently some studies showed low serum amylase and lipase values in metabolic syndrome and Diabetes. In contrast there are some studies which showed an increased levels of serum amylase and lipase in Diabetes Mellitus.

Hence the study is designed to estimate the levels of serum amylase and lipase in both type II diabetes mellitus patients and compared than healthy controls.

## THE ENDOCRINE PANCREAS

The pancreas is a secretory structure with an internal hormonal role (endocrine). The endocrine part is composed of hormonal tissue distributed along the pancreas in discrete units called islets of Langerhans. Its pancreatic islets—clusters of cells formerly known as the islets of Langerhans—secrete the hormones glucagon, insulin, somatostatin, and pancreatic polypeptide (PP).<sup>(21)</sup>

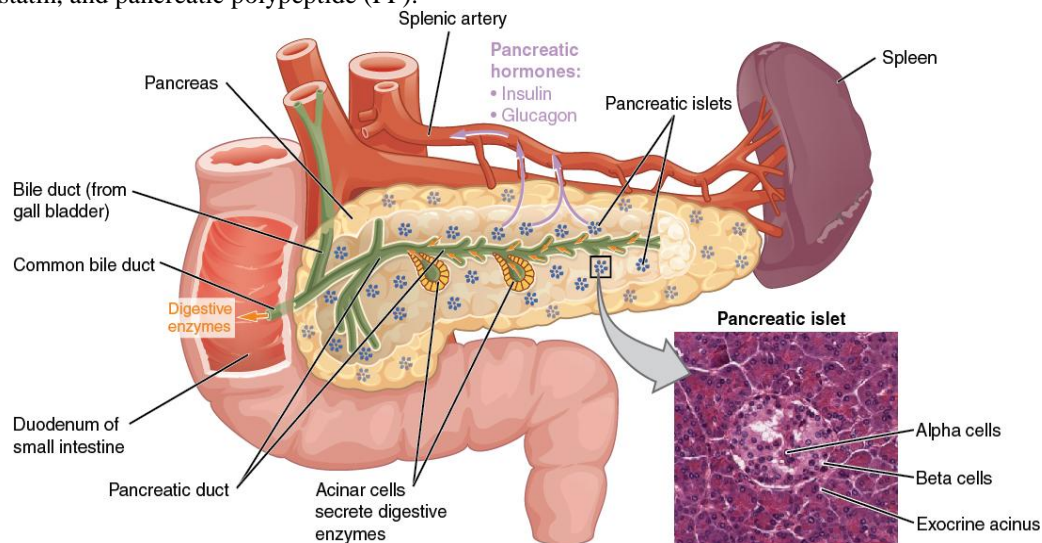


Figure 1. Pancreas. The pancreatic exocrine function involves the acinar cells secreting digestive enzymes that are transported into the small intestine by the pancreatic duct. Its endocrine function involves the secretion of insulin (produced by beta cells) and glucagon (produced by alpha cells) within the pancreatic islets. These two hormones regulate the rate of glucose metabolism in the body. The micrograph reveals pancreatic islets.<sup>(12)</sup>

### Cells and Secretions of the Pancreatic Islets

The pancreatic islets each contain four varieties of cells:

- The **alpha cell** produces the hormone glucagon and makes up approximately 20 percent of each islet. Glucagon plays an important role in blood glucose regulation; low blood glucose levels stimulate its release.
- The **beta cell** produces the hormone insulin and makes up approximately 75 percent of each islet. Elevated blood glucose levels stimulate the release of insulin.
- The **delta cell** accounts for four percent of the islet cells and secretes the peptide hormone somatostatin. Recall that somatostatin is also released by the hypothalamus (as GHIH), and the stomach and intestines also secrete it. An inhibiting hormone, pancreatic somatostatin inhibits the release of both glucagon and insulin.
- The **PP cell** accounts for about one percent of islet cells and secretes the pancreatic polypeptide hormone. It is thought to play a role in appetite, as well as in the regulation of pancreatic exocrine and endocrine secretions. Pancreatic polypeptide released following a meal may reduce further food consumption; however, it is also released in response to fasting.<sup>(21)</sup>

### REGULATION OF BLOOD GLUCOSE LEVELS BY INSULIN AND GLUCAGON

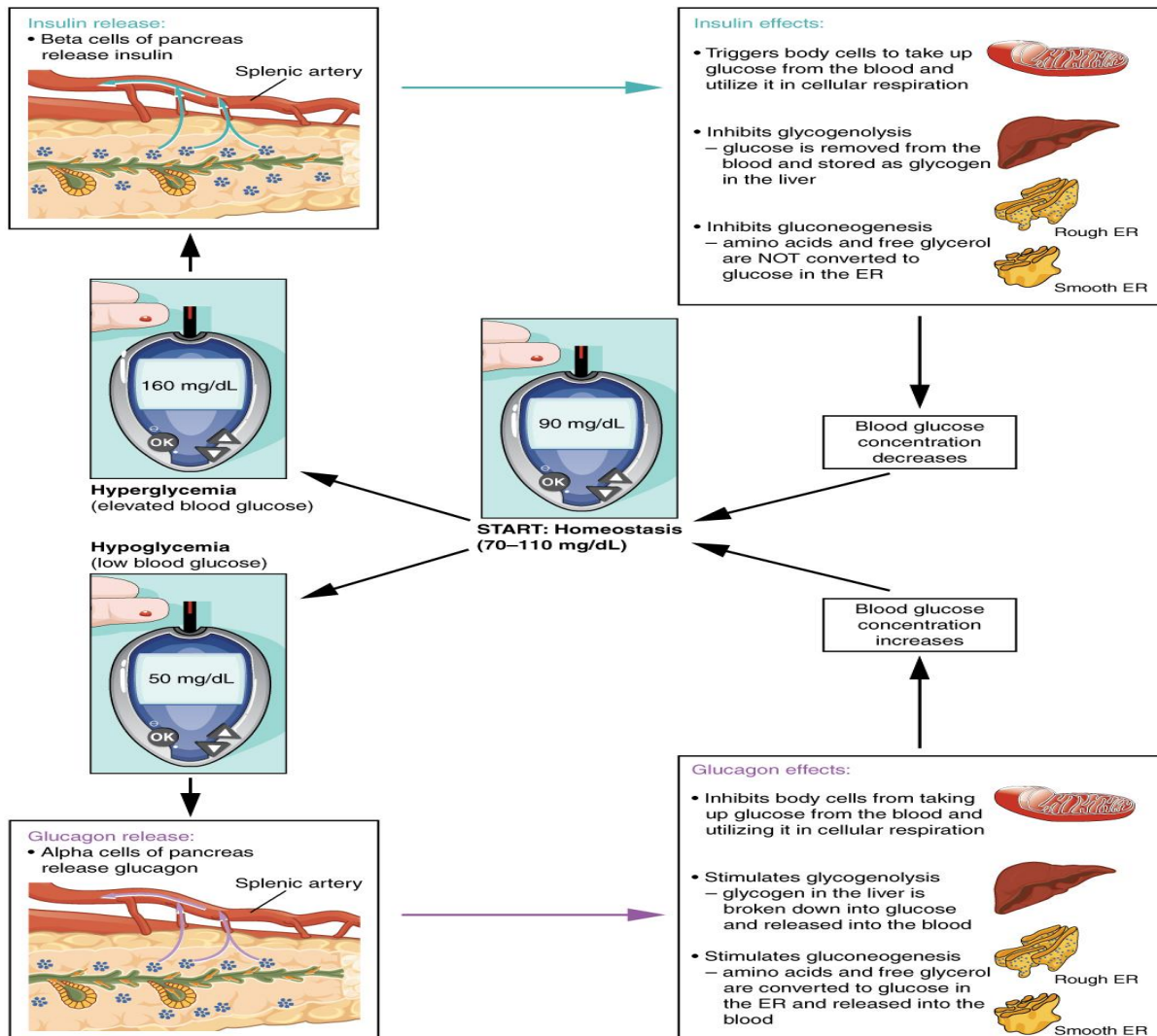
Glucose is required for cellular respiration and is the preferred fuel for all body cells. The body derives glucose from the breakdown of the carbohydrate-containing foods and drinks we consume. Glucose not immediately taken up by cells for fuel can be stored by the liver and muscles as glycogen, or converted to triglycerides and stored in the adipose tissue. Hormones regulate both the storage and the utilization of glucose as required. Receptors located in the pancreas sense blood glucose levels, and subsequently the pancreatic cells secrete glucagon or insulin to maintain normal levels.<sup>(21)</sup>

### GLUCAGON

Receptors in the pancreas can sense the decline in blood glucose levels, such as during periods of fasting or during prolonged labor or exercise (Figure 2). In response, the alpha cells of the pancreas secrete the hormone **glucagon**, which has several effects:

- It stimulates the liver to convert its stores of glycogen back into glucose. This response is known as glycogenolysis. The glucose is then released into the circulation for use by body cells.
- It stimulates the liver to take up amino acids from the blood and convert them into glucose. This response is known as gluconeogenesis.

- It stimulates lipolysis, the breakdown of stored triglycerides into free fatty acids and glycerol. Some of the free glycerol released into the bloodstream travels to the liver, which converts it into glucose. This is also a form of gluconeogenesis. Taken together, these actions increase blood glucose levels. The activity of glucagon is regulated through a negative feedback mechanism; rising blood glucose levels inhibit further glucagon production and secretion. <sup>(21)</sup>



**Figure 2. Homeostatic Regulation of Blood Glucose Levels.**

Blood glucose concentration is tightly maintained between 70 mg/dL and 110 mg/dL. If blood glucose concentration rises above this range, insulin is released, which stimulates body cells to remove glucose from the blood. If blood glucose concentration drops below this range, glucagon is released, which stimulates body cells to release glucose into the blood. <sup>(21)</sup>

## INSULIN

The primary function of **insulin** is to facilitate the uptake of glucose into body cells. Red blood cells, as well as cells of the brain, liver, kidneys, and the lining of the small intestine, do not have insulin receptors on their cell membranes and do not require insulin for glucose uptake. Although all other body cells do require insulin if they are to take glucose from the bloodstream, skeletal muscle cells and adipose cells are the primary targets of insulin.

The presence of food in the intestine triggers the release of gastrointestinal tract hormones such as glucose-dependent insulinotropic peptide (previously known as gastric inhibitory peptide). This is in turn the initial trigger for insulin production and secretion by the beta cells of the pancreas. Once nutrient absorption occurs, the resulting surge in blood glucose levels further stimulates insulin secretion.

Precisely how insulin facilitates glucose uptake is not entirely clear. However, insulin appears to activate a tyrosine kinase receptor, triggering the phosphorylation of many substrates within the cell. These

multiple biochemical reactions converge to support the movement of intracellular vesicles containing facilitative glucose transporters to the cell membrane.<sup>(21)</sup>

In the absence of insulin, these transport proteins are normally recycled slowly between the cell membrane and cell interior. Insulin triggers the rapid movement of a pool of glucose transporter vesicles to the cell membrane, where they fuse and expose the glucose transporters to the extracellular fluid. The transporters then move glucose by facilitated diffusion into the cell interior.<sup>(21)</sup>

Insulin also reduces blood glucose levels by stimulating glycolysis, the metabolism of glucose for generation of ATP. Moreover, it stimulates the liver to convert excess glucose into glycogen for storage, and it inhibits enzymes involved in glycogenolysis and gluconeogenesis. Finally, insulin promotes triglyceride and protein synthesis. The secretion of insulin is regulated through a negative feedback mechanism. As blood glucose levels decrease, further insulin release is inhibited. The pancreatic hormones are summarized in Table 7.<sup>(21)</sup>

<b>Hormones of the Pancreas (Table 7)</b>		
<b>Associated hormones</b>	<b>Chemical class</b>	<b>Effect</b>
Insulin (beta cells)	Protein	Reduces blood glucose levels
Glucagon (alpha cells)	Protein	Increases blood glucose levels
Somatostatin (delta cells)	Protein	Inhibits insulin and glucagon release
Pancreatic polypeptide (PP cells)	Protein	Role in appetite

### **DIABETES MELLITUS**

Diabetes mellitus is a metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action.<sup>(25,28)</sup> diabetes is a serious chronic disease that occurs either when the pancreas does not produce enough insulin (a hormone that regulates blood sugar or glucose). Or when the body cannot effectively use the insulin it produces. Diabetes is an important public health problem, one of four priority non communicable diseases (NCDS) the majority of the people with diabetes are affected by type2 diabetes. This used to occur nearly entirely among adults, but now occurs in children too. Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. The global prevalence (age standardized) of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population. Diabetes caused 1.5 million deaths in 2012 higher than – optimal blood glucose caused by an additional 2.2 million deaths.<sup>(29)</sup>

**Table 1: ETIOLOGIC CLASSIFICATION OF DIABETES MELLITUS**

<b>1. TYPE 1 diabetes</b> (beta cell destruction , usually leading to absolute insulin deficiency ) A. Immune-mediated B. Idiopathic
<b>2. Type 2 diabetes</b> (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretory defect with insulin resistance )
<b>3. Other specific types of diabetes</b> A. Genetic defects of $\beta$ –cell function characterized by mutation B. Genetic defects in insulin action C. Diseases of the exocrine pancreas D. Endocrinopathies E. Drug or chemical induced F. Infections G. Uncommon forms of immune – mediated diabetes H. Other genetic syndromes sometimes associated with diabetes
<b>4. Gestational diabetes mellitus(GDM)</b>

Courtesy: Harrison’s Principle of internal Medicine<sup>(31)</sup>

### **COMPLICATION OF DIABETES MELLITUS:**

Diabetes mellitus causes acute and chronic complications.

**Table 2: Acute and Chronic Complications**



<b>ACUTE COMPLICATIONS</b>
Diabetic keto acidosis (DKA) and hyperglycemic hyperosmolar state(HHS) are acute complications of diabetes <sup>(31)</sup>
<b>CHRONIC COMPLICATIONS</b>
<b>Micro vascular</b> Eye disease Retinopathy (non-proliferative/proliferative) Macular edema Neuropathy Sensory and motor (mono- and poly neuropathy) Autonomic Nephropathy
<b>Macro vascular</b> Coronary artery disease Peripheral vascular disease Cerebrovascular disease
<b>Non Vascular</b> Gastrointestinal (gastro paresis, diarrhea) Genitourinary (uropathy / sexual dysfunction) Dermatologic Infectious Glaucoma

**Courtesy: Harrison’s Principle of internal Medicine<sup>(22)</sup>**

**The laboratory diagnosis of diabetes:**

The laboratory diagnosis of diabetes is made exclusively by the demonstration of Hyperglycemia. <sup>(32)</sup>

**Table 3: Criteria for the Diagnosis of Diabetes Mellitus <sup>(32)</sup>**

<p><b>A. GLUCOSE</b> 1. Fasting plasma glucose (FPG) <math>\geq 7.0</math> mmol/L (126 mg/dl) OR 2. Symptoms of hyperglycemia and casual plasma glucose <math>\geq 11.1</math> mmol/L (200mg/dl) 3. 2 Hour plasma glucose <math>\geq 11.1</math> mmol/L (200 mg/dl) During an oral glucose tolerance test (OGTT) <b>B. Hemoglobin A1c (HbA1c)</b> HbA1c <math>\geq 6.5\%</math> In the absence of unequivocal hyperglycemia, these criteria should be confirmed by repeating the same test on a different day. Mixing different methods to diagnosis diabetes should be avoided.</p>
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**Courtesy: Tietz Text book of clinical Chemistry.**

**PANCREATITIS**

Inflammatory disorders of the pancreas range in severity from mild, self-limited disease to life-threatening, widely destructive process, and are accordingly associated with deficits that may be trivial and transient or serious and permanent.<sup>(22)</sup>

**Pancreatitis is of two types:**

1. Acute pancreatitis
2. Chronic pancreatitis

**1. Acute pancreatitis**

Acute pancreatitis is a reversible inflammatory disorder that varies in severity, ranging from focal edema and fat necrosis to widespread hemorrhagic parenchymal necrosis. Acute pancreatitis is relatively common, with an annual

incidence of 10 to 20 per 100,000 people in the Western world. Approximately 80% of cases are attributable to either biliary tract disease or alcoholism (Table 16–1). Roughly 5% of patients with gallstones develop acute pancreatitis, and

gallstones are implicated in 35% to 60% of cases overall Excessive alcohol intake has been reported as a cause of acute pancreatitis at variable rates, from 65% of cases in the United States to 5% or less in the United Kingdom. Other causes of acute pancreatitis include

- Non–gallstone-related obstruction of the pancreatic ducts (e.g., due to periampullary neoplasms such as pancreatic cancer, pancreas divisum, biliary “sludge,” or parasites, particularly *Ascaris lumbricoides* and *Clonorchis sinensis*)<sup>(22)</sup>
- Medications including anticonvulsants, cancer chemotherapeutic agents, thiazide diuretics, estrogens, and more than 85 others in clinical use
- Infections with mumps virus or coxsackievirus
- Metabolic disorders, including hypertriglyceridemia, hyperparathyroidism, and other hypercalcemic states<sup>(22)</sup>

**Table 4:**

**Table 16–1** Etiologic Factors in Acute Pancreatitis

<b>Metabolic</b>
Alcoholism*
Hyperlipoproteinemia
Hypercalcemia
Drugs (e.g., azathioprine)
<b>Genetic</b>
Mutations in the cationic trypsinogen ( <i>PRSS1</i> ) and trypsin inhibitor ( <i>SPINK1</i> ) genes
<b>Mechanical</b>
Gallstones*
Trauma
Iatrogenic injury
Perioperative injury
Endoscopic procedures with dye injection
<b>Vascular</b>
Shock
Atheroembolism
Polyarteritis nodosa
<b>Infectious</b>
Mumps
Coxsackievirus

\*Most common causes in the United States.

- Ischemia due to vascular thrombosis, embolism, vasculitis, or shock
- Trauma, both blunt force and iatrogenic during surgery or endoscopy<sup>(22)</sup>
- Inherited mutations in genes encoding pancreatic enzymes or their inhibitors (e.g., *SPINK1*). For example, hereditary pancreatitis is an autosomal dominant disease with 80% penetrance that is characterized by recurrent attacks of severe pancreatitis, usually beginning in childhood. It is caused by mutations in the gene *PRSS1*, which encodes trypsinogen, the pro enzyme of pancreatic trypsin. The pathogenic mutations alter the site through which trypsin cleaves and inactivates itself, abrogating an important negative feedback mechanism. This defect leads not only to the hyper activation of trypsin, but also to the hyper activation of many other digestive enzymes that require trypsin cleavage for their activation. As a result of this unbridled protease activity, the pancreas is prone to auto digestion and injury. Of note, 10% to 20% of cases of acute pancreatitis have no identifiable cause (idiopathic pancreatitis), although a growing body of evidence suggests that many may have an underlying genetic basis.<sup>(22)</sup>

#### **Clinical Features of acute pancreatitis:**

- i. Severe upper abdominal pain
- ii. Nausea and vomiting
- iii. Loss of appetite and weight
- iv. Fever
- v. Shock.

Laboratory findings include markedly elevated serum amylase during the first 24 hours, followed (within 72 to 96 hours) by rising serum lipase levels. Hypocalcemia can result from precipitation of calcium in areas of fat necrosis; if persistent, it is a poor prognostic sign.

#### **Chronic Pancreatitis**

Chronic pancreatitis is characterized by long-standing inflammation, fibrosis, and destruction of the exocrine pancreas; in its late stages, the endocrine parenchyma also is lost. Although chronic pancreatitis can result from recurrent bouts of acute pancreatitis, the chief distinction between acute and chronic pancreatitis is the irreversible impairment in pancreatic function in the latter. The prevalence of chronic pancreatitis is difficult to determine but probably ranges between 0.04% and 5% of the U.S. population. By far the most common cause of chronic pancreatitis is long-term alcohol abuse; middle-aged men constitute the bulk of patients in this etiologic group. Less common causes of chronic pancreatitis include<sup>(22)</sup>

- Long-standing pancreatic duct obstruction (e.g., by pseudocysts, calculi, neoplasms, or pancreas divisum)
- Tropical pancreatitis, a poorly characterized heterogeneous disorder seen in Africa and Asia, with a subset of cases having a genetic basis
- Hereditary pancreatitis due to mutations in the pancreatic trypsinogen gene (PRRS1) (see Table 16–1 earlier), or the SPINK1 gene encoding a trypsin inhibitor
- Chronic pancreatitis associated with CFTR mutations. As discussed in detail in Chapter 6, cystic fibrosis is caused by mutations in the CFTR gene; the CFTR protein also is expressed in pancreatic ductal epithelium, and CFTR mutations decrease bicarbonate secretion and increase the viscosity of the secretions, thereby promoting protein plugging. As many as 40% of persons with chronic pancreatitis have no recognizable predisposing factors.<sup>(22)</sup>

As with acute pancreatitis, however, a growing number of these “idiopathic” cases are associated with inherited mutations in genes important for normal pancreatic exocrine function. For example, genetic testing reveals that 25% to 30% of patients with “idiopathic” pancreatitis harbor germline mutations in the CFTR gene, albeit distinct from the ones that lead to classic multisystem cystic fibrosis.<sup>(22)</sup>

### **CAUSES OF CHRONIC PANCREATITIS**

- i. Longtime consumption of alcohol
- ii. Chronic obstruction of ampulla of Vater by gallstone
- iii. Hereditary cause (passed on genetically from one generation to another)
- iv. Congenital abnormalities of pancreatic duct
- v. **Cystic fibrosis**, a generalized disorder affecting the functions of many organs such as lungs (due to excessive mucus), exocrine glands like pancreas, biliary system and immune system
- vi. Malnutrition (poor nutrition; mal = bad)
- vii. Idiopathic pancreatitis (due to unknown cause).<sup>(22)</sup>

### **FEATURES OF CHRONIC PANCREATITIS**

- i. Complete destruction of pancreas: During the obstruction of biliary ducts, more amount of trypsinogen and other enzymes are accumulated. In spite of the presence of trypsin inhibitor in acini, some trypsinogen is activated. Trypsin in turn activates other proteolytic enzymes. All these enzymes destroy the pancreatic tissues completely
- ii. Absence of pancreatic enzymes: Pancreatitis is more dangerous because the destruction of acinar cells in pancreas leads to deficiency or total absence of pancreatic enzymes. So the digestive processes are affected; worst affected is fat digestion that results in steatorrhea
- iii. Severe pain in upper abdominal region, which radiates to the back
- iv. Fever, nausea and vomiting
- v. Tender and swollen abdomen
- vi. Weight loss.<sup>(22)</sup>

Although chronic pancreatitis is usually not acutely life threatening, the long-term outlook is poor, with a 50% mortality rate over 20 to 25 years. Severe pancreatic exocrine insufficiency and chronic malabsorption may develop, as can diabetes mellitus. In other patients, severe chronic pain may dominate the clinical picture. Pancreatic pseudocysts (described earlier) develop in about 10% of patients. Persons with hereditary pancreatitis have a 40% lifetime risk of developing pancreatic cancer. The degree to which other forms of chronic pancreatitis contribute to cancer development is unclear.<sup>(22)</sup>

## **V. MATERIAL AND METHODOLOGY**

### **SUBJECT SELECTION:**

The study was conducted prospectively with the approval from the institutional Ethics committee. This study was conducted in the Clinical Biochemistry Laboratory, Saveetha Medical College & Hospital. 30 type II Diabetes mellitus patients and 30 healthy control were included in the study. These patients and healthy control were selected from people attending various departments of Saveetha Medical College and Hospital.

**INCLUSION CRITERIA:** 20-60 years both genders

Known type II Diabetes Mellitus

**EXCLUSION CRITERIA:** Cardiovascular disease,

Hepatic disorders and alcohol abuse

**ARMAMENTARUM REQUIRED:**

- Sodium fluoride tubes
- Polypropylene EDTA tubes
- Plain tube
- Tourniquet
- Syringes (5 ml)
- Cotton
- Spirit
- Band aid

**SPECIMEN USED:** Venous blood

**SAMPLE COLLECTION:**

Fasting venous blood was collected from the cases (diabetic patients) and controls (healthy subjects).The blood samples were centrifuged and the plasma/serum was separated for analysis on the same day for the blood glucose, the Fasting Plasma Glucose (FPG), Post prandial Plasma Glucose (PPPG) which included the estimation of serum amylase, lipase and same time EDTA whole blood sample also collected in Glycated hemoglobin (HbA<sub>1c</sub>) activity as per the routine procedure which was followed in the department.

**PARAMETER:**

Parameter	Method	Reference range
Fasting Plasma Glucose	Glucose Oxidase-Peroxidase	74-106 mg/dl
Post prandial Plasma Glucose	Glucose Oxidase-Peroxidase	< 140 mg/dl
HbA <sub>1c</sub>	HPLC	Normal: 4.2 – 6.2 % Good Control: 5.5-6.8 % Fair control : 6.8-7.6 % Poor control : > 7.6 %
Serum. Amylase	CNP-G3	32-110 IU/L
Serum. Lipase	Enzymatic	23-300 U/L

**Analytical procedures conducted according to the following methods**

The sample analysis was carried out on a fully automated analyser by using different reagent kits as per the procedure which was defined by the manufacturer. The estimation of fasting blood glucose and post prandial blood glucose was done by the glucose oxidase-peroxidase method. The amylase was done by CNP-G3 and lipase activity was measured by a photometric enzymatic method. The HbA<sub>1c</sub> was measured by High performance liquid chromatography [HPLC] method.

**GLUCOSE**

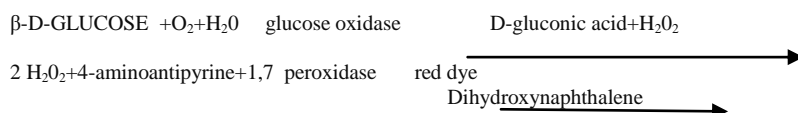
**VITROS 250 PRINCIPLE**

The <sup>(48)</sup> VITROS GLU Slide method is performed using the VITROS GLU Slides and the vitros chemistry products calibrator kit 1 on VITROS 250/350/950/5.1 FS and 4600 Chemistry systems and the VITROS 5600 integrated system. The VITROS GLU Slide is a multilayered, analytical element coated on a polyester support. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. The oxidation of sample glucose is catalyzed by glucose oxidase to form hydrogen peroxide and gluconate. This reaction is followed by an oxidative coupling catalyzed by peroxidase in the presence of dye precursors to produce a dye. The intensity of the dye is measured by reflected light. The dye system used is closely related to the first reported by Trinder. <sup>(49)</sup> The chemistry of the glucose slides has been described by curme,et al. <sup>(50)</sup>

**TEST TYPE AND CONDITIONS**

TEST TYPE	VITROS SYSTEM	APPROXIMATE INCUBATION TIME	TEMPERATURE	WAVELENGTH	REACTION SAMPLE VOLUME
Colorimetric	5600, 250/350	5 MINUTES	37 <sup>o</sup> C,98.6 <sup>o</sup> F	540nm	10µl

**REACTION SCHEME**



**VITROS INSTRUMENT**



**REAGENTS**

**SLIDE INGREDIENTS**

**Reactive ingredients per cm<sup>2</sup>**

Glucose oxidase (*Aspergillus* sp) 0.77 U; peroxidase (horseradish root) root) 3.6 U; 1, 7-Dihydroxynaphthalene (dye precursor) 67 µg and 4-aminoantipyrine hydrochloride (dye precursor) 0.11 mg

**Other ingredients:** Pigment, binders, buffer, surfactants, stabilizers and coss; linking agent.

**REAGENT HANDLING**

1. Inspect the packaging for signs of damage.
2. Be careful when opening the outer packaging with a sharp instrument so as to avoid damage to the individual product packaging.

**REAGENT PREPERATION**

1. Remove the slide cartridges from storage.
2. Warm the wrapped cartridge at room temperature for 30 minutes when taken from the refrigerator or 60 minutes from the freezer.
3. Unwrap and load the cartridge in to the slide supply. <sup>(36, 37, 51)</sup>

**SPECIMEN COLLECTION & PREPERATION AND STORAGE**

**Specimens Recommended**

Plasma for sodium fluoride/potassium oxalate (See Reagent Storage and Stability table for slide storage when using this specimen type)

## **PLASMA**

### **Patient preparation**

No special patient preparation is necessary.<sup>(52)</sup>

### **SPECIAL PRECAUTIONS**

1. For the effect of sample hemolysis on test results. Refer to “Limitation of the procedure
2. Grossly lipemic samples must be diluted twofold prior to analysis. Refer to “Sample Dilution for dilution instructions.
3. In vitro glycolysis decreases glucose by 5-7% per hour at room temperature.
4. Heparin, EDTA or Sodium fluoride/potassium oxalate plasma:
  - Follow manufacturer’s recommendation for mixing anticoagulant with specimens.
  - Separate plasma from cellular material according to the specimen collection tube manufacturer’s instructions. Additional guidance on specimen handling and processing can be found in CLSI H 18 – A4.<sup>(53)</sup>

### **TESTING PROCEDURE**

#### **Materials Required but Not Provided**

1. VITROS Chemistry products calibrator kit 1.<sup>(54)</sup>
2. Quality control materials, such as VITROS Chemistry products performance verifier I and II for serum and plasma tests or VITROS Chemistry products liquid performance verifier I and II for CSF tests.
3. VITROS Chemistry products 7% BSA
4. Isotonic saline or reagent –grade water
5. VITROS Chemistry products FS Diluent pack 2 (BSA/Saline) (for on – analyzer dilution)
6. VITROS Chemistry products FS Diluent pack 3 (BSA/Saline) (for on – analyzer dilution)

### **SAMPLE DILUTION**

#### **Plasma**

If glucose concentration exceed the system’s measuring (reportable or dynamic) range or if the sample is grossly lipemic :<sup>(55)</sup>

### **QUALITY CONTROL**

#### **QUALITY CONTROL PROCEDURE RECOMMENDATIONS**

1. Choose control levels that check the clinically relevant range.
2. Analyze quality control materials in the same manner as patients samples, before and during patient sample processing.
3. To verify system performance, analyze control materials:
  - After calibration.
  - According to local regulations or at least once each day that day that the test is being performed.
  - After specified service procedures are performed. Refer to the operating instructions for your system.
4. If control results fall outside your acceptable range, investigate the cause before deciding whether to report patient results.
5. For general quality control recommendations, refer to statistical quality control for quantitative measurements.

### **QUALITY CONTROL MATERIAL PREPERATION, HANDLING AND STORAGE**

Refer to the instructions for use for VITROS Chemistry products performance verifier I and II or to other manufacture’s product literature.

### **EXPECTED VALUES**

These reference intervals are based on external studies for serum<sup>16</sup>.

	<b>Conventional units(mg/dl)</b>	<b>SI units (mmol/L)</b>	<b>Alternate units (g/L)</b>
Fasting adults	74-106	4.1-5.9	0.7-1.1

Glucose concentration (mg/dl) ×24-hour volume (dl) =mg/day

### **LIMITATION OF THE PROCEDURE**

#### **Known interferences**

##### **Plasma**

- In fresh specimens, catalase released from the lysis of red blood cells causes a negative bias in glucose results. The degree of bias is proportional to the degree of hemolysis in fresh samples, a negative bias of up to 10% may be observed with a level of hemolysis associated with a hemoglobin concentration of 250 mg/dl (2.5 g/L).
- The VITROS GLU Slide method was screened for interfering substances following NCCLS Protocol EP7.<sup>(47,56,57)</sup>

## **GLYCATED HEMOGLOBIN (HbA<sub>1c</sub>)**

### **Estimation of HbA<sub>1c</sub> Methods:**

Various methods are available for the measurement of HbA<sub>1c</sub>. The principle of all methods is to separate the glycosylated and non glycosylated forms of hemoglobin (H-56). Methods of GHb assays have primarily evolved High Pressure Liquid Chromatography methodologies:

### **1. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY:**

**Principle:** This method is based on chromatographic separation of hemoglobin fractions by ion-exchange HPLC with a gradient mobile phase and spectrophotometric detection.

### **SAMPLE SELECTION :**

Whole blood collected with EDTA

### **Specimen storage :**

Whole blood specimen may be stored for 3 days at room temperature (15-30°C) and up to 7 days at 2-8 °C .

### **Sample preparation:**

1. Allow sample tubes to reach room temperature (15-30°C) before performing the assay.
2. This technique requires no predilution, or manual handling of patient's samples

### **REAGENTS:**

1. Dual kit recorder pack contains whole blood primer
2. Calibrator 1 and Calibrator 2
3. Calibrator diluents
4. Wash reagent
5. Elution buffer 1 & 2
6. Analytical cartridge

### **Storage instructions and reagent stability:**

The reagents are stable up to the end of the indicated month of expiry, if stored at 2-8 °C and contamination and evaporation are avoided. The reagents should be protected from light.

### **Reagent preparation :**

The reagents are ready to use.

### **ASSAY PROCEDURE :**

This technique requires no pre-dilution or manual handling of patient samples.

- The samples are directly introduced in their primary tubes following calibrators and control samples.
- The instrument draws sample directly from the EDTA vacutainer and all processing of the sample is performed internally.
- Samples are automatically mixed, diluted and injected into the cartridge.
- The analyzer delivers a programmed buffer gradient of increasing ionic-strength to the cartridge, where the haemoglobins are separated on the basis of their ionic-interactions with the cartridge material.
- The separated haemoglobin's are then passed through the flow cell of the filter photometer, where changes in the absorbance at 415 nm are measured.
- The run time is approximately 3 min per sample with a throughput of 20 samples per hour.
- A sample report and a chromatogram are generated for each sample.

### **REFERENCE VALUE :**

#### **Hemoglobin A<sub>1c</sub> ranges**

<b>Hemoglobin A<sub>1c</sub> (%)</b>	<b>Degree of Glucose Control</b>
> 8	Action Suggested <sup>+</sup>
< 7	Goal <sup>‡</sup>
< 6	Non-Diabetic level

<sup>+</sup> high risk of developing long-term complications such as retinopathy, nephropathy, neuropathy, and cardiopathy. Action suggested depends on individual patient circumstances.

<sup>‡</sup> Some danger of hypoglycemic reaction in Type 1 diabetes. Some glucose intolerant individuals and “sub-clinical” diabetics may demonstrate (elevated) HbA<sub>1c</sub> in this area.

### **AMYLASE**

Vitros chemistry products amylase slides quantitatively measure amylase (AMYL) activity in serum, plasma and urine using VITROS 250 integrated system.

**SUMMARY AND EXPLANATION OF THE TEST**

Amylase is an amylolytic digestive enzyme produced by the exocrine pancreas and salivary gland. Amylase is increased in acute pancreatitis, pancreatic abscess or pseudocyst, pancreatic trauma, amyloidosis, pancreatic neoplasm, common bile-duct obstruction, and after thoracic surgery. Increased amylase activity may be found in mumps parotitis and renal insufficiency.<sup>(24)</sup>

**PRINCIPLE OF THE PROCEDURE**

The VITROS AMYL slide is multilayered, analytical element coated on a poly ester support.

A drop of patient sample is deposited on the slide and evenly distributed by the spreading layer to the under layers. The spreading layer contains the dyed starch substrate (dye covalently linked to amylopectin ) for the reaction. The amylase in the sample catalyzes the hydrolysis of the dyed starch into smaller dyed saccharides. These dyed saccharides diffuse into the underlying reagent layer.

The reflection density of the dyed saccharides in the reagent layer is measured by reflectance spectrophotometry at 2.3 and 5 minutes. The difference in the slides reflection density between the two reading is proportional to sample amylase activity.

**TEST TYPE AND CONDITION**

Test Type	Approximate Incubation time	Temperature	Wavelength	Reaction sample volume
Two-point rate	5 minutes	37	540 nm	10 UI

**REACTION SCHEME**

Dyed amylopectin      amylase      dyed saccharides

**REAGENTS**

Slide ingredients

Reactive ingredients per cm<sup>2</sup>

Dyed amylopectin 340 ug.

Other ingredients

Pigment, binder, buffer, mordent, Surfactant & stabilizer

**Reagent Handling**

- Caution
- Do not use slide cartridge with damaged or incompletely sealed packing.
- Inspect the packaging for sign of damage.
- Be careful when the opening the outer packaging with a sharp instrument so as to avoid damage to the individual product packaging.

**Reagent Preparation**

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

**Important:** The slide storage must reach room temperature, 18 – 28 °C (64-82 °F)

Before it's unwrapped and loaded into the slide supply.

**Reagent Storage and Stability**

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

Reagent	Storage condition	Stability
Unopened	Refrigerated    2 – 8 °C (36-46 °F)	≤ 4 weeks
	Frozen            ≤ – 8 °C (≤ °F)	until the expiration date
Opened	On-analyzer    System turned on	≤ 2 weeks
	On-analyzer    System turned off	≤ 2 hours

Verify performance with quality control material:

- If the system is turned off for more than 2 hours.
- After reloading cartridges that have been removed from the slide supply and stored for later use.

**Specimen Collection, Preparation and Storage**

Specimen Recommended

- Serum



- Plasma : Heparin <sup>(35)</sup>
- Urine

Note: Plasma activities are approximately 20 U/L higher than serum activities<sup>(35)</sup>

**Specimen Not Recommended**

- Plasma
  - Citrate
  - EDTA
  - Fluoride oxalate
- Urine
  - Boric acid/Sodium formate
  - Glacial acetic acid
  - Concentrated hydrochloric acid

**Serum and Plasma**

Specimen Collection and Preparation

Collect specimen using standard laboratory procedure<sup>(36,37)</sup>

No special patient preparation is necessary

**Specimen Storage and Stability: Serum and Plasma**

Storage	Temperature	Stability
Room temperature	18–28 °C (64-82 °F)	≤ 7 days
Refrigerated	2–8 °C (36-46 °F)	≤ 1 month
Frozen	≤ -18 °C (≤ 0 °F)	Not recommended

**Urine**

Specimen Collection and Preparation

Collect specimen using standard laboratory procedure<sup>(38)</sup>

No special patient preparation is necessary.

**Specimen Storage and Stability: Urine**

Storage	Temperature	Stability
Room temperature	18–28 °C (64-82 °F)	≤ 7 days
Refrigerated	2–8 °C (36-46 °F)	≤ 1 month
Frozen	≤ -18 °C (≤ 0 °F)	Not recommended

**Testing Procedure**

- Take the slide cartridges from storage.
- Add 10ul of serum sample
- Approximate Incubate for 37 °C 5 minutes
- Take reading 540 nm

**Measuring Range (Reportable or Dynamic)**

	Conventional and SI Units ( U/L )	Alternate Units (ukat/L)
Serum	30-1200	0.5 – 20.0
Urine	30-1200	0.5 – 20.0

For out-of range samples, refer to “sample Dilution”

**Traceability of Calibration**

Values assigned to the VITROS Chemistry Products Calibrator Kit 3 for Amylase are traceable to the paranitrophenol matopentaoside method <sup>(39)</sup> at 37 °C

**Limitation of the Procedure**

**Known interferences**

The VITROS AMYL slide method was screened for interfering substance following NCCLS Protocol EP7. <sup>(31,32)</sup>None have been identified

For substance that were tested and did not interfere, refer to “Specificity”

**Reference Value**

	Conventional and SI Units (U/L)	Alternate Units ( $\mu$ kat/L)
Serum	30 -110	0.5 – 1.8
Urine	32 – 641	0.5 – 10.7

**LIPASE**

**Principle of the Procedure**

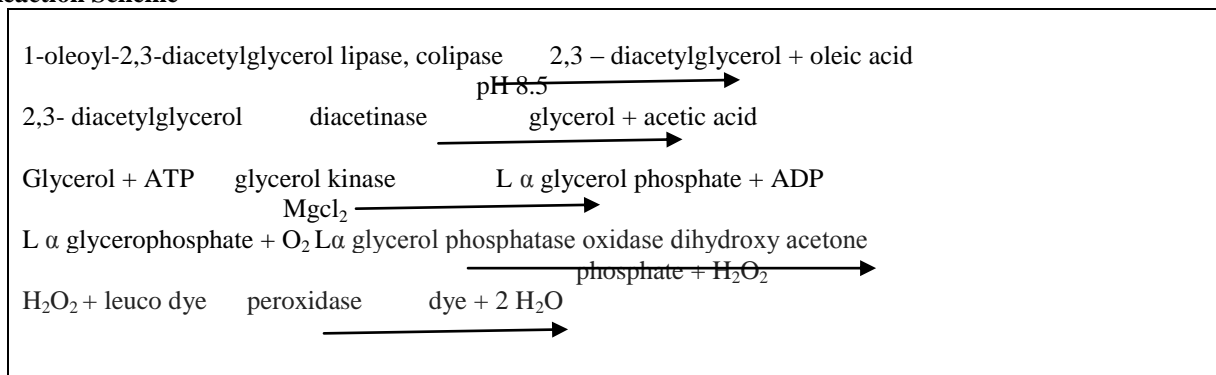
Lipase slide is a multilayered, analytical element coated on a poly ester support. The analysis is based on an enzymatic method described by Mauck <sup>(44)</sup>

A drop a patient sample is deposited on the slide and evenly distributed by the spring layer to the under laying layers. The method incorporates colipase which facilitates the adsorption of lipase to the substrate micelles in the presence of the bile salts. Lipase then catalyzes the hydrolysis of water soluble triacylglycerol ester. The method uses the enzyme diacetinase to convert the substrate 2,3-diacetylglycerol. Glycerol kinase converts the glycerol to L-alpha peroxidase. Peroxidases oxidize a leuco dye to produce a colored dye. The resulting change in reflection density is measured at the 2 times points. The difference in reflection density is proportional to the activity of lipase present in the simple.

**Test Type and Condition**

Test Type	Approximate Incubation Time	Temperature	Wavelength	Reaction Sample Volume
Two-point rate	5 minutes	37°C (98.6°F)	540 nm	5.5 $\mu$ L

**Reaction Scheme**



**Reagents**

Slide ingredients

Reactive Ingredients per cm

Diacetinase (Bacillus sp) 0.54U; glycerol kinase (Coli or Cellulomonas sp)

0.32 U; L-  $\alpha$ - glycerol phosphate oxidase (Aerococcus sp) 0.39U; peroxidase (horseradish root) 0.62U; colipase (porcine pancreas) 5.9 U; adenosine triphosphate

0.16 mg; 1-oleoyl-2,3-diacetyl glycerol 0.80 mg and 2-(3,5-dimethoxy-4-hydroxyphenyl)-4,5-bis(dimethylaminophenyl)imidazole (leuco dye) 33 $\mu$ g.

**Other ingredients**

Pigment, binders, surfactants, enzyme cofactor, stabilizers, buffer,

Dye solubilizes, scavenger ,scavenger and cross linking agent.

**Reagent Handling**

Caution: Do not use slide cartridge with damaged or incompletely sealed packaging

- Inspect the packaging for sign of damage.
- Be careful when the opening the outer packaging with a sharp instrument so as to avoid damage to the individual product packaging.

**Reagent Preparation**

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

**Important:** The slide storage must reach room temperature, 18 – 28 ° C (64-82 °F)

Before it’s unwrapped and loaded into the slide supply

**Reagent Storage and Stability**

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

Reagent	Storage condition	Stability
Unopened	Frozen $\leq -18\text{ }^{\circ}\text{C}$ ( $\leq 0\text{ }^{\circ}\text{F}$ )	until the expiration date
Opened	On-analyzer System turned on	$\leq 1$ weeks
	On-analyzer System turned off	$\leq 2$ hours

Verify performance with quality control material:

- If the system is turned off for more than 2 hours.
- After reloading cartridges that have been removed from the slide supply and stored for later use.

**Specimen Collection, Preparation and Storage**

Specimen Recommended

- Serum
- Plasma : Heparin

**Specimen Not Recommended**

Do not use grossly lipemic specimen. Refer to “Limitation of the procedure”

**Serum and Plasma**

Specimen Collection and Preparation

Collect specimen using standard laboratory procedure<sup>(36,37)</sup>

No special patient preparation is necessary

**Specimen Storage and Stability**

Storage	Temperature	Stability
Room temperature	18–28 °C (64-82 °F)	$\leq 7$ days
Refrigerated	2–8 °C (36-46 °F)	$\leq 3$ weeks
Frozen	$\leq -18\text{ }^{\circ}\text{C}$ ( $\leq 0\text{ }^{\circ}\text{F}$ )	$\leq 5$ months

**Testing Procedure**

- Vitros chemistry products LIPA slides
- Quality control performance verifier I & II
- 7 % BSA
- FS Diluent Pack 2 (BSA/Saline)

**Operation instructions**

Bring all fluids and samples to room temperature 18–28 °C (64-82 °F), prior to analysis.

**Sample Dilution**

If Lipase activity exceeds the systems measuring range:

**Manual sample dilution**

1. Dilute the samples with 7% BSA
2. Reanalyze.
3. Multiply the results by the dilution factor to obtain an estimate of the original samples lipase activity.

**Measuring Range (Reportable or Dynamic)**

Conventional and SI Units ( U/L )	Alternate Units ( ukat/L)
10-2000	0.2 – 33.4

For out of range samples, refer to “sample dilution”

**Traceability of Calibration**

Values assigned to the VITROS Chemistry Products Calibrator Kit 3 for Lipase activity in standard triolein emulsion with a pH- Stt analyzer<sup>(45)</sup>

**Limitation of the Procedure**

**Known interferences**

The VITROS AMYL slide method was screened for interfering substance following NCCLS Protocol EP7. <sup>(40,41)</sup>None have been identified

For substance that were tested and did not interfere, refer to “Specificity”

Interferent	Interferent Concentration	Lipase Activity		Average Basis	
		Conv.and SI Alternative Units		Conv.and SI Alternative Units	
		U/L	(ukat/L)	U/L	(ukat/L)
5-Aminosalicylate	23 mg/dl (1.5mmol/L)	200	3.34	-28	-0.47
Hemoglobin	600 mg/dl (6g/L)	283	4.73	-35	0.58

**Other limitation**

- Normal endogenous concentrations of glycerol do not interfere with this method; however, samples containing high concentration of glycerol will be flagged with DP code .samples flagged with a DP code should be diluted and reanalyzed. Refer to the “sample dilution”. Highly elevated glycerol concentration are usually caused by contamination the final lipase result may be normal.

- Certain drugs and clinical condition are known to alter lipase activity  
In vivo. For additional information, refer to one of the published summaries<sup>(46,47)</sup>

**Reference Interval**

Conventional and SI Units ( U/L )	Alternate Units (ukat/L)
23 – 300	0.4 – 5.0

**VI. RESULTS**

In our study Fasting, post prandial plasma glucose ,Hba1c ,Serum amylase and lipase are estimated for 30 healthy controls and 30 type II Diabetes mellitus patients and the values are Tabulated in Table 1 & 2.

**TABLE 1:**

Parameter	Sample size (N)	Control	Minimum	Maximum
		Mean ± SD		
Fasting Plasma Glucose	30	91.90 ± 8.57	76	112
Post prandial Plasma Glucose	30	110.37 ± 18.35	74	168
Hba1c	30	5.48 ± 0.267	4.8	5.9
Amylase	30	70.73 ± 13.28	48	102
Lipase	30	79.53 ± 13.97	59	116

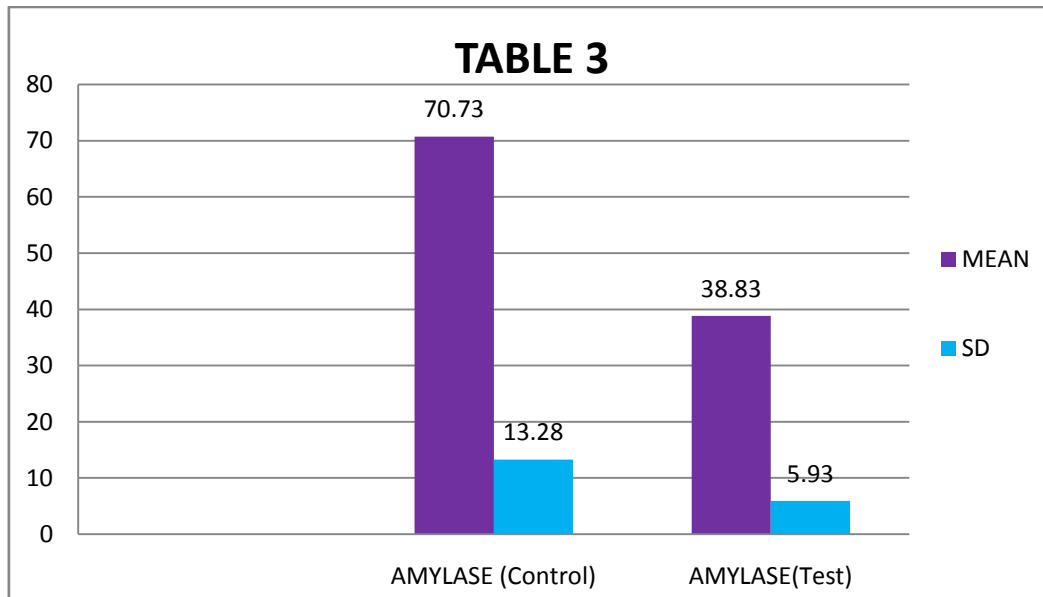
Parameter	Sample size(N)	Case	Minimum	Maximum
		Mean ± SD		
Fasting Plasma Glucose	30	226.43 ± 97.12	85	592
Post prandial Plasma Glucose	30	337.23 ± 119.28	159	627
Hba1c	30	12.357 ± 2.235	9.0	18.0
Amylase	30	38.83 ± 5.93	28	49
Lipase	30	31.5 ± 4.99	21	43

**TABLE 2:**

**TABLE 3:** Comparison of Serum amylase between healthy controls and type II diabetes mellitus patients.

Parameter	Group	N	Mean ± SD	Minimum	Maximum	P value
Amylase	Control	30	70.73 ± 3.28	48	102	<0.001
	Case	30	38.83 ± 5.93	28	49	

Serum amylase for healthy control ranged between 48 to 102.

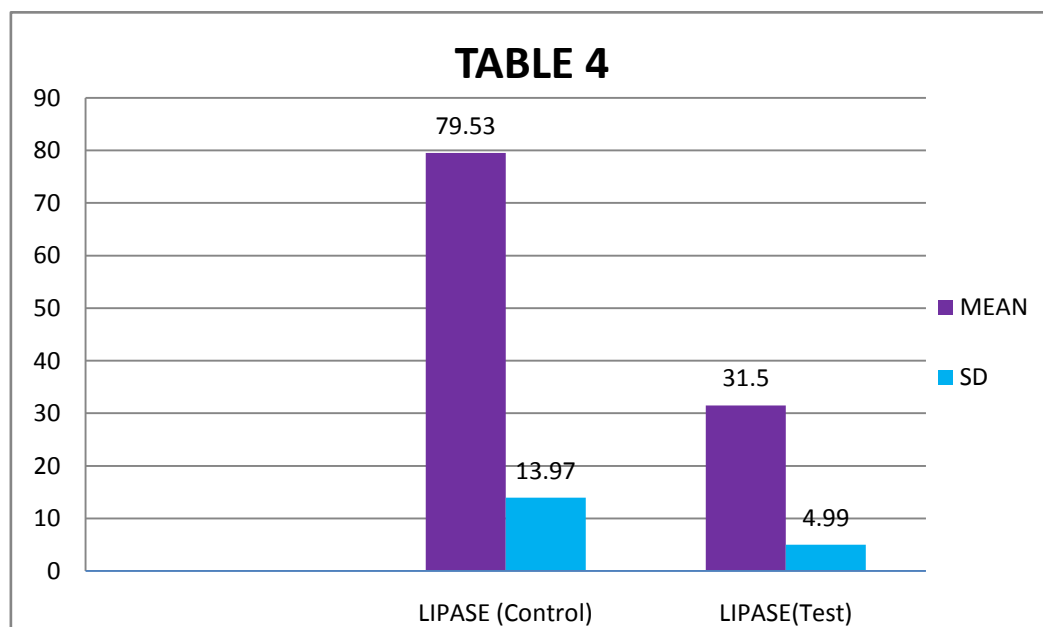


There is a statically significant decrease in serum amylase of diabetic patients when compared to healthy controls ( P value <0.001)

**Table 4:** Comparison of Serum Lipase between healthy controls and type II diabetes mellitus patients.

Parameter	Group	N	Mean ± SD	Minimum	Maximum	P value
Lipase	Control	30	79.53 ±13.97	28	49	<0.001
	Test	30	31.5 ± 4.99	21	43	

Serum Lipase for healthy control ranged between 48 to 102.



There is a statically significant decrease in serum lipase of diabetic patients when compared to healthy controls ( P value <0.001)

## VII. DISCUSSION

In our study we have estimated serum amylase and lipase in 30 healthy controls and 30 Type II Diabetes Mellitus patients. There is a statistically significant decrease in serum amylase & lipase values in Type II Diabetes Mellitus patients and healthy controls. The serum amylase ranged between 28-49 Iu/L in type II Diabetes Mellitus patients and 48-102 Iu/L in healthy controls. The serum lipase ranged between 21- 48 Iu/L in type II Diabetes Mellitus patients and 48-102 Iu/L in healthy controls.

### **This is in consistent with the following studies**

There is a statistically significant decrease in serum amylase and lipase levels in type II Diabetes Mellitus patients than in healthy controls.

1. Rakhee yadav, Jai prakash bhartiya, Sunil Kumar verma, Manoj Kumar verma, Manoj Kumar nandkeoliar. The Evaluation of Serum Amylase in the patients of Type 2 Diabetes Mellitus, with a possible Correlation with the Pancreatic Function. JCDR, 2013 Jul, Vol-7(7);1291-12942.
2. Nakajima K. Low serum amylase and obesity, diabetes and metabolic syndrome: [15]A novel interpretation. *World J Diabetes*. 2016;7(6):112–21. <sup>(58)</sup>
3. Snehanakar K, Rumi D, Risha G, Kailash B. Serum amylase and lipase activities in [17] newly diagnosed patients with type 2 diabetes mellitus. *International Journal of Advanced Research*. 2016;4(7):1476-83. <sup>(59)</sup>
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In all the above 6 studies the authors found a decrease in serum amylase and lipase in Type I and Type II Diabetes Mellitus Patients than healthy controls.

The decrease in serum amylase and lipase in Diabetes Mellitus could be due to the following reasons.

1. In diabetes there will be pancreatic fibrosis, atrophy, fatty infiltration and loss of Exocrine acinar cells.
2. The hormones like insulin and glucagon secreted by pancreas, influence the enzyme synthesis and its release from the exocrine pancreas. Insulin has a trophic/stimulatory effect on the acinar cells, whereas glucagon has an inhibitory influence on the exocrine secretions which leads to decrease in the sensitivity of the diabetic pancreatic acini to secretagogues. So, the deficiency of insulin and the excess of glucagon in diabetes affect the internal milieu of the pancreas and decreases the secretion of enzymes Amylase and Lipase.
3. Diabetic neuropathy may lead to impaired entero-pancreatic reflexes and exocrine dysfunction
4. Reduced cytosolic free calcium concentration and gene expression for amylase.
5. Due to cytokines such as TNF-alpha, TGF-alpha 2 Beta and gastrin.

## VIII. CONCLUSION

From the results and the discussion held so far the following are concluded.

1. Serum amylase and lipase are decreased in Type II Diabetes Mellitus patients than healthy controls.
2. Hence there is derangement of exocrine function of pancreas in Diabetes Mellitus
3. Estimation of serum amylase and lipase should be made mandatory in all Diabetes Mellitus patients to assess the pancreatic exocrine function.

### **FUTURE SCOPE OF THE STUDY**

1. The study can be extended with larger population
2. The study can be done in both Type I and Type II Diabetes Mellitus patients.
3. Can be done along with the estimation of insulin.
4. Can be done at different levels of Glucose.

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#### **ABBREVIATION**

ADA	: American Diabetes Association
CCK	: Cholecystokinin
CLSI	: Clinical and Laboratory Standards Institute
DM	: Diabetes Mellitus
DKA	: Diabetic Keto Acidosis
EDTA	: Ethylene Di amino Tetra Acetic acid
FPG	: Fasting PlasmaGlucose
GDM	: Gestational Diabetes Mellitus
GOD POD	: Glucose oxidase-peroxidase
HbA <sub>1c</sub>	: Glycated hemoglobin
HPLC	: High Performance Liquid Chromatography
HHS	: Lyperglycemic Hyperosmolar State
OGTT	: Oral Glucose Toleranc Test
PPPG	: Postprandial PlasmaGlucose
PP	: Pancreatic Polypeptide
NCDS	: Non Communicable Diseases
NCCLS	:National Committee for Clinical Laboratory Standards
TNF	: Tumer Necrosis Factor
TGF	