

ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
DEPARTMENT OF MEDICAL LABORATORY SCIENCES



Assessment of Serum Amylase and Lipase Levels and associated factors among Type 2 Diabetes Mellitus follow up Patients at Hakim Gizaw Teaching Hospital Debre Berhan, Ethiopia

By: Afewerk Habtamu Aseme

Advisors: Samuel Kinde (MSc, PhD fellow)
Gobena Dedefo (MSc)

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This is to certify that the thesis prepared by Afewerk Habtamu, entitled:

Assessment of Serum Amylase and Lipase Levels in Controlled vs. Uncontrolled Type 2 Diabetes Mellitus follow up Patients at Hakim Gizaw Teaching Hospital Debre Berhan, Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Clinical Chemistry) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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External Examiner _____ Signature _____ Date _____

Internal Examiner _____ Signature _____ Date _____

Advisor _____ Signature _____ Date _____

Advisor _____ Signature _____ Date _____

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Abbreviations

ADA	American Diabetic Association
AUC	Area Under the Curve
BAI	Body Adiposity Index
BMI	Body Mass Index
BP	Blood pressure
FBS	Fasting Blood Sugar
HbA1c	Hemoglobin A1c
IDF	International Diabetic Federation
NAD	Nicotineamide Adenine Dinucleotide
NADH	Nicotineamide Adenine Dinucleotide
ROC	Receiver Operating Characteristics
SSA	Sub-Saharan Africa
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus

Abstract

Background: Diabetes mellitus is the leading non communicable disease in most low and middle income countries. Due to lack of proper follow up tools in most peripheral areas, majority of patients attending follow-up appointment at diabetic clinics failed to maintain good glycemic control causing destruction of the exocrinal cells of pancreas resulting in impaired secretion of pancreatic amylase and lipase. Therefore, assessment of serum amylase and lipase in uncontrolled and controlled type 2 diabetes may possibly help to effectively manage diabetes mellitus.

Objective: To assess the level of serum amylase and lipase and associated factors among type 2 diabetic follow-up patients, 2025.

Methods: A hospital based comparative cross-sectional study design from February 05 to March 30, 2025 was conducted at Hakim Gizaw Teaching Hospital Debre Berhan, Ethiopia with a total of 112(56 controlled and 56 uncontrolled) type 2 diabetes mellitus (T2DM) patients recruited using convenient sampling technique. Sociodemographic data was collected by face to face interview using pre tested questionnaire. HbA1c, FBS, Serum amylase, and Serum lipase was measured using Siemens Dimension EXL 200 clinical chemistry analyzer. IBM SPSS Version 27 was utilized for data entry, clearing and analysis with a significance level of $P < 0.05$.

Result: The mean level of serum amylase and lipase were significantly decreased in uncontrolled T2DM than controlled T2DM ($p < 0.001$). Serum amylase level showed statistically significant negative correlation with duration of DM ($r = -0.599$, $p < 0.001$), Waist to Hip ratio (WHR) ($r = 0.249$, $p = 0.008$), SBP ($r = -0.251$, $p = 0.008$), DBP ($r = -0.257$, $p = 0.006$), FBS ($r = -0.478$, $p = < 0.001$) and HbA1c ($r = -0.575$, $p = < 0.001$). Serum lipase had a strong negative correlation with duration of DM ($r = -0.484$, $p < 0.001$), FBS ($r = -0.625$, $p = < 0.001$) and HbA1c ($r = -0.679$, $p = < 0.001$). Increased HbA1c, taking OHA, abdominal discomfort were key associated factors for decreased serum amylase and lipase. The AUC from ROC curve for serum amylase and lipase was 0.849 and 0.955 respectively indicating the effectiveness of these enzymes to investigate glycemic status in T2DM.

Conclusion: The study demonstrated that uncontrolled T2DM patients have significantly decreased serum amylase and lipase this might be due to destruction of exocrine compartment of pancreas. Therefore, these enzymes might be additional biomarkers to manage T2DM and further large scale studies are recommended to investigate their diagnostic role to predict glycemic control among T2DM.

Key words: Diabetes mellitus, Amylase, Lipase, HbA1c, Blood glucose, Pancreas

1. Introduction

1.1. Background

Diabetes mellitus is a cluster of metabolic disorders characterized by a chronic hyperglycemia resulted either from lack of insulin secretion by pancreas or decreased sensitivity of target cells to the secreted insulin or both (1). Depending on its etiology, Diabetes mellitus can be grouped as Type 1 diabetes mellitus (T1DM), Type 2 diabetes mellitus (T2DM), Gestational diabetes mellitus (GDM) and other types of diabetes mellitus with Type 2 diabetes mellitus accounting the highest (more than 90%) prevalence and incidence followed by type 1 diabetes mellitus worldwide (2, 3).

Pancreas serves our body as endocrine and exocrine gland with clusters of the endocrinal islets cell covering the exocrinal acinar cells. The endocrinal cells secrete hormones (insulin and glucagon) to regulate the blood glucose level and the exocrinal acinar cells produce digestive enzymes (amylase and lipase) (4). In patients with type 2 diabetes mellitus, connection between the exocrine and endocrinal cells is lost and the insulin-acinar-ductal-incretin gut hormonal axis will be dysfunctional resulting in defective signaling and secretion of insulin; in conjunction with other defects the pancreas will face alteration in secretion and release of pancreatic exocrinal enzymes including amylase and lipase (5). Furthermore, the pancreatic juice output is controlled by the central nervous system, the naturally occurring gut hormones, cholecystokinin and secretin; this interconnected regulation of pancreatic juice secretion is disturbed by the common autonomic neuropathy and micro vascular diabetic complications (6).

Recent studies reported that the mean activity of pancreatic amylase and lipase were decreased among patients with diabetes mellitus as compared with normal individuals. The most important enzyme for breaking down dietary fat, decreasing the buildup of fat in adipose tissue, and regulating weight gain is pancreatic lipase, all of which have positive effects on overweight and obesity, which are common in diabetes patients. Triglycerides, which make up 90 to 95% of the ingested fats, are broken down and absorbed by pancreatic lipase (7).

Several factors showed association with decreased serum amylase and lipase level among diabetes mellitus patients. A recent animal study and other human studies showed that sedentary life styles without regular physical exercise leading to obesity was strongly associated with a decreased pancreatic

amylase and lipase (8, 9). Various studies investigated that population aging, sedentary lifestyles and high-calorie diets contributed for the decreased serum amylase and lipase among diabetic patients (10, 11). Prolonged duration of diabetes mellitus also showed impaired exocrinal activity of the pancreas while a large scale trial on the effect and action of diabetic therapeutic agents in diabetes showed that both serum amylase and lipase was slightly elevated above the upper normal limit. This was explained as the acute inflammation of pancreas due to the adverse effect of the oral anti-glycemic agents with the exception of metformin which causes no difference on serum amylase and lipase levels (12).

The majority of previous studies have investigated the metabolic imbalance caused by impaired insulin action and chronic hyperglycemia without any attempt to associate pancreatic exocrine enzyme activity with glycemic control status of diabetic patients. However, altered pancreatic endocrine activity among diabetic patients may result in derangement of the pancreatic exocrine activity; which may aid the management of diabetes mellitus by correlating with glycation status (13). Hence, the current study aimed to assess serum amylase and lipase level in controlled and uncontrolled type 2 diabetics in relation with their glycemic control status.

1.2. Statement of the problem

Non communicable diseases including diabetes mellitus are currently increasing in alarming rate remaining to be a major public health concern worldwide. The 2022 World Health Organization (WHO) reports showed that the incidence of new diabetes mellitus cases as well as its prevalence has been steadily increasing over the past few decades affecting the lives of majorities living in low and middle-income countries. According to this report, the total number of patients with diabetes mellitus increased from 180 million in 1980 to 455 million in 2014 (14).

In the Sub-Saharan Africa (SSA), 15.5 million peoples were estimated to live with diabetes mellitus in 2017 (15). Ethiopia is one of the countries in the SSA with high burden and mortality rate of diabetes mellitus. According to the first national WHO Steps survey of 2015, the overall prevalence of diabetes mellitus was 3.2% (16). Another systematic review and meta-analysis by Bishu.et.al in 2019 reported the prevalence of DM ranged from 2-6.5% (17). Some other researches also reported the prevalence of DM in the range of 0.5-6.5% (18, 19).

Diabetes mellitus is now a days a major non communicable disease with subclinical destruction of the exocrinal portion of the pancreas resulting in impaired secretion and activity of pancreatic enzymes, amylase and lipase with reported prevalence of about 50% and 30-50% exocrine pancreatic insufficiency among type 1 and type diabetes mellitus patients respectively (20). Recent study also explained that these important pancreatic enzymes are significantly decreased among type 2 diabetics with poor control as compared with the normal control (21). To date, with no large scale trials conducted, some of the existing studies correlated decreased serum amylase and lipase with metabolic disorders caused by insulin hypo secretion or resistance with diabetes mellitus its complications as a major disorder (22, 23).

Diabetic subjects with pancreatic exocrine insufficiency commonly presents with gastrointestinal symptoms such as loose bowel movement, abdominal discomfort and flatulence (24). Around 44% of Type 1 diabetes mellitus and 18% of Type 2 diabetes mellitus patients demonstrated to had exocrine insufficiency which caused deficiency of macronutrients, steatorrhea and consequent malnutrition among the patients (25, 26).

From histological and imaging (MRI, CT and Ultrasound) examination of diabetic patients several structural and functional derangements of pancreatic exocrinal cells were reported including fibrosis, atrophy, change in size and morphology. These exocrinal damages are associated with different causes,

involvement of the acinar cells in the autoimmune destruction of islets which is the primary cause of pancreatic exocrine insufficiency in type 1 diabetic patients (10). Obesity is common in type 2 diabetics imposed by accumulation of fats and adipose tissues in the central abdomen resulting in increased production of free fatty acids and deregulation of adipokine production which leads to impaired tropic action of insulin on the exocrinal cells (11). Moreover, autonomic diabetic neuropathy resulting in enteropancreatic reflex impairment and sufferance of exocrinal cells from hypoxia of micro vascular complications of diabetes mellitus are other ways of acinar cell destruction in diabetics patients (27, 28).

Even though the previous studies demonstrated the relation between the exocrine pancreatic function with diabetes mellitus by measuring the activity of serum amylase and lipase on diabetics and diabetic free study subjects, the association between glycemic control and serum amylase and lipase and the prognostic role of these pancreatic enzymes in diabetic patients is still under question. Furthermore, there is no study conducted in Ethiopia to investigate the exocrinal function of pancreas among diabetes mellitus patients and the association between glycemic control and these important pancreatic enzymes is not investigated.

However, complications of diabetes mellitus combined with the increasing sedentary life style in majority of the country including the current study area may result in increased destruction of pancreatic enzymes and assessment of these enzymes may have good prognostic value than the classic follow up methods. Therefore, we aimed to assess the activity of serum amylase and lipase among controlled and uncontrolled type 2 diabetes mellitus patients.

1.3. Significance of the study

There are limited studies conducted to evaluate the clinical relevance of serum amylase and lipase in diabetes mellitus patients though their results were inconsistent with some contradiction on the serum lipase. Therefore, assessing serum amylase and lipase among controlled and uncontrolled type 2 diabetes mellitus patients and establishing their association with glycemic control will enhance the management of diabetes mellitus.

The findings of this study clearly established the association between the level of serum amylase and lipase with glycemic control status as well as key factors associated with decreased level of the enzymes among T2DM patients was identified. Therefore, T2DM patients with abnormally decreased level of these enzymes could easily suspect poor glycemic control. The findings might be also utilized by health professionals as aid to consider assessment of these enzymes as a routine examination among T2DM patients to predict glycation status. Moreover, the scientific society might utilize the finding of this study as a baseline to perform large scale study on the prognostic relevance of serum amylase and lipase among T2DM patients.

2. Literature review

Anatomically, pancreas is comprised of the endocrinal islets cells (2%) which are responsible to secrete insulin and glucagon the two vital hormones involved in blood glucose regulation; the exocrinal acinar cells (84%) which are involved in secretion of digestive enzymes including amylase, lipase and proteases. The remaining portions of the pancreas are made up from extracellular matrix (10%) and the ductal cells as well as blood vessels (4%). Various studies investigated that the exocrine and endocrine cells of the pancreas are structurally and functionally interrelated with the so called islet-duct-acinar axis. The two peptides of endocrinal islets, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic hormone (GIP) collectively known as incretins are secreted in the insulo-acinar portal vascular pathway, thus controlling and maintaining the activities of the ducts and acinar cells (5, 6).

2.1. Glycemic control in diabetic patients

Glycemic control is a clinical term to indicate the status of the diabetic patient in his/her blood sugar level. Even though measuring fasting plasma glucose (FBS) and HbA1c in the blood is used to assess glycemic control, the latter is recommended which provides an individual's average blood glucose level over the past three weeks to 3 months. Therefore, HbA1c is a reference method to decide on the control status of patients and it is also valuable in the diagnosis of new diabetic patients (29, 30).

The American Diabetic Associations (ADA, 2022) recommended that the goals in adult diabetes mellitus population is maintaining HbA1c below 7% which is regarded as good control that suggests lower risk of developing micro vascular and macro vascular diabetic complications while, HbA1c above 7% is considered as poor control and increases the risk of diabetes mellitus complications (31). Various epidemiological and large scale clinical trials including the Diabetes Control and Complication Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) also reported HbA1c as a gold standard to declare new diabetes mellitus cases and to predict the risk of diabetic complications (32, 33).

2.2. Association of serum amylase and lipase with DM and glycemic control status

A systematic review and meta-analysis was performed to evaluate the clinical relevance of low serum amylase, lipase and trypsin as biomarkers for metabolic disorders in 2020 by Ko J. et al. The pooled analysis reported that type 2 diabetic patients had 3.1 and 2.9 times lower mean serum amylase and lipase activity than the healthy individuals respectively (34).

A facility based comparative study conducted by Tanvi NE and his colleagues in Bangladesh (2017) assessed the mean activity of serum amylase and lipase among diabetic patients and healthy controls. The results showed that serum amylase and lipase were significantly lower in diabetic patients than the healthy controls ($p < 0.001$) with mean \pm SD of serum amylase (IU/L) 33.37 ± 2.36 and 56.37 ± 1.84 as well as serum lipase (IU/L) 21.58 ± 1.69 and 29.34 ± 0.43 for diabetics and healthy controls respectively. They also reported significant negative correlation between serum amylase and lipase with duration of diabetes mellitus with Pearson's correlation coefficient $r = -0.321$, $p = 0.023$ and $r = -0.204$, $p = 0.015$ respectively (35).

A hospital based comparative cross-sectional study conducted in Jordan in 2005 to examine pancreatic exocrine insufficiency among healthy and diabetic patients reported that reduction of serum amylase among type 2 diabetes mellitus patients (49% of type 2 diabetic patients) while there was no reduction in serum lipase among type 2 diabetes mellitus patients. The study also reported that prolonged duration of type 2 diabetes mellitus showed a remarkable correlation with reduction of serum amylase (59%) and no significant reduction was reported in serum lipase with prolongation of type 2 diabetes mellitus (36).

Another case control study from Gujarat, India in 2016 reported significantly decreased ($p < 0.001$) serum amylase and lipase in type 2 diabetes mellitus patients than the healthy controls with mean \pm SD (IU/L) of 56.37 ± 23.43 and 80.40 ± 20.26 as well as 26.13 ± 13.23 and 38.67 ± 12.42 respectively. The correlation result of their study reported that serum amylase and lipase showed a strong negative correlation ($p < 0.001$) with fasting blood sugar (FBS) level ($r = -0.734$ and -0.891 respectively) (37).

Pancreatic amylase and lipase function were impaired in type 2 diabetes mellitus patients depending on the report of a cross-sectional comparative study in India by Bhadarge et al, 2021. Significant decrease in serum amylase ($p < 0.001$) were reported with 38.6 ± 16.0 IU/L and 66.7 ± 18.1 IU/L in type 2 patients compared with healthy subjects respectively as well as significantly decreased serum lipase ($p < 0.001$) were reported with 21.0 ± 2.8 IU/L in type 2 patients and 26.2 ± 4.1 IU/L in healthy subject (38).

A recent case control research conducted in Babylon Iraq (Abdulhakeem, 2023) reported that, the mean levels of serum amylase and lipase in type 2 diabetes mellitus patients were (71.4 ± 29.11) and (49.7 ± 14.69) (U/L) while these values were (72.7 ± 27.19) and (42.0 ± 7.35) (U/L) among the healthy controls. In this study, the mean serum amylase was nearly similar within the cases and control groups

while the mean serum lipase showed significant increase ($p < 0.05$) among type 2 diabetes mellitus patients than the healthy controls (39).

Another case control study was conducted in New Delhi, India to assess the serum amylase and lipase among diabetic patients with regard to healthy controls. The study reported that serum amylase among diabetic patients was significantly lower than that of the healthy controls (48.13 ± 9.16 and 73.9 ± 23.1 respectively) with a p value of < 0.001 . Significant negative correlation between serum amylase and fasting blood sugar was also reported by the study ($r = -0.185$, $p < 0.05$) and concluded that diabetic patients having poor glycemic control showed decreased serum amylase activity when compared with those having good glycemic control (40).

In a case control study conducted at Bangalore medical college and research Centre, Bangalore, India by Vishwanath HL, Shwetha 30 age and sex matched diagnosed cases of type 2 DM between 40-80yr were included in the study and 30 healthy individuals as controls. Study was biochemical analysis of serum amylase and serum lipase levels in patients of type II diabetes mellitus. It was concluded that there was a considerable decline in serum amylase ($p < 0.0001$) and serum lipase ($p = 0.00661$) in diabetic patients compared to controls who were age and sex matched. It showed pancreatic exocrine destruction in type 2 DM. Serum pancreatic enzymes can be used as an extra explanatory parameter for the evaluation of progression of the disease and response to treatment (41).

3. Objectives

3.1. General objective

- To assess the level of serum amylase and lipase and associated factors in type 2 diabetic follow-up patients at Hakim Gizaw Teaching Hospital Debre Berhan, Ethiopia, 2025

3.2. Specific objectives

- To compare the level of serum amylase activity in T2DM patients with poor glycemic control and good glycemic control at Hakim Gizaw Teaching Hospital Debre Berhan, Ethiopia, 2025
- To compare the level of serum lipase activity in T2DM patients with poor glycemic control and good control at Hakim Gizaw Teaching Hospital Debre Berhan, Ethiopia, 2025
- To assess factors associated with serum amylase and lipase in uncontrolled and controlled type 2 DM patients at Hakim Gizaw Teaching Hospital Debre Berhan, Ethiopia, 2025

4. Hypothesis

- Null hypothesis: Serum amylase and lipase level have no significant difference in controlled and uncontrolled type 2 diabetes mellitus patients

5. Methods and Materials

5.1. Study area

The study was conducted at Hakim Gizaw Teaching Hospital. The Hospital is found in Debre Berhan city which is around 130 kilometers away in the north from the capital city of Ethiopia, Addis Ababa. It is a teaching hospital which provides health care services for the community and training to its students for biomedical and clinical departments. The hospital serves around 101,902(\approx 41,000/year) out patients since its establishment May 9, 2022. The hospital has also a number of outpatient follow up clinics for chronic illnesses including diabetic follow-up cases. Diabetes mellitus follow up is under the non-communicable diseases (NCD) clinic which have around nine internal medicine specialists that makes the hospital strong enough to effectively manage the burden of chronic illnesses including diabetes mellitus.

5.2. Study design and period

A hospital based comparative cross-sectional study was conducted from February 05 to March 30, 2025

5.3. Population

5.3.1. Source population

All T2DM patients living in and around Debre Berhan city attending follow-up appointment at Hakim Gizaw Teaching Hospital

5.3.2. Study Population

T2DM patients coming to Hakim Gizaw Teaching Hospital for follow-up appointment during the study period who fulfilled the eligibility criteria and gave consent to participate in the study

5.4. Eligibility criteria

5.4.1. Inclusion Criteria

- According to their medical record, confirmed T2DM patients
- Who have regular follow up pattern for at least 1 year and who come to Hakim Gizaw Teaching Hospital for follow-up appointment during the study period
- Willing to participate in the study was included to the study.

5.4.2. Exclusion Criteria

- Diabetic patients with pregnancy
- Have currently acute infection
- Have a known history of cancer
- Have confirmed acute or chronic pancreatitis
- Have confirmed gall stones
- Diabetic patients diagnosed with other class of diabetes were excluded from the study

5.5. Study variables

5.5.1. Dependent variables

- ✓ Serum amylase level, Serum lipase level

5.5.2. Independent variables

- | | |
|-------|------------------------|
| ✓ Age | ✓ FBS |
| ✓ Sex | ✓ HbA1c |
| ✓ BMI | ✓ Duration of diabetes |
| ✓ BAI | ✓ Mode of treatment |
| ✓ BP | |

5.6. Sample size calculation and Sampling technique

5.6.1. Sample size calculation

Sample size is calculated using appropriate comparative based study design for quantitative data by taking 95% confidence level and 80% power of the study using the formula;

$$n=r+1 (Z_{\alpha/2}+Z_{\beta})^2(SD_1^2+ SD_2^2)/r (d)^2 \quad (42).$$

Where;

- ✓ n=minimum sample size for each group
- ✓ SD_1 =Standard deviation among group 1 from previous studies
- ✓ SD_2 =Standard deviation among group 2 from previous studies

- ✓ $Z_{\alpha/2} = Z_{0.05/2} = Z_{0.025} = 1.96$ -from Z table(at 95% confidence interval & 5% type 1 error)
- ✓ $Z_{\beta} = Z_{0.20} = 0.84$ -from Z table (at 80% power of the study and 20% type II errors)
- ✓ $d =$ Effect size (difference between the mean values)
- ✓ $r =$ The ratio of group 1 to group 2

As there is no study conducted in Ethiopia on serum amylase and lipase among uncontrolled and controlled diabetic patients, a study conducted in Dhiraj Hospital, Sumandeep Vidyapeeth institute, Pipariya, Vadodara, India that shows; the mean of serum amylase in uncontrolled type 2 diabetes ‘40.63 IU/L’ with standard deviation of ‘10.54’ and mean of serum amylase in well controlled diabetic patients is ‘52.80 IU/L’ with standard deviation of ‘18.44’ are taken to calculate the estimated sample size for each group (43).

By substituting using the above formula;

$$n=2 \times (1.96+0.84)^2 (18.44^2 +10.54^2) / (52.80-40.63)^2=47.76 \approx 48 \text{ (for each group)}$$

In order to eliminate any potential outliers the sample size is increased by 15% as $(15 \times 48/100 + 48)$ to make it up to 56 participants for each group; 56 for group 1 (uncontrolled type 2 diabetes), 56 for group 2 (controlled type 2 diabetes). As a result, a total of 112 participants were recruited for the study.

5.6.2. Sampling technique

- ✓ Convenient sampling technique was used

5.7. Measurement and Data collection

5.7.1. Data collection procedure

After written informed consent obtained, Sociodemographic data was collected by face to face interviews and reviewing medical records by a principal investigator and a trained data collector with the aid of a structured and pre-tested questionnaire. Anthropometric measurements (Hip circumference, Waist circumference, height and weight) of all the study participants were measured to calculate their Waist to Hip circumference Ratio (WHR), body mass index (BMI) and Body Adiposity Index (BAI). The participants were allowed to sit and rest for about 5 min and blood pressure reading was taken after allowed to seat in the upright position and supine position using a mercury sphygmomanometer apparatus.

5.7.2. Grouping study participants

The study participants were grouped based up on the glycemic control status into two groups: group I- uncontrolled T2DM patients (HbA1c >7%) and group II- controlled T2DM patients having good glycemic control (HbA1c ≤7%) (31). Furthermore, participants were categorized based on WHO guideline as: Obesity when BMI ≥ 30kg/m², overweight when BMI between 25-29.9 kg/m², Normal when BMI between 18.5-24.9 kg/m² and underweight when BMI < 18.5 kg/m² (44). According to the seventh report of the Joint National Committee (JNC7) participant's blood pressure was classified as normal (<120 mmHg), pre-hypertensive (120-139 mmHg) and hypertensive (≥140 mmHg) for systolic blood pressure and <80 mmHg, 80-89 mmHg and ≥90 mmHg for diastolic blood pressure respectively (45). The BAI of respondents was calculated and study participants were considered obese for a BAI of >22% and >35% for male and female respectively (46).

5.7.3. Laboratory Sample Collection and processing

After providing written informed consent and answering the questionnaire, all the participants given a code number registered on the notebook. After overnight fasting, the principal investigator and a competent laboratory technologist collected around 5 ml of venous blood from medial cubital vein from each participant using standard operating procedure of venous blood sample collection. Around 3 ml was transferred to serum separator (SST) tube for amylase, lipase and fasting blood sugar (FBS) tests. The remaining 2 ml was transferred to ethylene diamine tetra acetic acid (EDTA) tube for glycosylated hemoglobin (HbA1c) measurement. The sample in SST tube was at room temperature until for about 15-20 minutes and centrifuged for 5 minutes at 4000 revolution per minute (RPM).

5.7.4. Laboratory analysis method and principle of each test

Serum amylase, serum lipase, HbA1c and FBS was analyzed according to the standard operating procedure (SOP) format of each biochemical analytes standardized against the International Federation of Clinical Chemistry (IFCC) recommendations using Siemens Dimension EXL 200 clinical chemistry analyzer.

A. HbA1c

Freshly drawn whole blood was taken and analyzed using Siemens Dimension EXL 200 clinical chemistry analyzer. The Dimension® HB1C assay measures both HbA1c and total hemoglobin.

Method: The HbA1c measurement is based on a turbidimetric inhibition immunoassay (TINIA) principle, and the measurement of total hemoglobin is based on a modification of the alkaline hematin

reaction. Using the values obtained for each of these two analytes, the relative proportion of the total hemoglobin that is glycosylated is calculated and reported.

Principle: A sample of whole blood is added to the first cuvette containing lysing reagent. This reagent lyses the red blood cells and simultaneously converts the released hemoglobin to a derivative that has a characteristic absorbance spectrum. Concentration is measured at 405 nm and 700 nm. The same aliquot of the lysed whole blood that is transferred from the first cuvette to the second cuvette for the Hb measurement is also used for the measurement of HbA1c. The second cuvette contains an anti-HbA1c antibody in a buffered reagent. Hemoglobin A1c in the sample reacts with anti-HbA1c antibody to form a soluble antigen-antibody complex. A polyhapten reagent containing multiple HbA1c epitopes is then added to this cuvette. The polyhapten reacts with excess (free) anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex. The rate of this reaction is measured turbidimetrically at 340 nm and blanked at 700 nm and is inversely proportional to the concentration of HbA1c in the sample.

B. FBS

Method: Serum was taken to analyze blood glucose level using Siemens Dimension EXL 200 clinical chemistry analyzer by following the manufacturer's instruction using a principle of glucose hexokinase (HK) method.

Principle: HK catalyzes the phosphorylation of glucose in the presence of adenosine-5'-triphosphate (ATP) and magnesium to form glucose-6-phosphate (G-6-P) and adenosinediphosphate (ADP). G-6-P is then oxidized by glucose-6-phosphate dehydrogenase (G-6-PDH) in the presence of nicotinamide adenine dinucleotide (NAD) to produce 6-phosphogluconate and NADH. One mole of NAD is reduced to one mole of NADH for each mole of glucose present. The absorbance due to NADH (and thus the glucose concentration) is determined using a bichromatic (340 and 383 nm) endpoint technique.

C. Serum amylase

Method: Serum was taken to analyze amylase level using Siemens Dimension EXL 200 clinical chemistry analyzer by kinetic monitoring.

Principle: α -amylase catalyzes the hydrolysis of a defined synthetic substrate, 2-chloro-4-nitrophenyl- α -D-maltotriose (CNPG3), to yield 2-chloro-4-nitrophenol (CNP), 2-chloro-4-nitrophenyl- α -D-maltoside (CNPG2), maltotriose (G3) and glucose. After an incubation of 70 seconds at 37 °C, the absorbance due to the formation of 2-chloro-4-nitrophenol (CNP) is measured using a bichromatic (405, 577 nm) rate

technique.

D. Serum lipase

Method: Serum was taken to analyze lipase level using Siemens Dimension EXL 200 clinical chemistry analyzer by kinetics monitoring.

Principle: Lipase catalyzes the hydrolysis of 1, 2-O-dilauryl-rac-glycero-3-glutaric acid (6'-methylresorufin) ester in the presence of colipase, bile salt, and CaCl₂ at alkaline pH. The hydrolysis produces 1, 2-O-dilauryl-rac-glycerol and glutaric acid 6'-methylresorufin ester. Glutaric acid-6'-methylresorufin ester is an unstable reaction intermediate and breaks down to yield chromogenic free methylresorufin in proportion to the activity of lipase in the sample. The rate of production of methylresorufin is measured by a bichromatic rate reaction at 577 and 700 nm.

5.7.5. Biological variation, reference Ranges and clinical interpretation of Biochemical parameters

According to the European Biological Variation Study (EuBIVAS), the estimated within-subject and between-subject biological variation of serum amylase was 6.8% and 30.4% while the estimate for serum lipase was 7.75 and 23.8% (47). ADA criteria were used to set Reference ranges for HbA1c and FBS as healthy (HbA1c < 5.7% and FBS 70-99 mg/dl), Pre-diabetes (HbA1c 5.7-6.4% and FBS 100-125 mg/dl) and diabetes mellitus (HbA1c ≥ 6.5% and FBS ≥ 126 mg/dl) (48). The International Federation for Clinical Chemistry (IFCC) recommends the reference ranges for serum amylase and lipase as 28-100 U/L and 13-60 IU/L respectively (49).

5.8. Statistical analysis and interpretation

Data was entered to and analyzed by using IBM-SPSS version 27 statistical software package. After testing the data for normality, the descriptive data like frequency, percentage, mean and standard deviation was calculated and independent 't' test was used to compare mean ± SD of clinical and biochemical parameters among each group. Pearson correlation coefficient (r), simple linear regression and multiple linear regression analysis was used appropriately. Receiver operating characteristics (ROC) curve was plotted to evaluate the effectiveness of serum amylase and lipase for glycemic control status investigation. Besides this the level of significance P value < 0.05 was considered as statically significant for the statistical out puts.

5.9. Data quality assurance

Before the actual data collection carried out the pre-test was done at Debre Berhan Comprehensive Specialized Hospital with 10% of the sample size from January 20 to January 30 2025. To reduce misleading answers, the purpose of the study was informed to the study participants and sufficient time was taken to collect data. Anthropometric measurements were taken by trained professionals to maintain the reproducibility of measurements. To assure the reliability of test result, SOP was followed throughout the process. The blood samples was collected after optimal fasting and the serum was separated immediately and stored in refrigerator at 4-8^oc for a maximum of 8 hours only until analysis. The performance verification of clinical chemistry analyzer was checked daily by using standard controls for each test. Generally pre analytical, analytical and post analytical quality assurance was maintained.

5.10. Operational definitions

- **Good glycemic control**-adult non-pregnant diabetic patients whose HbA1c $\leq 7\%$ based on the American Diabetic Association (ADA, 2020) (50).
- **Poor glycemic control**-adult non-pregnant diabetic patients whose HbA1c $>7\%$ based on the American Diabetic Association (ADA, 2020) (50).
- **Uncontrolled diabetes**-diabetic following patients who are unable to achieve good glycemic status during follow-up appointment for the last 3 month.
- **Controlled diabetes**-diabetic patients having good glycemic status during their follow- up appointments.

5.11. Ethical consideration

Before data collection, ethical clearance (DRERC/788/24) was obtained from the Research and Ethics Review Committee (RERC) of Addis Ababa University College of Health Science, Department of Medical Laboratory Science and an official permission letter was submitted to Debre Berhan University Hakim Gizaw Teaching Hospital Chronic Diseases OPD and Medical Laboratory Department for communication. Furthermore, informed written consent was obtained from each study participant before the actual data collection and the purpose of the study was explained to the study participant and they were informed about the information obtained in course investigation will be kept confidential using codes and they do have right to get their results without payment and any clinically important result were immediately linked to the responsible physician for further diagnosis and treatment.

5.12. Dissemination of the Results

The study finding will be submitted to Addis Ababa University, College of Health Sciences Department of Medical Laboratory Sciences and Debre Berhan University Hakim Gizaw Teaching Hospital Department of Medical Laboratory Sciences. Furthermore, the findings of the study will be submitted to national or international peer reviewed journal for publication. The findings will be presented at national and international scientific conferences.

6. Results

6.1. Descriptive Characteristics of study participants

6.1.1. Sociodemographic characteristics of study participants

The study was conducted on a total of 112 Type 2 diabetes mellitus (T2DM) patients of which 56 having controlled T2DM and 56 uncontrolled T2DM. Around 66 (58.9%) and 46 (41.1%) of the study participants were male and female respectively. Majority of the study participants were in the age group of 34-49 years (64, 57.1%) with a median age of 44 years. Study participants who attended higher education were 39 (34.8%), secondary education 28 (25.0%), primary education 16 (14.2%) and with no formal education were 29 (26.0%). The proportion of study participants who have urban residence, sedentary life style and consume meat and meat products 1-3/month were 84 (75%), 49 (43.7%) and 68 (60.7%) respectively (**Table 1**).

6.1.2. Clinical characteristics of study participants

More than half of the study participants were included in a BMI range of 25-29.9 kg/m² (33/56, 58.9%) and 18.5-24.9 kg/m² (33/56, 58.9%) among Uncontrolled T2DM and Controlled T2DM patients respectively. The proportion of T2DM patients with a Systolic Blood Pressure (SBP) of ≥140 mmHg and a Diastolic Blood Pressure (DBP) of ≥90 mmHg were 17(15.2%) and 18(16.2%) respectively whereas 59(52.7%) and 47(41.9%) had a SBP of 120-139 mmHg and a DBP of 80-89 mmHg respectively. Majority of T2DM patients in this study were taking Oral Hypoglycemic Agents (OHA) (78, 69.6%) and had DM duration of 5-10 years (77, 68.7%) (**Table 1**).

Table 1: Sociodemographic and clinical characteristics of study participants at Hakim Gizaw Hospital, Debre Berhan, Amhara, Ethiopia, 2025 (N=112)

Variable	Category	Uncontrolled T2DM N (%)	Controlled T2DM N (%)	Total N (%)
Sex	Male	34 (60.7)	32 (57.1)	66 (58.9)
	Female	22 (39.3)	24 (42.9)	46 (41.1)
Educational Status	No formal Education	15 (26.8)	14 (24.9)	29 (26.0)
	Primary Education	4 (7.1)	12 (21.4)	16 (14.2)
	Secondary Education	14 (25.0)	14 (25.0)	28 (25.0)
	Higher Education	23 (41.1)	16 (28.7)	39 (34.8)
Age group	18-33 years	11 (19.6)	8 (14.2)	19 (17.0)
	34-49 years	35 (62.5)	29 (51.7)	64 (57.1)
	>49 years	10 (17.9)	19 (34.1)	29 (25.9)

Residence	Rural	10 (17.8)	18 (32.1)	28 (25.0)
	Urban	46 (82.2)	38 (67.9)	84 (75.0)
Life style	Sedentary	39 (69.6)	10 (17.8)	49 (43.7)
	Non sedentary	17 (30.4)	46 (82.2)	63 (56.3)
Meat consumption	Never	7 (12.5)	6 (10.7)	13 (11.6)
	1-3/Month	29 (51.8)	39 (69.6)	68 (60.7)
	1-3/Week	20 (35.7)	11 (19.7)	31 (27.7)
BMI (Kg/m ²)	<18.5 kg/m ²	1(1.9)	1(1.9)	2(1.9)
	18.5-24.9 kg/m ²	18(32.1)	33(58.9)	51(45.5)
	25-29.9 kg/m ²	33(58.9)	19(33.9)	52(46.4)
	≥ 30 kg/m ²	4(7.1)	3(5.3)	7(6.2)
SBP (mmHg)	<120 mmHg	10(17.8)	26(46.4)	36(32.1)
	120-139 mmHg	32(57.2)	27(48.2)	59(52.7)
	≥140 mmHg	14(25.0)	3(5.4)	17(15.2)
DBP (mmHg)	<80 mmHg	14(25.0)	33(58.9)	47(41.9)
	80-89 mmHg	26(46.4)	21(37.5)	47(41.9)
	≥90 mmHg	16(28.6)	2(3.6)	18(16.2)
Mode of Therapy	OHA	37(66.1)	41(73.2)	78(69.6)
	Insulin injection	3(5.3)	6(10.7)	9(8.1)
	Combination	16(28.6)	9(16.1)	25(22.3)
Duration of DM (Years)	0-5 years	13(23.2)	28(50.0)	41(36.6)
	5-10 years	28(50.0)	27(48.3)	55(49.1)
	>10 years	15(26.8)	1(1.7)	16(14.3)
Family history of DM	No	29(51.8)	27(48.2)	56(50.0)
	Yes	27(48.2)	29(51.8)	56(50.0)
Abdominal discomfort	No	25(44.6)	37(66.1)	62(55.3)
	Yes	31(55.4)	19(33.9)	50(44.7)

*No formal education: have no classrooms for education, primary education from grade 1-8, Secondary Education from grade 9-12 and Higher Education includes College and Universities.

6.2. Data distribution Assessment of Dependent variables

Normal Q-Q plot, Histogram and Shapiro-Wilk test were used to check the distribution of dependent variables (Serum amylase and Lipase level). The Q-Q plot and histogram plots indicated that the two outcome variables (serum amylase and lipase level) were normally distributed as indicated by the graph (**Figure 1**). Furthermore, the statistics from Shapiro-Wilk normality test indicates the distribution of serum

amylase and serum lipase was normal with a p value of 0.080 and 0.070 respectively. Therefore, the overall statistical analyses performed were parametric tests.

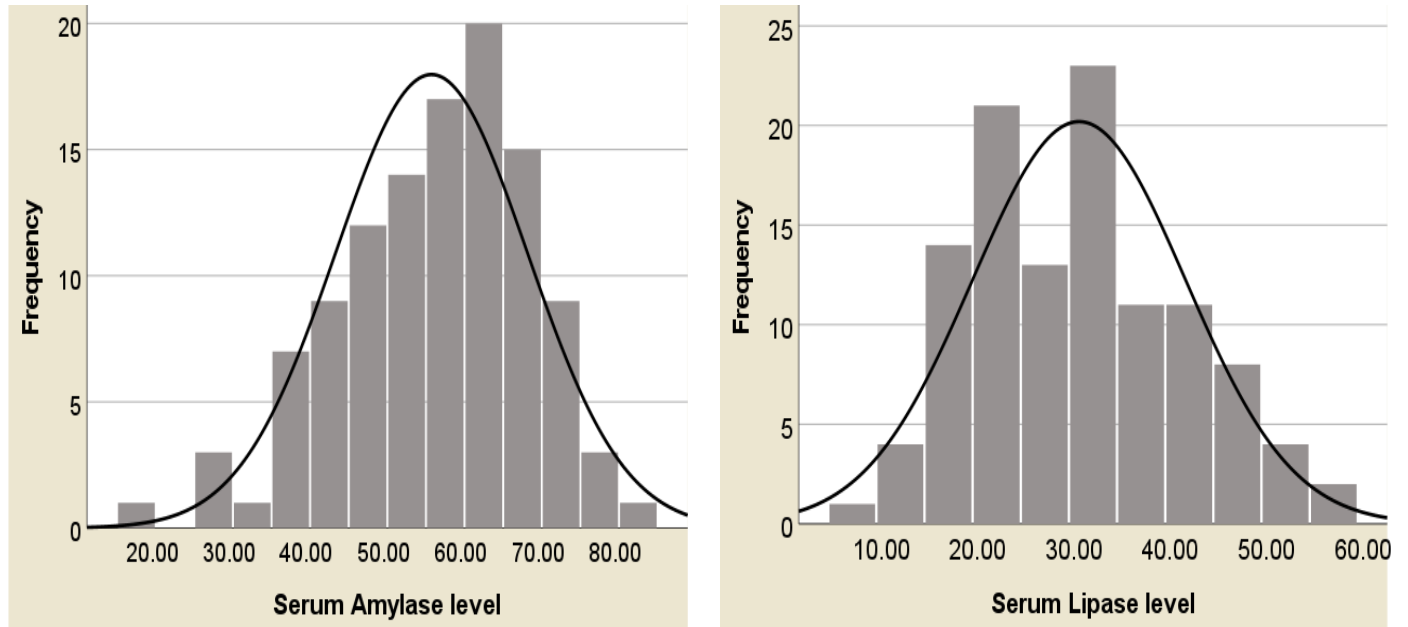


Figure 1: Distribution of Serum Amylase and Serum lipase among T2DM patients, 2025 (N=112)

6.3. Comparison of biochemical and clinical parameters among Controlled and Uncontrolled T2DM patients

Independent ‘t’ test was performed to compare the mean values of anthropometric and biochemical parameters. The mean FBS (mg/dl), HbA1c (%), Serum Amylase (IU/L) and Serum Lipase (IU/L) among Uncontrolled and Controlled T2DM were (192.64 ± 39.70 and 122.55 ± 23.02 ; 8.73 ± 1.27 and 6.04 ± 0.41 ; **48.85 ± 11.86** and **63.58 ± 7.76** ; **22.14 ± 6.41** and **38.96 ± 7.85**) respectively. These mean differences in serum biochemical parameters of Uncontrolled and Controlled T2DM patients were statistically significant ($p < 0.001$).

6.4. Relation of serum amylase and lipase with biochemical and clinical parameters

The Pearson correlation analysis demonstrated that serum amylase had strong negative correlation with FBS ($r = -0.478$, $p = 0.001$) and HbA1c ($r = -0.575$, $p = < 0.001$). The study also revealed statistically significant negative correlation of serum amylase with duration of DM ($r = -0.599$, $p < 0.001$), SBP ($r = -0.251$, $p = 0.08$) and DBP ($r = -0.257$, $p = 0.006$). Serum lipase had a strong negative correlation with FBS ($r = -0.655$, $p = < 0.001$) and HbA1c ($r = -0.717$, $p = < 0.001$). The Pearson correlation revealed that serum lipase was negatively correlated with DM duration ($r = -0.477$, $p < 0.001$), BAI ($r = -0.248$, $p = 0.008$), SBP ($r = -0.260$, $p = 0.006$) and DBP ($r = -0.302$, $p = 0.007$) (**Table 2**).

Table 2: Correlation of serum amylase and lipase with some biochemical and clinical parameters among patients with T2DM at Hakim Gizaw Hospital, Debre Berhan, Amhara, Ethiopia, 2025 (N=112)

Tested Parameters	Serum Amylase (IU/L)		Serum Lipase (IU/L)	
	r	p-value	r	p-value
Duration of DM (years)	-0.599**	<0.001	-0.477**	<0.001
BMI (kg/m ²)	-0.144	0.130	-0.264**	0.005
BAI (%)	-0.139	0.145	-0.248**	0.008
WHR	-0.249**	0.008	-0.249**	0.008
SBP(mmHg)	-0.251**	0.008	-0.260**	0.006
DBP(mmHg)	-0.257**	0.006	-0.302**	0.001
FBS (mg/dl)	-0.478**	<0.001	-0.655**	<0.001
HbA1c (%)	-0.575**	<0.001	-0.717**	<0.001

**Correlation is significant at the 0.01 level & *Correlation is significant at the 0.05 level (2-tailed).

6.5. Factors associated with decreased serum amylase and lipase among T2DM patients

Simple linear regression was performed by enter method and variables with p value less than 0.25 were considered for multiple linear regression. The multiple linear regression analysis revealed that taking oral hypoglycemic agents (OHA) and experiencing abdominal discomfort were statistically associated factors for decreased serum amylase level among T2DM patients. T2DM patients taking oral hypoglycemic agents (OHA) are 9.72 times more likely to have decreased serum amylase level than those taking insulin injection (AOR (95% CI) = 9.72(-13.52-(-5.90)), P <0.001). Abdominal discomfort increase the likelihood of having decreased serum amylase level by 4.31 among T2DM patients (AOR (95% CI) = 4.31(-7.67-(-0.95)), P = 0.012). A 1% increase in HbA1c decreases the level of serum amylase by 3.02 IU/L (AOR (95% CI) = -3.02(-5.04-(-1.01)), P = 0.004) among T2DM patients (**Table 3**).

Table 3: Factors Associated with decreased serum amylase level among patients with T2DM at Hakim Gizaw Hospital, Debre Berhan, Amhara, Ethiopia, 2025 (N=112)

Covariates	Category	Bivariate Analysis		Multivariate Analysis	
		COR (95% CI)	P	AOR(95% CI)	P
Sex ^b	Male	1			
	Female	1.91(-2.83-6.64)	0.426		
Life style ^a	Non Sedentary	1		1	
	Sedentary	-13.53(-17.49-(-9.58))	<0.001	-3.42(-7.91-1.08))	0.135
Mode of Therapy ^a	Insulin injec.	1		1	
	OHA	-9.99(-14.28-(-5.70))	<0.001	-9.72(-13.52-(-5.90))	<0.001*
	Combination	-4.04(-10.36-2.28))	0.208	-5.59(-11.18-(-0.12))	0.050
Abdominal discomfort ^a	No	1		1	
	Yes	-8.53(-12.95-(-4.11))	<0.001	-4.31(-7.67-(-0.95))	0.012*
DM Duration ^a	6.38±2.56 years	-2.91(-3.65-(-2.17))	<0.001	-1.16(-2.02-(-0.28))	0.009*
FBS (mg/dl) ^a	157.59±47.78	-0.11(-0.15-(-0.06))	<0.001	0.05(-0.02-0.12)	0.123
HbA1c (%) ^a	7.38±1.64	-4.06(-5.26-(-2.85))	<0.001	-3.02(-5.04-(-1.01))	0.004*

^avariables included in multivariate analysis and ^bvariables not included in the multivariate analysis

Type 2 DM patients who are taking OHA were 5.93 times more likely to have decreased serum lipase level when compared with T2DM patients taking insulin injection (AOR (95%CI) = -5.93(-9.25-(-2.59)), P <0.001). The result also demonstrated that taking combined therapy increases the risk of having impaired serum lipase level by 7.94 fold than taking insulin injection to monitor their glucose level (AOR (95%CI) = -7.94(-12.82-(-3.05)), p = 0.002). Every 1% elevation in glycated hemoglobin is resulted to decrease the level of serum lipase by 3.55 IU/L (AOR (95%CI) = -3.55(-5.31-(-1.79)), P <0.001) (**Table 4**).

Table 4: Factors Associated with decreased serum Lipase level among patients with T2DM at Hakim Gizaw Hospital, Debre Berhan, Amhara, Ethiopia, 2025 (N=112)

Covariates	Category	Bivariate Analysis		Multivariate Analysis	
		COR (95% CI)	P	AOR(95% CI)	P
Sex ^b	Male	1			
	Female	1.05(-3.17-5.27)	0.622		
Life style ^a	Non Sedentary	1		1	
	Sedentary	-9.62(-13.40-(-5.84))	<0.001	1.94(-1.99-5.87)	0.330
Mode of Therapy ^a	Insulin injection	1		1	
	OHA	-9.75(-13.98-(-5.51))	<0.001	-5.93(-9.25-(-2.59))	<0.001*
	Combination	-13.1(-18.85-(-7.35))	<0.001	-7.94(-12.82-(-3.05))	0.002*
Abdominal discomfort ^a	Yes	-5.34(-9.39-(-1.27))	0.011	-1.77(-4.71-1.16)	0.235
	No	1		1	
DM Duration ^a	6.38±2.56 years	-2.06(-2.78-(-1.34))	<0.001	-0.36(-1.12-0.39)	0.345
FBS (mg/dl) ^a	157.59±47.78	-0.15(-0.18-(-0.12))	<0.001	-0.03(-0.08-0.03)	0.373
HbA1c (%) ^a	7.38±1.64	-4.83(-5.71-(-3.94))	<0.001	-3.55(-5.31-(-1.79))	<0.001*

^avariables included in multivariate analysis and ^bvariables not included in the multivariate analysis

6.6. Evaluation of Serum amylase and lipase as biomarker for glycemc control

Receiver operating characteristics (ROC) curve was plotted for serum amylase and lipase to evaluate the role of these enzymes as a biomarker of glycemc control in T2DM. The area under the curve for serum amylase was 0.849 and for serum lipase was 0.955. The highest diagnostic sensitivity and specificity of serum amylase to differentiate controlled T2DM from uncontrolled T2DM was at a cut off value of 58.5 IU/L (sensitivity = 75% and specificity = 80.4%) while it was found to be 30.5 IU/L for serum lipase (sensitivity = 89.3% and specificity = 87.5%). Measuring the level of amylase and lipase had demonstrated to be a good biomarker of investigating glycemc control status in T2DM (Figure 2).

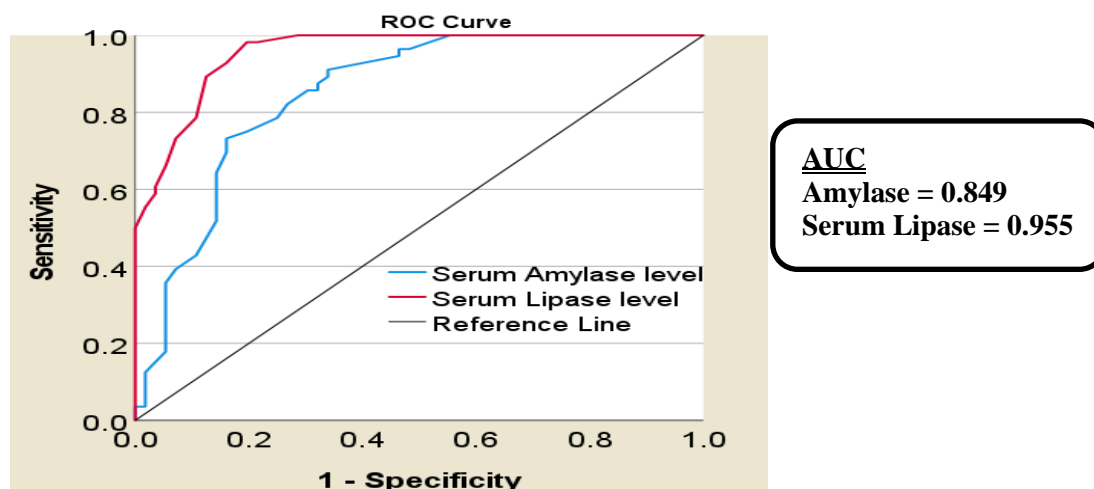


Figure 2: ROC curve for serum amylase and lipase among T2DM to assess glycemc control

7. Discussion

The current study evaluated the level of serum amylase and lipase among type 2 diabetic patients in relation to their glycemic control status. Overall, the results of the study revealed that the mean serum amylase and lipase level were statistically decreased among uncontrolled type 2 diabetes mellitus patients when compared with patients having controlled type 2 diabetes mellitus. Serum amylase and lipase demonstrated a significant negative correlation with waist to hip ratio, systolic blood pressure, diastolic blood pressure, DM duration, FBS and HbA1c. A rise in HbA1c, prolonged duration of DM, having OHA and combined therapy options and experiencing abdominal discomfort were found to be a key associated factors for decreased level of serum amylase and lipase among T2DM patients.

Our study revealed that the mean level of both serum amylase and lipase were significantly decreased among uncontrolled T2DM patients than controlled T2DM patients. This decreased level of serum amylase and lipase may be due to the progressive destruction of the pancreatic exocrine cells attributable to prolonged insulin resistance through different mechanisms. Even though the current study did not demonstrate the exact pathways of pancreatic destruction, insulin resistance in uncontrolled T2DM leads to disturbance of the normal function of pancreatic cells through atrophy and fibrosis (35, 37). The finding of the current study is consistent with a systematic review and meta-analysis performed in 2020 (34) and other various cross-sectional as well as case control studies conducted in different parts of the world including Jordan, Bangladesh and India (35-38, 40, 41).

This finding is contrasted with the results of a study conducted in Babylon, Iraq which reported a slightly decreased serum amylase with significantly increased serum lipase level among T2DM patients than healthy controls (39). Even though the previous study did not classified T2DM patients as controlled and uncontrolled, the result demonstrated no significant decrease in serum amylase and serum lipase level related with their glycemic status. The possible reason for this contradiction could be attributed to the increased secretion of amylase and lipase by pancreatic exocrine cells among newly diagnosed T2DM patients (51). Since our study included T2DM patients with a follow up history of at least one year, the serum level of both enzymes decreased as the duration of DM increases.

The Pearson correlation analysis also revealed that serum amylase ($r = -0.599$, $p < 0.001$) and lipase ($r = -0.484$, $p < 0.001$) level showed statistically significant negative correlation with Duration of DM. This negative association was also demonstrated by multiple linear regression model after adjusting for confounding variables, a single year increase in duration of DM was associated with 1.16 IU/L decrease in serum amylase (AOR (95%CI) = $-1.16(-2.02-(-0.28))$, $P = 0.009$). This finding was in line with the previous

studies study conducted in Saudi, India and Nepal. These all previous studies collectively reported significant negative correlation of DM duration with serum amylase and lipase level among T2DM patients (36, 38, 52).

T2DM patients taking oral hypoglycemic agents were more likely to have decreased serum amylase and lipase level than those taking insulin injection as a treatment option. Some oral hypoglycemic agents like sulfonylureas reduces blood glucose by directly initiating insulin production but prolonged stimulation of pancreatic beta islets may lead to exhaustion of the whole pancreas and impairment of amylase and lipase secretory acinar cells (53). On the other hand insulin injection directly exerts its effect on tropical tissues and cells to take up circulatory glucose as well as due to the autocrine activity of insulin stimulation on the pancreatic acinar cells to produce amylase and lipase, the level of serum amylase and lipase may be elevated than those taking OHA (54).

Abdominal discomfort was negatively associated with the level of serum amylase among T2DM patients (AOR (95%CI) = -4.31(-7.67-(-0.95), P = 0.0012). The possible reason for these digestive problems including nausea, bloating and abdominal discomfort could be due to the decreased secretion of serum amylase and lipase which have significant role in digestion. The current study demonstrated that T2DM patients with abdominal pain and discomfort had significantly decreased serum amylase (p <0.001) and lipase (p = 0.011) level attributed for the digestive problems encountered (35).

The study demonstrated the usefulness of serum amylase and serum lipase as a biomarker of glycemic control in T2DM by using receiver operating characteristics (ROC) curve. The area under the curve (AUC) was used as a metric to decide on the effectiveness of these enzymes to investigate glycemic control status of T2DM patients (55). Serum amylase was excellent in distinguishing T2DM patients with good glycemic control from those having poor glycemic control [AUC (95%CI) = 0.849 (0.777-0.922)]. The study also revealed that serum lipase level has an excellent ability to detect uncontrolled T2DM patients from those having good glycemic control [AUC (95%CI) = 0.955 (0.921-0.988)]. The cut off values with highest sensitivity and specificity to discriminate the two groups were determined for serum amylase at 58.5 IU/L (sensitivity = 75.5% and specificity = 80.4%) and at 30.5 IU/L for serum lipase (sensitivity = 89.3% and specificity = 87.5%). The study strongly suggests the use of serum levels of these enzymes to investigate the glycemic control status of T2DM patients with serum lipase being more effective than serum amylase depending on the AUC illustrated by the ROC analysis.

7.1. Strength and Limitation of the study

The major strength of this study was the application of fully automated Siemens Dimension EXL 200 clinical chemistry analyzer for laboratory analysis of tests and investigating the level of serum amylase and lipase level among uncontrolled T2DM by comparing with controlled T2DM patients. However, the cross-sectional nature of the study did not allow establishing causal relationship of glycemic status with serum amylase and lipase level among T2DM patients. Moreover, the study included small number of study participants this may reduce the representativeness and generalizability of the study conclusion.

8. Conclusion and Recommendation

8.1.1. Conclusion

The study demonstrated that serum amylase and lipase levels were significantly decreased in uncontrolled T2DM than controlled one. The level of serum amylase and lipase were showed strong negative correlation with duration of diabetes mellitus, body mass index, body adiposity index, fasting blood sugar, systolic blood pressure and diastolic blood pressure. Prolonged duration of Diabetes, abdominal discomfort and taking oral hypoglycemic agent treatment options were key factors associated with decreased level of both enzymes. The receiver operating characteristics (ROC) curve demonstrated that assessment of these enzymes have an excellent diagnostic effectiveness to discriminate uncontrolled and controlled T2DM.

8.1.2. Recommendation

As the study established negative association of serum amylase and lipase with glycemic control, investigation of these enzymes may have significant role to understand the glycemic control status of T2DM patients. Therefore, these enzymes should be investigated among T2DM patients in regular manner. Health policy makers and responsible stake holders are recommended to incorporate assessment of these enzymes among T2DM follow up patients to assess their glycemic control. This study indicated further large scale studies to assess the prognostic role of these enzymes among T2DM patients.

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