ASSESSMENT OF SERUM LIVER ENZYMES AND ASSOCIATED FACTORS AMONG DIABETIC PATIENTS ATTENDING AMBO PUBLIC HOSPITALS, AMBO, ETHIOPIA

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ABSTRACT

Background: *Diabetes is one of the largest health emergencies of the 21st century. Diabetes is associated with various liver disorders. The elevations of liver enzymes are the primary indicator of hepatic abnormalities. However, the studies that conducted on the prevalence of elevated liver enzymes and associated factors among diabetic patients are scarce and inconsistent particularly in Ethiopia.*

Objective: *To assess serum liver enzymes and associated factors among diabetic patients attending Ambo public hospitals, Ambo, Ethiopia from July 20 to October 20, 2020.*

Methods: *Institutional-based cross-sectional study and systematic random sampling technique were carried out to select 250 diabetic patients attending Ambo public hospitals. The socio-demographic and clinical data were collected using the structured questionnaires and patient's medical record review. Venous blood was collected after overnight fasting. Then fasting blood glucose, alanine aminotransferase (ALP), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma glutamyl-transferase (GGT) were measured using Cobas c311 automated clinical chemistry analyzer. Data were analyzed using Statistical package for social science version 26 and logistic regression was used to assess associated factors.*

Result: *Among 250 diabetic patients, 43.2% of them were found to have one or more elevated liver enzymes. The prevalence of elevated serum ALT, ALP, AST, and GGT was 26.4%, 23.6%, 20.8%, and 20.8% respectively. Having high waist to hip ratio was significantly associated with the elevation of all selected liver enzymes ($p<0.05$). While living with diabetes for more than eleven years, presence of hypertension, and treatment with both insulin and oral hypoglycemic agent were significantly associated with the elevation of one or more liver enzymes ($p<0.05$).*

Conclusion and Recommendation*: This study found high prevalence of one or more and individual elevated liver enzymes in diabetic patients than many studies. Routine assessment of serum liver enzymes might be beneficial for diabetic patients to control and follow up liver dysfunction in diabetic patients. Furthermore, researches that justify this results, clarify causative factors and mechanisms of liver damage in diabetic patients should be conducted.*

Key words*: Elevated, Liver Enzymes, Diabetes, Ambo*

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ABBREVIATIONS AND ACRONYMS

AGH Ambo General Hospital

ALP Alkaline phosphatase

ALT Alanine aminotransferase

AOR Adjusted odds ratio

AST Aspartate aminotransferase

AURH Ambo University Referral Hospital

BMI Body mass index

DBP Diastolic blood pressure

GGT Gamma-glutamyltransferase

HBsAg Hepatitis B surface antigen

HCV Hepatitis C virus

IDF International Diabetic Federation

U/L International unit per liter

LFTs Liver function tests

NAFLD Non-Alcoholic Fatty Liver Disease

OHA Oral hypoglycemic agent

SBP Systolic blood pressure

SD Standard deviation

SOP standard operating procedure

SPSS Statistical package for social science

T1DM Type 1 diabetes mellitus

T2DM Type 2 diabetes mellitus

ULN Upper limit of normal value

WHO World health organization

WHR Waist to hip ratio

OPERATIONAL DEFINITION

Liver enzymes are enzymes include alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma glutamyl-transferase (GGT).

Elevated serum liver enzymes refers to an enzyme which is above the upper limit of normal values (ULN) based on reference ranges of Cobas c311 analyzer kit; for male ALT > 40U/L, AST > 40 U/L, ALP > 129, and GGT > 60 U/L. For female ALT > 33U/L, AST > 32 U/L, ALP > 104, and GGT > 40 U/L

Duration of diabetes: is the duration of diabetes after diagnosis which is measured as years or months.

Body Mass Index (BMI): BMI is calculated by dividing the body weight in kilograms by height in meters squared (kg/m^2), and the underweight < 18.5 kg/m2, normal range is 18.5-24.9 kg/m^2, overweight is 25-29.9 kg/m^2, and obesity ≥ 30 kg/m^2 (1).

Waist to hip ratio (WHR): Was calculated as waist circumference (cm) dividing by hip circumference (cm). Normal WHR < 0.9 for male and <0.85 for female. High WHR was defined as ≥0.90 for male and ≥0.85 for female (2).

Hypertension: Hypertension is defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or current use of antihypertensive medication (3).

CHAPTER ONE: INTRODUCTION

1.1. Background

The liver is the largest and most complex organ which is involved in several excretory, synthetic, storage and metabolic functions (4,5). It is responsible for protein synthesis, amino acid and nucleic acid metabolism, lipoprotein synthesis and metabolism, xenobiotic metabolism, storage of iron and, and synthesis of hormones. It is also the site of clearance of many hormones such as insulin, estrogens, parathyroid hormone, and cortisol. Exclusively, the liver is the site of metabolism of ammonia to urea (5).

The liver plays a pivotal role in glucose homeostasis by regulating various pathways of glucose metabolism, including glycogenesis, glycogenolysis, glycolysis, and gluconeogenesis (6,7). About 30 to 60% of all glucose absorbed in the gastrointestinal tract undergoes hepatic processing with subsequent storage as glycogen or metabolism into amino acids or fatty acids (6). In physiological conditions, liver provides about 90–95% of circulating glucose (8).

Liver contains high levels of several enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamayl-transferase (GGT), alkaline phosphatase (ALP), and 5′-nucleotidase. With liver injury, when the cell membrane damaged these enzymes released into plasma and can be used for liver function tests (4,5,9) that commonly used in clinical practice to screen for liver disease, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs (10).

Elevated serum liver enzymes can reflect abnormalities in liver cells or the bile duct. AST and ALT are more elevated in patients with hepatocellular injury, whereas ALP and GGT are more elevated in cholestatic injury (11). Mild chronic elevations of transaminases often reflect underlying insulin resistance (10). Elevated ALP and aminotransferases can indicate a mixed pattern of injury (12). The AST: ALT ratio can identify a specific disease or give perception into liver disease severity. An AST: ALT ratio more than 2 indicates alcoholic liver disease. On the other hand, NAFLD was associated with a ratio less than 1 (13).The most common causes of

elevated transaminase levels are alcoholic liver disease and nonalcoholic fatty liver disease (12–14).

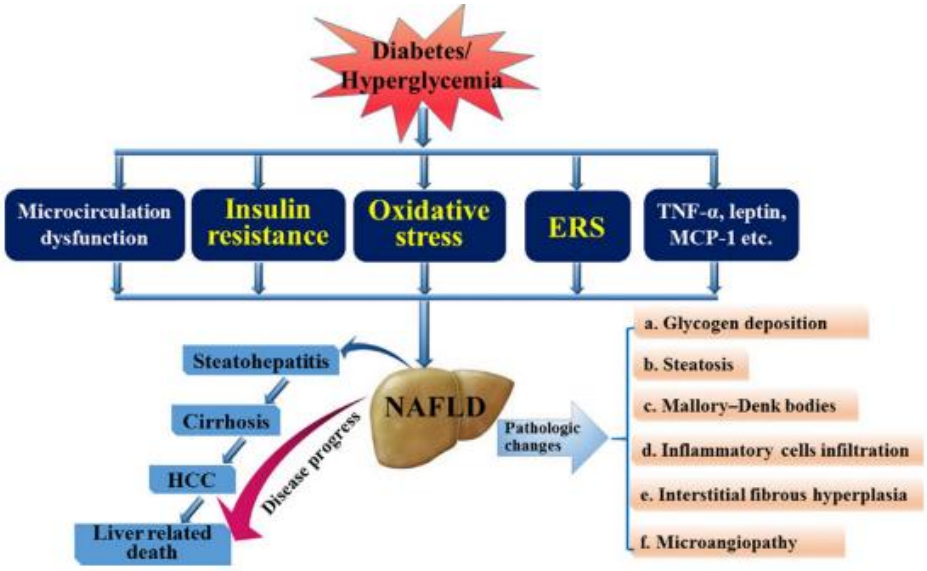
Diabetes is a group of metabolic disorders characterized and known by the presence of hyperglycemia due to underused glucose (4,5,15). The heterogeneous etiopathology of diabetes includes defects in insulin secretion, insulin action, or both, and disturbances of carbohydrate, fat and protein metabolism (15).

The major categories of diabetes are T1DM and T2DM. T1DM is characterized by absolute insulin deficiency and beta-cell destruction which accounts for 5% to 10% of all cases of diabetes (4,5). T2DM, a common and serious worldwide health problem is characterized by relative insulin deficiency and resistance to insulin action. It accounts for between 90% and 95% of all diabetes, which is most common in adults, but an increasing number of children and adolescents are also affected (5,15)

Diabetes damages multiple vital organs, thereby leading to more severe and irreversible pathological conditions such as nephropathy, retinopathy, vasculopathy, neuropathy and cardiovascular diseases, as well as hepatopathy. Liver damage is a serious complication among diabetic patients (9). DM is associated with several liver disorders, such as viral hepatitis, abnormal glycogen deposition, NAFLD, fibrosis, cirrhosis and hepatocellular carcinoma (9,16–18). The important factors for diabetic liver injury include insulin resistance, oxidative stress, inflammation, and endoplasmic reticulum stress (19). Insulin resistance, the main cause of compensatory hyperinsulinemia and hyperglycemia is the leading cause of liver damage in diabetes (9). Although insulin resistance is usually associated with T2DM, it can also be a feature of patients with T1DM (20).

In diabetic patients, insulin resistance causes adipose tissue to undergo lipolysis and the release of free fatty acids to the bloodstream which eventually accumulated in the liver, and also promotes de novo liponeogenesis. The reduced secretion of very low-density lipoproteins from hepatocytes leads to steatosis. Simultaneously, adipocytokines release tumour necrosis factor-α and leptin, worsening the hepatocyte damage by increasing mitochondrial oxidative stress. The combination of mitochondrial oxidative stress, hyperinsulinaemia and hyperglycaemia produce

free radicals which in turn induce inflammation and cellular necrosis. Hepatic stellate cells stimulated as the tissue inflammation, and produce collagen, leading to fibrosis, cirrhosis and, finally, hepatocellular carcinomas (9,18).



Adopted from reference number (19)

Figure 1: The mechanisms and pathological changes of liver injury induced by diabetes.

Generally, the risk of liver injury in diabetic patient is higher as the result of deleterious effects of several factors like insulin resistance, inflammation, and oxidative stress. Injured hepatocytes release enzymes such as ALT, AST, ALP, and GGT to blood circulation those act as the initial and most important indicators of liver abnormality (9). This study is aimed to assess serum liver enzymes and associated risk factors in diabetic patients attending Ambo public hospitals, Ambo, Ethiopia.

1.2. Statement of Problem

Diabetes is one of the fastest growing global health emergencies of the 21st century, with the greatest rise in low- and middle-income countries compared to high-income countries, and with an increasing number of children and young adults (15,21,22). It is one of chronic disease that responsible for significant morbidity, mortality, and increased health care cost (5).

According to WHO estimation, there were about 422 million adults with diabetes worldwide in 2014. Also, it was estimated that 1.1 million children and adolescents aged 14–19 years have T1DM (15). International Diabetic Federation estimated 463 million (9.3%) adults aged 20–79 years were living with diabetes globally in 2019. This will be predicted to rise to 578 million (10.2%) by 2030 and to 700 million (10.9%) by 2045. About 20.4 million (15.8%) of live births were affected by hyperglycemia during pregnancy in 2019. Two-thirds of diabetic patients live in the urban areas and three- forth are in working age. The estimated number of deaths resulting from diabetes related causes in 2019 is 4.2 million, and the estimated global annual health care expenditure for diabetes among adults was 760 billion USD (23).

An estimated 19.4 million adults (3.9%) aged 20–79 years have diabetes in the Africa which might rise to 28.6 million by 2030, and to 47.1 million by 2045. In 2019 the estimated health care expenditure for diabetes in Africa was 9.5 billion USD (23). In Ethiopia, diabetes and associated complications are major causes of morbidity and mortality with consequential economic impact (24). There were about 2,567,900 cases of diabetes; 7.5% of adults aged 20-79 years in Ethiopia in 2017 (15). The recent systematic review shows the prevalence of diabetes in Ethiopia ranged 2.0%–6.5% (24).

DM is a silent killer, and a trigger of multiple diseases, both cardiovascular and liver-related (25). The relationship of DM with liver diseases is complex and bidirectional (26). The prevalence of liver disease among DM patients is estimated to be 17% to 100% (27). The entire spectrum of liver diseases seen in patients with type 2 diabetes are nonalcoholic fatty liver disease (NAFLD), cirrhosis, hepatocellular carcinoma, chronic viral hepatitis, and acute liver failure (28,29).

From liver diseases seen in diabetic patients, NAFLD is a major risk factor of morbidity and mortality in type 2 diabetes (30). It is the most dominant liver diseases worldwide that cover a spectrum of diseases, including simple steatosis, non-alcoholic steatohepatitis (NASH), liver cirrhosis and hepatocellular carcinoma (29,31,32). It is recognized that NAFLD predicts the development of diabetes and vice versa (29). Several studies had been proven that NAFLD is highly prevalent (ranged 34% to 74%) in T2DM (33–35), and also it can occur in type 1 diabetes mellitus (T1DM) (34). Studies show that the prevalence of fatty liver disease in children and adolescents with type 1 diabetes is about 10% (36).

DM is also closely associated with hepatitis C virus (35,37), which contributes 25% of hepatocellular carcinoma (HCC) (38). The risk of developing T2DM among patients infected with hepatitis C is about 3.7 times higher than those without hepatitis C (35).

DM is an independent risk factor of hepatocellular carcinoma (HCC), which is the sixth most common cancer worldwide and accounts eleventh of cancer-related deaths. The prevalence of HCC is increased 2 to 4 times in patients with diabetes as well as the prevalence of diabetes also increased in patients with HCC (28,38). The mortality of patients with HCC and DM is higher than patients with HCC without DM (18).

DM is one of the causes of liver cirrhosis and vice versa (35,37). It increases the morbidity and mortality of patients with liver cirrhosis (18). Moreover, the class or dose of anti-diabetic drugs may be associated with the risk of cirrhosis (37). The association of cirrhosis and diabetes is complicated by the fact that cirrhosis itself is associated with insulin resistance (28). The prevalence of diabetes in cirrhosis is ranging from 12.3 to 57% (28).

DM can be result in glycogenic hepatopathy, a rare diabetic complication that characterized by the temporary liver function abnormalities with elevated liver enzymes and associated hepatomegaly caused by the reversible accumulation of excess glycogen in the hepatocytes. It is mainly seen in patients with T1DM for a long time and rarely reported in association with T2DM. The true prevalence and incidence of glycogenic hepatopathy in diabetes is unknown (27).

Generally, patients with diabetes are more likely susceptible to liver injuries that lead to the releasing of liver enzymes into plasma. Even though, elevation of liver enzymes are the primary indicator of hepatic abnormalities, the prevalence of elevated liver enzymes and associated factors in diabetic patients are inconsistent in different literatures. Additionally, no current data that indicate the prevalence of elevated liver enzymes in diabetic patients in Ethiopia particularly at study area. Therefore, this study aimed to assess serum liver enzymes and associated risk factors in diabetic patients attending Ambo General Hospital and Ambo University Referral Hospital.

1.3. Significance of the Study

Diabetes mellitus is one of the risk factors for the development of liver function impairment. The initial and most important indicators in assessing liver injury are levels of serum ALT, AST, ALP and GGT. This study was aimed to assess serum liver enzymes and associated factors in diabetic patients attending Ambo public hospitals.

This study provides evidence-based information on the elevation of liver enzymes and associated factors in diabetic patients. The finding of the study might be used for health care policymakers to develop strategies to reduce morbidity and mortality from diabetes-associated liver diseases. Additionally, it is important to create awareness among diabetic population and health care professionals to take care of liver function impairment. Furthermore, it might be used as a baseline reference for other researchers those have interest to conduct similar or related studies.

CHAPTER TWO: LITERATURE REVIEW

The institutional-based, cross-sectional study conducted among 13,200 adults with T1DM over the age of 20 years in German showed that prevalence of increased liver enzymes was 20%. It also found that poorer glycemic control; less favorable cardiovascular risk profile and a higher prevalence of late complications of diabetes were associated with elevation of liver enzymes. Moreover, the study showed that patients with increased ALT were older patients, had a higher BMI and waist circumference, and had higher HbA1c value and abnormal lipid profile (39).

Another cross-sectional study carried out on 453 T2DM patients in health care center in Ramallah district, Palestine from March 2018 to January 2019 showed that 13.9 % of patients with T2DM had abnormal ALT, and 3.8% had abnormal AST. It indicated that abnormal serum hepatic enzymes were significantly associated with poorly controlled diabetes (HbA1c≥7%), and higher triglyceride, higher BMI, total cholesterol, and low density lipoprotein value (40).

Moreover, the population-based cross-sectional study constituted 1198 type 2 diabetic patients aged >35 years was conducted in rural areas of China from July 2012 to August 2013. According to the study, the prevalence of elevated ALT and elevated AST was 10.3% and 6.1% in diabetic patient respectively. In addition to this, high triglyceride was positively associated with both elevated ALT and elevated AST, while taking anti-diabetes medicine was inversely related to both elevated ALT and elevated AST. The risk of elevated ALT in diabetic patients increased with the presence of obesity, and was lower in women. Hypertension, current drinking status were risk factors for elevated AST (41).

Similarly, the hospital-based cross-sectional study constituted 210 patients with T2DM was conducted between September 2009 and December 2010 at Bir Hospital, Nepal was found significant ($p < 0.001$) higher ALT, but not significant AST, ALP and GGT levels in patient with NAFLD. The study showed that the prevalence of ALT, AST, and ALP was 40.4%, 17%, and 16% respectively. Moreover, it was indicated that the duration of T2DM was significantly associated with elevated liver enzymes, irrespective of the presence of a fatty liver (42).

Another descriptive cross-sectional study was conducted among 162 T2DM patients and 138 non-diabetic controls aged above 40 years at Manmohan Memorial Teaching Hospital, Nepal,

during the study period in 2018 and the study revealed that there was an increased level of ALT (57%), AST (46%) and ALP (7 %) among patients with T2DM. Furthermore, a significant level of elevation in AST and ALT was observed among the patients with DM compared to non-diabetic controls ($p<0.001$). But, no significant the level of ALP was also high among the diabetic group of patients compared to controls ($p=0.17$). According to this study, the values of transaminase were no significant differences in gender-wise distribution (43).

Another comparative cross-sectional study was conducted among 30 T2DM patients and 30 normal individuals in India in 2015. The study revealed that the prevalence of increased activity of AST was 56.1%, ALT was 19.8% and ALP was 33% in T2DM patients. The study suggested that the liver enzymes (ALT, AST, and ALP) had shown higher activity with T2DM patients than individuals who do not have T2DM (44).

Moreover, retrospective study carried out on data from 320 type 2 DM patients in Shillong, Meghalaya, India from December 2014 and March 2015 showed that 71.25% subjects had an abnormality in at least one liver function test (LFT). The prevalence of elevated ALT, elevated AST, and elevated ALP was 42.2%, 24.5% and 7.5%, respectively. This study also showed that AST, ALT, and ALP were found to be independently associated with fasting glucose values (45).

Furthermore, the retrospective, cross-sectional study included data of 300 T2DM subjects' ≥18 years old was done in a tertiary hospital in Malaysia from January 2011 to December 2014. The study resulted in the prevalence of abnormal ALT and ALP was 27.3% and 13%; with 90.2% and 97.4% having mild ALT and ALP elevations, respectively. Moreover, it noted that significant associations for age, body mass index (BMI) and duration of T2DM for ALT whereas for ALP, anti-diabetic medication was significant between groups of normal and abnormal levels. Elevated liver enzymes were associated significantly with dyslipidemia (46).

Another cross sectional study conducted on 30 T2DM patients and 20 individuals without diabetes from March 2016 and August 2017 in Kirkuk, Iraq was revealed that elevated AST and ALT in T2DM was 46.7% and 43.3 respectively (47).

Furthermore, cross-sectional study performed among 1014 T2DM patients age ranged between 26 and 85 years from August-December 2007; in Jordan was showed that the prevalence of

elevated ALT level was 10.4% with the gender-wise prevalence being 12.8% in men and 7.4% in women. The prevalence of elevated AST levels was 5.4% with the gender-wise prevalence being 5.6% in men and 5.4% in women. Male gender (OR=2.35), and high waist circumference (OR=1.9) were associated with an increased risk of elevated ALT levels. Additionally, the study showed that younger patients had a higher tendency to have elevated ALT compared to those over 65 years (OR=12.4 for patients aged 25-45years, and OR=5.8 for those who were 45-65 years old). Non-insulin use was associated with a high odds ratio for elevated ALT levels (OR=1.7) (48).

Similarly, a cross-sectional study carried among 453 T2DM patients in Palestine from March 2018 through January 2019 revealed that the prevalence of abnormal ALT and AST was 13.9% and 3.8% respectively. The study showed that poorly- controlled diabetes, BMI, triglyceride, and total cholesterol were significantly associated with abnormal ALT (40).

Another retrospective, cross section study carried out on 211 T2DM patients in Jeddah, Saudi Arabia in 2017 was showed that the prevalence of elevated ALT was 7.58%, and elevated AST was 6.16%. It also revealed that higher ALT and AST was no significant association with elevated levels and gender, age, BMI, HbA1c, TG, TC, HDL-C, LDL-C, smoking, or hypertension (49).

Moreover, the retrospective study carried out based on the data of 313 T2DM patients from January 2004-October 2007 in South Africa was found that 46.6 % of patients had liver function test abnormalities. The study showed that the prevalence of elevated ALT, GGT, and ALP was 15.3%, 25.2%, 23.3%, respectively. Patients with abnormal liver enzymes had significantly higher mean serum total triglycerides and LDL-cholesterol (p-value < 0.05) but, no difference was observed between those with abnormal and normal liver enzymes about age, duration of diabetes, HbA1C and other lipid levels. Additionally, fibrate drug use was associated with abnormal liver enzyme prevalence (50).

Furthermore, cross-sectional study carried out in Wad Medani, Abo Agla Diabetes Centre, Sudan on 50 T2DM patients aged 43 to 79 years and 30 non-diabetic controls showed that mean values of ALT, AST and GGT were significantly higher in patients than in the controls ($P<0.001$). It

indicated that 22% of patients had at least one or more abnormal liver function test enzymes. Out of 50 T2DM patients elevated ALT, elevated AST, and elevated GGT were equal (12%) (51).

Moreover, the cross-sectional study conducted on 192 T2DM patients and 192 non-diabetic control at North West of Ethiopia from May to August 2017 was revealed that the prevalence of elevated AST, ALT, and GGT in T2DM was 48.4%, 40.1%, and 1.6% respectively (52).

Another comparative cross-sectional study conducted at the University of Gondar Comprehensive Specialized Hospital in 2018 among 159 T2DM patients and 159 non-diabetic controls showed that 33.3% of T2DM patients had one or more liver function test abnormality. The study also revealed that the prevalence of elevated ALT and AST was 23.3% and 21.4% among T2DM respectively. Furthermore, it showed that high blood pressure, alcohol drinking, older age and being male were significantly associated with liver enzymes abnormality (53).

Likewise, a cross-sectional study conducted on 80 T2DM patients and 60 non diabetic controls from May, 2012 to April, 2013 at the Diabetic Center in Tikur Anbessa Specialized Teaching Hospital, Ethiopia was revealed that the mean values of liver enzymes (ALT, AST, ALP) were significantly higher in diabetic patients compared with the non-diabetic controls ($P<0.05$). Factors assumed to affect liver function tests were waist to hip ratio, age, BMI and Diabetic Duration but, those factors had no strong correlation with most of liver function tests (54).

In addition to this institution-based cross-sectional study conducted among 376 diabetic patients from March-June, in 2014 at Jimma University Specialized Hospital, Ethiopia was revealed that 57.7% of diabetic patients were found to have one or more abnormal liver function tests. The study found that the prevalence of abnormal serum activities of AST, ALT, ALP, and GGT was 23.3%, 20.5%, 20.5%, and 3.2% respectively. Elevated ALT test result was a statistically significant association ($p <0.05$) with increasing body mass index (BMI) (55).

Generally, the prevalence of abnormal serum liver enzymes and associated factors in diabetic patients are inconsistent in different literatures. Also, there is a scarcity of recent kinds of literature on this area in Ethiopia, particularly in Ambo. This study was aimed to assess serum liver enzymes and associated factors in diabetic patients attending Ambo Public Hospitals.

2.1 Conceptual Framework

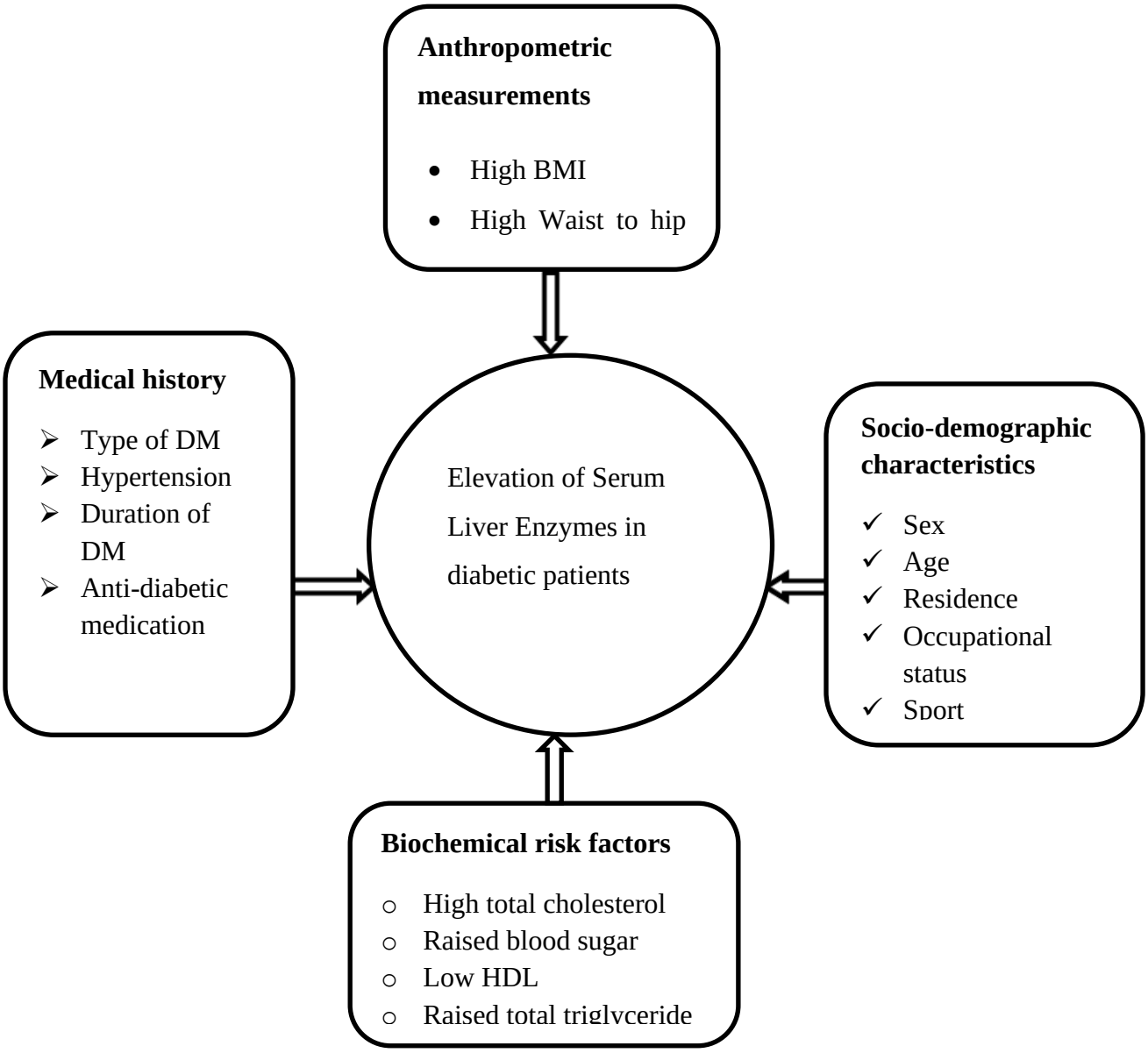


Figure 2: Conceptual framework that indicates serum liver enzymes level is associated with different variables positively or negatively based on the literature review.

CHAPTER THREE: OBJECTIVES

3.1. General Objective

- To assess serum liver enzymes and associated factors among diabetic patients attending Ambo public hospitals, Ambo, Ethiopia from July 20 to October 20, 2020.

3.2. Specific Objectives

1. To assess the prevalence of elevated serum liver enzymes among diabetic patients attending Ambo public hospitals, Ambo, Ethiopia from July 20 to October 20, 2020.
2. To determine factors associated with elevated serum liver enzymes among diabetic patients attending Ambo Public Hospitals, Ambo, Ethiopia from July 20 to October 20, 2020.

CHAPTER FOUR: MATERIALS AND METHODS

4.1. Study Area and Period

The study was conducted at Ambo General Hospital and Ambo University Referral Hospital from July 20 to October 20, 2020. Ambo General Hospital and Ambo University Referral Hospital are located in Ambo town in the West Shoa Zone of the Oromia Region which is located at 115 kilometers to west of Addis Ababa, the capital of Ethiopia. AGH and AURH give different health care services such as obstetric care, medical and surgical care, pharmacy, and laboratory service for patients coming from both urban and rural areas approaching 1.4 million per year. Diabetic patients served at chronic care unit of AGH and AURH.

4.2. Study Design

The institutional-based prospective cross-sectional study was carried out from July 20 to October 20, 2020.

4.3. Source Population

All DM patients attending AGH and AURH during the study period were source population.

4.4. Study population

All diabetes patients those attending AGH and AURH that fulfill the inclusion criteria were used as the study population

4.5. Eligibility Criteria

4.5.1. Inclusion Criteria

The patients with confirmed diabetes mellitus or newly diagnosed diabetes mellitus by WHO criteria (15); fasting plasma venous glucose of ≥ 7 mmol/l (126 mg/dl) or random or two hour postprandial plasma venous glucose of ≥ 11.1 mmol/l (200 mg/dl) at least two times in different occasion of times with the presence of signs and symptoms.

4.5.2. Exclusion Criteria

- Age <18 years.
- Pregnant women.
- History of liver disease or clinical evidence of acute hepatitis
- Subjects seropositive for HBsAg, and HCV antibodies.
- Patients taking ART drugs

4.6. Study Variables

4.6.1. Dependent variables

- ✓ Liver enzymes

4.6.2. Independent variables

- ➢ Age
- ➢ Gender
- ➢ Residence
- ➢ Type of diabetes
- ➢ Duration of diabetes
- ➢ Body mass index (BMI)
- ➢ Raised waist to hip ratio (WHR)
- ➢ Elevated fasting blood glucose (FBS)
- ➢ Hypertension
- ➢ Anti-diabetic medication
- ➢ Physical activity

4.7. Sample Size Determination

The sample size was calculated using single-population proportion formula, with the following assumptions:

The estimated proportion (p=20.5%) was taken from reviewed literature, which was the prevalence of abnormal ALT activity among diabetic patients (55). The prevalence of abnormal ALT was selected to determine sample size because it is most liver specific enzyme. Whereas margin of error (d=0.05), 95% confidence interval (CI), Zα/2 = the standard normal value for 95% confidence interval (1.96) and n=total sample size

$$n = \frac{(Z\alpha/2)^2 P (1-P)}{d^2}$$

$$n = \frac{(1.96)^2\ 0.205(0.795)}{0.05^2}$$

$$n = \frac{3.842 \times 0.163}{0.0025}$$

n= 250

A total of 250 diabetic patients those fulfill inclusion criteria were included in this study.

4.8. Sampling Techniques

The systematic random sampling technique was used to select a total of 250 diabetic patients, according to inclusion criteria during the study period. The numbers of participants were selected proportionally from both hospitals. The patient's Medical registration numbers from both hospitals were listed serially. Then first study subject was selected by the lottery method and selected by every third interval. The participants were waited until they came for their medical follow-up. From the total of 250 study subjects the 138 were selected from 420 DM patients attended AGH, and the 112 were selected from 340 DM patients attended AURH.

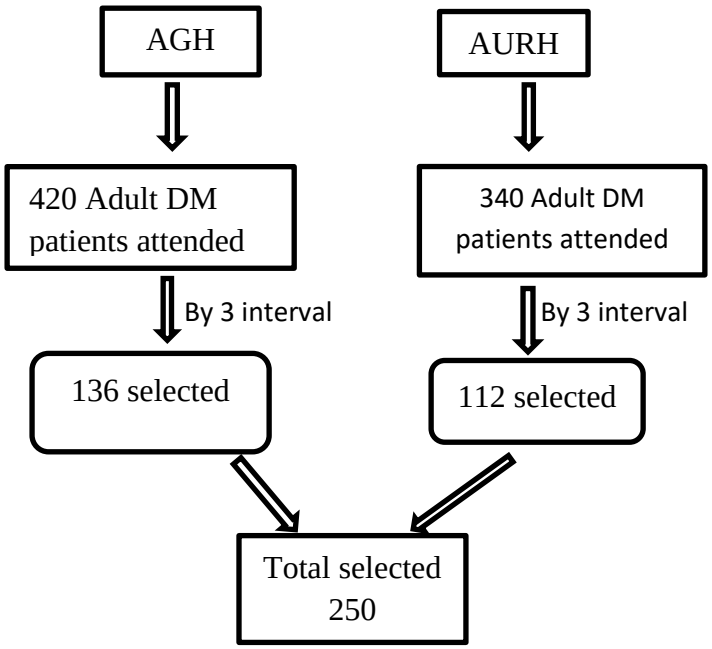


Figure 3, Systematic and proportional selection of study participants

4.9. Data Collection Techniques and Instruments

4.9.1. Socio-demographic and clinical related data collection

Structured questionnaire and patient's medical record review were used for socio-demographic and other related clinical data of study participants.

Blood pressure measurement: Blood pressure was measured by using an aneroid sphygmomanometer and a stethoscope from the upper left arm. SBP and DBP were measured by a trained nurse working at chronic illness clinic after patients take rest for 10 minutes. The participants was sit comfortably, with back supported, legs uncrossed, the feet on ground and upper arm positioned at the heart level during blood pressure measurement.

Hypertension diagnosis for DM patients were made based on the average of the two blood pressure measurements of SBP ≥ 140 mmHg and /or DBP ≥ 90 mmHg according to World Health Organization hypertension diagnosis criteria (3).

4.9.2. Data collection for anthropometric measurements

Weight and height were measured by using a digital weighing machine and height scale (EPWS SH-8007, China), respectively. BMI was calculated as weight divided by height square in meters. During the height measurement, the study participant's shoes and any hats or hair ornaments were removed. With the subject looking straight ahead, the projection was placed at the crown of the head and with the reader's eye at the level of the headpiece. Then the height was taken in meter (56).

Waist circumference was measured in centimeter using the tape to circle of waistline which is located above belly button and below rib cage. Participants was informed not suck hi/her stomach during measurement. Hip circumference was measured in centimeter using the tape by starting at one hip and wrapping the tape measure around rear, around the other hip, and back to where started. Any bulky clothing that can add padding around abdomen was removed during both waist and hip circumference measurement. WHR was calculated by dividing waist to hip circumference (2).

4.10. Blood Sample Collection and Processing

After giving the information about the study, written consent was obtained from the study subject. About 5 ml of venous blood was drawn aseptically from the median vein into jell coated serum separator test tube or plain tube labeled with a unique ID number by trained nurses after overnight fasting of participants. The collected blood samples were placed at room temperature for 30 minutes to facilitate clotting. Then, the blood samples were centrifuged at 2000 revolution per minute (rpm) for 10 minutes to separate serum from formed elements.

4.11. Biochemical Analysis

Biochemical tests such as fasting blood glucose (FBS), serum alanine aminotransferase activity (ALT), serum aspartate aminotransferase activity (AST), serum alkaline phosphatase activity (ALP), and serum gamma-glutamyl transferase activity (GGT) were analyzed by Cobas c311 (Roche Diagnostic GmbH,. Mannheim Germany) automated clinical chemistry analyzer according to the manufacturer's instruction and AURH Laboratory standard operation procedures. Serum of all individuals were screened for HBsAg by using Onsite HBsAg rapid test cassette and for anti-HCV by using HCV rapid test Cassette in order to exclude patients those infected with hepatis B virus, and hepatitis C virus.

Serum glucose was analyzed on the Cobas c311 Clinical chemistry analyzer, using the glucose oxidase (GOD) method. Glucose is oxidized by glucose oxidase to gluconate and hydrogen peroxide. Phenol + 4-AAP + hydrogen peroxide, in the presence of peroxidase, produce a quinoneimine dye that is measured at 500nm. The absorbance at 500nm is proportional to the concentration of glucose in the sample.

Serum ALT activity was measured by an enzymatic rate method. In the reaction, ALT catalyzes the reversible transamination of L-alanine and α-ketoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of NADH to NAD. The catalytic activity of alanine aminotransferase is determined by measurements of the rate of NADH oxidation in reaction. The system monitors the rate of change in absorbance at 340 nm over a fixed time interval. The rate of change in absorbance is directly proportional to the ALT activity in the sample.

Serum AST activity was also measured by an enzymatic rate method on Cobas c311 analyzer in which AST catalyzes the transfer of the amino group from L-aspartate to α-Ketoglutarate to yield oxalacetate and L-glutamate. The oxalacetate undergoes reduction with simultaneous oxidation of NADH to NAD in the malate dehydrogenase (MDH) catalyzed indicator reaction. The resulting rate of decrease in absorbance at 340nm is directly proportional to the AST activity.

Serum ALP activity was determined by measuring the rate of hydrolysis of ρ-Nitrophenyl phosphate to ρ-nitrophenol and inorganic phosphate. The rate at which the ρ-Nitrophenyl is hydrolyzed, measured at 405 nm, is directly proportional to the ALP activity.

Serum GGT activity was determined by modified kinetic method in which GGT catalyzes the transfer of the glutamyl group from gamma-glutamyl-3-carboxy-4-nitroanilide to glycylglycine and 5-amino-2-nitrobenzoate. The change in absorbance at 410/480 nm is due to the formation of 5-amino-2-nitrobenzoate and is directly proportional to the GGT activity in the sample.

4.12. Data Quality Assurance and Management

Before data collection, English version questionnaires were translated to Afan Oromo and Amharic then back to English by authorized language experts to check its consistency. Training was given for data collectors before data collection by the principal investigator. Quality controls of biochemical analysis were done before performing tests on daily basis. The principal investigator was supervised the correct implementation of the data collection procedure and check for completeness and logical consistency of the study tool on a daily base. Double data entry was done to check the consistency and validity of data.

4.13. Data Processing and Analysis

The collected data were reviewed and checked for completeness and consistency by principal investigators on daily bases at the spot during the data collection time. After filtered and checked for their completeness, data were entered to epi-info version 7 and exported to excel then to SPSS. SPSS version 26 was used to analyze recorded data. Mean ± SD were used to summarize continuous variables, whereas frequencies and percentages were used to express categorical variables. Binary logistic regression was computed to assess statistical association via calculating

odds ratio to see the association of independent variables and dependent variables, and the significance of statistical association was assured or tested using 95% confidence interval and P-value (<0.05). During multivariable analysis model fitness has been checked by Hosmer-Lemeshow model fitness and which was insignificant. No multi-collinearity was detected.

4.14. Ethical Considerations

Ethics approval letter was obtained from the Institutional Research Review board (IRB) of Jimma University Institute of Health before the beginning of data collection. Then permission was obtained from the clinical directors of AGH, AURH and respective department heads. Data collectors were trained on how to approach the study participants, and on the contents of the study. Participation in the study was based on each participant's willingness. Voluntarily informed written and signed consent was taken from each participant after explanation of the study purpose, risks, benefits and rights given to them and they were declare with their signatures.

Participants were guaranteed confidentiality of the information collected. Questionnaires and data collection forms were assigned ID numbers and names of the patients was not used during data collection or entered into the computer for analysis.

Principal investigator and data collectors were give education/information/ based on the finding from the biochemical analysis, interview and review of their medical records and facilitate them to have better discussion with concerned health professionals based on their finding for better glycemic control in the future.

4.15. Result Dissemination plan

The result of this study will be presented to Jimma University Institute of Health, School of Medical Laboratory Sciences. Furthermore, the finding will be disseminated to Ambo General Hospital, Ambo University Referral Hospital, Jimma University post-graduate library and the manuscript of this research will be prepared and submitted to appropriate journals for publication.

CHAPTER FIVE: RESULTS

5.1. Socio-demographic, Anthropometric, and Clinical Characteristics

Socio-demographic characteristics of diabetic participants have been displayed in Table 1. Among 250 confirmed adult diabetic patients enrolled in this study, 136 (54.4%) were females and 114 (45.6%) were males. The mean age of participants was 49.64 ±18.15 years, ranged between 18 and 94 years. The 159 (63.6%) of participants live in urban, and 91(36.4%) live in rural. The majority of them belonged to the Oromo ethnic group, which accounts for 222 (88.8%). The Orthodox followers were taken great number 132 (52.8%), followed by protestants (44.4%), Muslims (1.6%) and Waqefatas (1.2%). Regarding marital status, the majority were married (86%) followed by single (12.4%). The 34.8% (87) of participants did not have formal education and 26.8% (67) of them were farmers. Most of the study subjects did not perform physical activity regularly.

The clinical and anthropometric characteristics of the patients were presented in Table 2. One hundred ninety-seven of study participants (78.8%) were T2DM patients, whereas the remaining 53 (21.2%) were T1DM patients. The mean measured FBS was 203.02 ± 94.27. Concerning diabetic treatment, 149 (59.6%) patients were treated with OHA, 86 (34.4%) patients were treated with insulin, and 15 (6.0%) were treated with both insulin and OHA. The duration of diabetes mellitus for the majority of the patients was less than five years (47.6%), whereas 27.6%, 9.6 %, and 15.2% of participants were living with DM for 5 to 10, 11 to 15, and greater than 15 years, respectively.

The calculated WHR from waist and hip circumference revealed that more than half of the participants (53.2%) had high WHR. The calculated BMI from height and weight revealed that the majority of the participants (49.2%) had BMI in the normal range (18.5-24.9 Kg/m^2). The underweight (BMI <18.5) patients were 6.8%, overweight (BMI 25-29.9) patients were 30%, and obese (BMI ≥ 30) patients were 14%. Patients' medical record review and blood pressure measurements indicated that 69 (27.6%) participants had hypertension. Two hepatitis B and three hepatitis C patients among participants were excluded upon screening. They were informed and connected to medical service for further investigation and treatment.

Table 1: Socio-demographic characteristics of sampled diabetic patients attending Ambo Public Hospitals, Ambo, Ethiopia, 2020

Variables	**Category**	**Frequency**	**Percentage**
Gender	Male	114	45.6%
	Female	136	54.4%
Age	< 55 years	148	59.2%
	≥ 55 years	102	40.8%
Residence	Urban	159	63.6%
	Rural	91	36.4%
Ethnic group	Oromo	222	88.8%
	Amara	24	9.6%
	Gurage	4	1.6%
Religion	Orthodox	132	52.8%
	Protestant	111	44.4%
	Muslim	4	1.6%
	Waqefata	3	1.2%
Marital status	Married	215	86%
	Single	31	12.4%
	Others	4	1.6%
Level of Education	No formal education	87	34.8%
	Grade 1-8th	77	30.8%
	Grade 9-12th	41	16.4%
	Level I-IV and Diploma	25	10%
	Degree and above	20	8%
Occupation	Farmer	67	26.8%
	Government employee	47	18.8%
	House wife	46	18.4%
	Merchant	39	15.6%
	Student	16	6.4%
	Retired	16	6.4%
	NGO employee	13	5.2%
	Daily Laborer	6	2.4%
Physical activity	Never	112	44.8%
	Sometimes	111	44.4%
	Regular	27	10.8%

Table 2: Clinical and anthropometric characteristics of sampled diabetic patients attending Ambo Public Hospitals, Ambo, Ethiopia, 2020

Variables	**Category**	**Frequency**	**Percentage**
Types of DM	T1DM	53	21.2%
	T2DM	197	78.8%
Anti-diabetic agent	Insulin	86	34.4%
	OHA	149	59.6%
	OHA and Insulin	15	6%
Duration of diabetes	< 5 years	119	47.6%
	5-10 years	69	27.6%
	11-15 years	24	9.6%
	>15 years	38	15.2%
WHR	Normal	117	46.8%
	High	133	53.2%
BMI (kg/m²)	Underweight (<18.5)	17	6.8%
	Normal (18.5-24.9)	123	49.2%
	Overweight (25-29.9)	75	30%
	Obese (≥ 30)	35	14%
Hypertension	Absent	181	72.4%
	Present	69	27.6%
FBS (mean ± SD)	203.02 ± 94.27 mg/dl		

NB: n- number, Kg/m²- kilogram per meter square, mg/dl-milligram per deciliter

5.2. Prevalence of Elevated Serum Liver Enzymes and Associated Factors in Diabetic Patients

The mean and prevalence of elevated liver enzymes in diabetic patients were shown in Table 3. From 250 DM patients, 108 (43.2%) of participants were found to have one or more elevated liver enzymes. The Mean ±SD was 36.88 ± 38.20 for ALT, 33.49 ± 35.03 for AST, 98.28 ± 50.71 for ALP, and 32.12 ± 28 for GGT. The prevalence of elevated serum ALT activity among diabetic patients was 26.4% (n=66) with gender-wise prevalence of 29.4% (n=40) in females and 22.8% (n=26) in men. The prevalence of both elevated serum AST and GGT activity was 20.8%

(n=52). The prevalence of elevated serum AST activity was 22.1% (n=30) in females and 19.3% (n=22) in men, while the prevalence of elevated serum GGT activity was 27.2% (n=37) in females and 13.2% (n=15) in men. The prevalence of elevated serum ALP activity was 23.6% (n=59) with gender-wise prevalence of 29.4% (n=40) in females and 16.7% (n=19) in men. In 46 (18.4%) of DM patients both serum ALT and AST activities were elevated while in 31(12.4%) of participants both serum ALP and GGT activities were elevated.

Table 3: Mean values and prevalence of elevated liver enzymes of sampled diabetic patients attending Ambo Public Hospitals, Ambo, Ethiopia, 2020

Liver Enzymes	Mean ± SD	Reference range	Prevalence of elevated liver enzymes
ALT	36.88 ± 38.20	0-40 U/L (M)	26.4%
		0-33 U/L (F)	
AST	33.49 ± 35.03	0-40 U/L (M)	20.8%
		0-32 U/L (F)	
ALP	98.28 ± 50.71	40- 129 U/L (M)	23.6%
		35- 104 U/L (F)	
GGT	32.12 ± 28.47	0-60 U/L (M)	20.8%
		0-40 U/L (F)	

NB: F-female, M-male, SD-standard deviation

The prevalence of elevated ALT, AST, ALP and GGT activities according to demographic, clinical, and anthropometric characteristics were shown in Table 4 and 5. In this study, living in the urban area, duration of diabetes for ≥ 11 years, presence of hypertension, having high WHR, and treatment with both insulin and OHA were significant predictors of elevated serum ALT in diabetic patients (P< 0.05). Diabetic patients from the urban area were 2.38 times (AOR=2.38, 95% CI: 1.02-5.54) more likely to have elevated serum ALT activity than those from the rural area. Moreover, patients who have lived with diabetes for 11 to15 years were 15.49 times (AOR=15.49, 95% CI: 3.82-62.79) more likely to have elevated serum ALT than those who have lived with diabetes for less than five years. On the other hand, the prevalence of elevated ALT was significantly higher in patients who had high WHR than those who had normal WHR (elevated ALT: 40.6% in high WHR and 10.3% in normal WHR, p=0.01). In addition,

hypertensive diabetic patients were 3.34 times (AOR=3.34, 95% CI: 1.39-8.05) more likely to have elevated ALT than non-hypertensive diabetic patients. Even though, the prevalence of elevated ALT was increased within BMI and FBS categories, there was no significant association with elevated ALT activity.

We observed that elevated AST was positively associated with duration of diabetes (for 11-15 years: AOR=12.87, 95% CI: 3.32-49.95, p<0.001, for >15 years: AOR=17.04, 95% CI: 3.23-89.81, p<0.001). The prevalence of elevated AST was significantly higher in patients that had high WRH (AOR=9.46, 95% CI: 2.82-31.73, p<0.001) than patients who had normal WHR. Moreover, participants who had FBS ≥301 mg/dl were 8.43 times (AOR=8.43, 95% CI: 1.69-41.89, p=0.01) more likely to have elevated AST than those had FBS < 125 mg/dl. This study revealed that gender, type of DM, residence, level of BMI, and hypertension were not significant predictors of elevated AST activity in diabetic patients (P> 0.05).

The prevalence and the association of elevated ALP and GGT activity with variables were shown in Table 5. Living with DM for more than fifteen years, being overweight (BMI= 25-29.9 kg/m^2), having high WHR, and being hypertensive were significantly associated with the elevated ALP activity in diabetic patients (p< 0.05). The patients who lived with diabetes for greater than fifteen years were 4.77 times (AOR= 4.77, 95% CI: 1.23-18.44) more probably to have elevated serum ALP activity than those who lived with diabetes for less than five years. Moreover, the patients with high WHR had 4 times (AOR=3.98, 95% CI: 1.55-10.23) more elevated ALP than patients with normal WHR. Furthermore, hypertension was significantly associated with elevated serum ALP activity in diabetic patients (p=0.004). Even though sex was not a significant predictor, the prevalence of both elevated serum ALT, AST and ALP were higher in females than men.

This study showed that female gender, treatment with both insulin and OHA, high WHR, and FBS ≥301 mg/dl were significant predictors of elevated serum GGT in diabetic patients (p<0.05). Female diabetic patients were 4 times (AOR=4, 95% CI: 1.62-9.91, p=0.003) more reasonably to have elevated GGT than male diabetic patients. DM patients treated with both insulin and OHA were 11 times (AOR=11.12, 95% CI: 1.67-74.13, p=0.01) more likely to have elevated serum GGT than who treated with insulin only. Elevated serum GGT was also significantly elevated in patients those had high WHR (p<0.001).

Table 4: Association between demographic, clinical characteristics and elevated ALT and AST in diabetic patients attending diabetes Ambo Public Hospitals, 2020

Variables		ALT				AST			
Gender		N	E	AOR (95% CI)	p-value	N	E	AOR (95% CI)	p-value
	Male	88(77.2%)	26 (22.8%)			92(80.7%)	22(19.3%		
	Female	96(70.6%)	40(29.4%)	1.63(0.72,3.78)	0.24	106(77.9%)	30(22.1%)	1.24(0.48,3.18)	0.67
Age	<55	118(79.7%	30(20.3%)			128(86.5%)	20(13.5%)		
	≥55	66(64.7%)	36(35.3%)	2.16(0.83,5.58)	0.11	70(68.6%)	32(31.4%)	1.77(0.57,5.47)	0.32
Residence	Rural	70(76.9%)	21(23.1%)			75(82.4)	16(17.6%)		
	Urban	114(71.7%)	45(28.3%)	2.38(1.02,5.54)	0.04	123(77.4)	36(22.6%)	2.7(0.96,7.6)	0.06
Type of DM	T1DM	40(75.5%)	13(24.5%)			45(84.9%)	8(15.1%)		
	T2DM	144(73.1)	53(26.9%)	2.70(0.67,10.86)	0.15	153(77.7%)	44(22.3%)	1.01(0.2,5.05)	0.99
Duration of DM	<5 yrs	106(89.1%)	13(10.9%)			113(95.0%)	6(5.0%)		
	5-10 yrs	54(78.3%)	15(21.7%)	1.81(0.70,4.65)	0.22	60(87.0%)	9(13.0)%)	1.67(0.5,5.61)	0.4
	11-15 yrs	16(66.7%)	8(33.3%)	3.54(1.08,11.6)	0.04	14(58.3%)	10(41.7%)	12.87(3.32,49.95)	<0.001
	>15 yrs	8(21.1%)	30(78.9%)	15.49(3.82,62.79)	<0.001	11(28.9%)	27(71.1%)	17.04(3.23,89.81)	0.001
Mode of treatment	Insulin	66(76.7%)	20(23.3%)			72(83.7%)	14(16.3%)		
	OHA	112(75.2%)	37(24.8%)	2.99(0,91,9.81)	0.71	119(79.9)	30(20.1%)	2.1(0.56,72)	0.27
	Insulin & OHA	6(40.0%	9(60.0%)	9.79(1.3,73.51)	0.03	7(46.7%)	8(53.3%)	5.31(0.58,48.87)	0.14
BMI (kg/m²)	<18.5	13(76.5%)	4(23.5%)			15(88.2%)	2(11.8%)		
	18.5-24.9	104(84.6%)	19(15.4%)	0.57(0.14,2.24)	0.42	105(85.4%)	18(14.6%)	1.83(0.3,11.17)	0.51
	25-29.9	57(76.0%)	18(24.0%)	0.97(0.23,4.18)	0.97	67(89.3%)	8(10.7%)	1.08(0.15,7.77)	0.94
	≥30	10(28.6%)	25(71.4%)	1.24(0.2,7.92)	0.82	11(31.4%)	24(68.6)	2.08(0.22,20.12)	0.53
WHR	Normal	105(89.7%)	12(10.3%			112(95.7%)	5(4.3%)		
	High	79(59.4%)	54(40.6%)	3.01(1.26,7.21)	0.01	86(64.7%)	47(35.3%)	9.46(2.82,31.73)	<0.001
Hypertension	No	149(82.3%)	32(17.7%)			154(85.1%)	27(14.7%)		
	Yes	35(50.7%)	34(49.3%)	3.34(1.39,8.05)	0.01	44(63.8%)	25(36.2%)	1.12(0.38,3.29)	0.83
FBS (mg/dl)	<125	47(78.3%)	13(21.7%)			53(88.3%)	7(11.7%)		
	126 – 200	60(80%)	15(20%)	1.07(0.39,2.94)	0.9	64(85.3%)	11(14.7%)	2(0.57,6.97)	0.28
	201-300	64(82.1%)	14(17.9%)	0.44(0.14,1.33)	0.15	67(85.9%)	11(14.1%)	1.21(0.33,4.53)	0.77
	≥301	13(35.7%)	24(64.9%)	2.66(0.67,10.50)	0.16	14(37.8%)	23(62.2%)	8.43(1.69,41.89)	0.01

Abbreviations: CI-confidence interval, kg/m²- kilogram per meter square, M-male, F-female, N-normal, E-elevated, mg/dl-milligram per deciliter,

Table 5: Association between demographic, clinical characteristics and elevated ALP and GGT in diabetic patients attending diabetes Ambo Public Hospitals, 2020

Variables		**ALP**				**GGT**			
Gender		**N**	**E**	**AOR (95% CI)**	**p-value**	**N**	**E**	**AOR (95% CI)**	**p-value**
	Male	95(83.3%)	19(16.7%)			99(86.8%)	15(13.2%)		
	Female	96(70.6%)	40(29.4%)	2.22(0.95,5.2)	0.07	99(72.8%)	37(27.2%)	4(1.62,9.91)	0.003
Age	<55	121(81.8%)	27(18.2%)			127(85.8%)	21(14.2%)		
	≥55	70(69.6%)	32(31.4%)	1.06(0.72,3.04)	0.91	71(69.6%)	31(30.4%)	0.85(0.33,2.12)	0.73
Residence	Rural	77(84.6%)	14(15.4%)			79(86.8%)	12(13.2%)		
	Urban	114(71.7%)	45(28.3%)	1.22(0.5,2.94)	0.67	119(74.8%)	40(25.2%)	1.12(0.47,2.67)	0.8
Type of DM	T1DM	37(69.8%)	16(30.2%)			46(86.8%)	7(13.2%)		
	T2DM	154(78.2%)	43(21.8%)	3.7(0.96,14.17)	0.06	152(77.2%)	45(22.8%)	0.34(0.08,1.79)	0.22
Duration of DM	<5 years	104(87.4%)	15(12.6%			107(89.9%)	12(10.1%)		
	5-10 years	57(82.6%)	12(17.4%)	0.8(0.29,2.22)	0.67	57((82.6%)	12(17.4%)	1.89(1,5.16)	0.21
	11-15 Years	20(83.3%)	4(16.7%)	0.94(0.24,3.69)	0.93	18(75%)	6(25%)	2.82(0.79,10.02)	0.11
	>15 Years	10(26.3%)	28(73.7%)	4.77(1.23,18.44)	0.02	16(42.1%)	22(57.9%)	3.18(0.79,12.86)	0.1
Mode of treatment	Insulin	62(72.1%)	24(27.9%)			75(87.2%)	11(25%)		
	OHA	122(81.9%)	27(18.1%)	0.83(0.25,2.68)	0.75	116(77.9%)	33(22.1%)	3.25(0.89,11.88)	0.07
	Insulin & OHA	7(46.7%)	8(53.3%)	2.52(0.36,17.71)	0.35	7(46.7%)	8(53.3%)	11.12(1.67,74.13)	0.01
BMI (kg/m²)	<18.5	12(70.6%)	5(29.4%)			14(82.4%)	3(17.6%)		
	18.5-24.9	103(83.7%)	20(16.3%)	0.28(0.07,1.13)	0.07	110(89.4%)	13(10.6%)	0.32(0.07,1.53)	0.15
	25-29.9	64(85.3%)	11(14.7%)	0.18(0.04,0.85)	0.03	62(82.7%)	13(17.3%)	0.44(0.09,2.23)	0.32
	≥30	12(34.3%)	23(65.7%)	0.54(0.08,3.43)	0.51	12(34.3%)	23(65.7%)	2.4(0.38,15.37)	0.35
WHR	Normal	107(91.5%)	10(8.5%)			108(92.3%)	9(7.7%)		
	High	84(63.9%)	49(36.8%)	3.98(1.55,10.23)	0.004	90(67.7%)	43(32.3%)	2.74(1.07,7.02)	0.036
Hypertension	No	154(85.1%)	27(14.9%)			153(84.5%)	28(15.5%)		
	Yes	37(53.6%)	32(46.4%)	2.99(1.24,7.25)	0.015	45(65.2%)	24(34.8%)	1.34(0.55,3.27)	0.52
FBS (mg/dl)	<125	51(85%)	9(15%)			48(80%)	12(20%)		
	126 – 200	64(85.3%)	11(14.7%)	1.31(0.41,4.12)	0.65	64(85.3%)	11(14.7%)	0.9(0.31,2.58)	0.85
	201-300	60(76.9%)	18(18.4)	1.18(0.38,3.68)	0.77	64(82.1%)	14(17.9%)	0.55(0.18,1.69)	0.3
	≥301	16(43.2%)	21(56.8%)	1.86(0.47,7.41)	0.38	22(59.5%)	15(40.5%)	0.56(0.13,2.3)	0.42

CHAPTER SIX: DISCUSSION

This study conducted on selected 250 of diabetic patients from Ambo University Referral hospital and Ambo General Hospital. Overall, 43.2% (108) of diabetic patients had at least one or more elevated liver enzymes. This result is higher than a study done in Sudan, in which 22% of diabetic patients had at least one or more elevated liver enzymes (51). The variation may be due to differences in the background of patients (environment, genetic) and sample size.

According to this study, serum ALT was elevated among 26.4% of diabetic patients. This result is slightly comparable with the study done in Malaysia (27.3%) (46). This result was higher than the finding of study conducted in China (10.3%) (41), Jordan (10.4%) (48), Palestine (13.9%) (40), India (19.8%) (44), Saudi Arabia (7.58%) (49), South Africa (15.3%) (57), Sudan (12%) (51), Gondar, Ethiopia (23.3%) (53) and Jimma, Ethiopia which was 20.5% (55). However, the prevalence of elevated ALT was less than study done in Iraq (43.3%) (47), India (42.2%) (45), and Nepal (57%) (43) and North West Ethiopia (40.1%) (52). This variation may be due to the difference in living style, environment, medical care and knowledge of the patients on the risk factors.

In our study**,** serum elevation of ALT was positively and significantly associated with the duration of DM for more than 11 years ($p< 0.05$). This result was in agreement with the study conducted by Mandal et al in Nepal (42), and by Thambiah et al in Malaysia (46). Even though there was no significant association of elevation of ALT with increased BMI, the prevalence of elevated ALT was increased with increased BMI. This result is consistent with a study conducted by Alzahrani et al in Jeddah (49). However, some earlier studies revealed that elevation of serum ALT activity was significantly associated with increase in BMI (17,39,40,46,55). This disagreement may be due to the difference in medical care and sample size. We have observed patients live in urban were more likely (OR=2.38, 95% CI: 1.02-5.54, p=0.04) to have elevated ALT than who live in rural. This may be due to the sedentary lifestyle of the urban population. Hypertension was a significant predictor of elevation of serum ALT in diabetic patients. This result is in line with a study carried out by Teshome et al at Gondar in 2019 (53).

This study indicated that the prevalence of elevated serum AST was 20.8% in diabetic patients. This result was in line with study carried out in Gondar, Ethiopia (21.4%) (53); less than the result of study carried out in Nepal (46%) (43), India (56.1%) (44), Meghalaya, India (24.5%) (45), Iraq (46.7%) (47), North West of Ethiopia (48.4%) (52), Jimma , Ethiopia (23.3%); greater than the result of study conducted in Palestine (3.8%) (40), China (6.1%) (41), Nepal (17%) (42), Malaysia (13%) (46), Jordan (5.4%) (48), Palestine (3.8%) (40), Saudi Arabia (6.16%) (49), and Sudan (12%) (51). The variations may be because of difference in environment, genetics, sample size and cut off point. We observed that the elevation of AST was positively and significantly associated with high WHR ($p<0.001$). Which is as similar as study conducted at Tikur Anbessa Specialized and Teaching Hospital, Ethiopia (54). Duration of DM for more than 11 years was a significant predictor of elevated AST ($P<0.05$). This finding was consistent with the study conducted by Mandal et al in Nepal in (42). Our study also indicates significant association of FBS $\geq$ 301mg/dl with elevation of activity of AST ($p<0.001$). This result is agreed with the study done in India from December 2014 and March 2015 (45).

The study found that the prevalence of elevated serum ALP in diabetic patients was 23.6%. A similar result was observed in study done in South Africa (58). However, this result is greater than the findings of various studies (42,43,45,46,55), and less than the study conducted by Mathur et al in India in 2016 (44). The variation may be due to differences in medical care, genetic, environment, study design, and cut-off value.

In our study the prevalence of elevated activity of GGT was 20.8% in diabetic patients. This result is greater than the findings of various studies (51,52,55), and less than the study conducted in South Africa which found that 25.2% of patients had elevated serum GGT (58). This variation may be attributed to the difference of study design, medical care, cut-off value and the knowledge of the patients on the risk factors. Being female, treating DM with both insulin and OHA, and having high WHR were significantly associated with the elevation of serum GGT in diabetic patients ($p<0.05$). We observed the elevation of GGT was not significantly associated with age, residence, type of DM, duration of DM, BMI, hypertension and FBS level.

This study indicated that the elevation of liver marker enzymes (ALT, AST, ALP and GGT) were positively and significantly associated with having high WHR ($p<0.05$). This indicates abdominal obesity was significantly associated with elevation of liver marker enzymes in serum.

High WHR indicates increased visceral adipose tissue which is associated with a range of metabolic abnormalities, including decreased glucose tolerance, reduced insulin sensitivity and adverse lipid profiles (2). The abdominal obesity may leads to an abnormal accumulation of fat in liver cells. This may either triggers inflammations or causes the swelling and bursting of cells and leakage of enzymes into serum that leads to the elevation of liver marker enzymes in serum.

We found that in 46 (18.4%) of diabetic patients both serum ALT and AST activities were elevated while in 21 (18.4%) of diabetic patients both serum ALP and GGT activities were elevated. This might be indicating the presence of more hepatocellular injury than cholestatic injury among diabetic patients but, this needs further investigation. AST and ALT are more elevated in patients with hepatocellular injury, whereas ALP and GGT are more elevated in cholestatic injury (11).

Our study revealed the presence of elevated serum liver marker enzymes among diabetic participants. This may be due to the deleterious effect of hyperglycemia and/or insulin resistance. Insulin resistance can lead to the pathologic accumulation of lipids in the liver due to increased hepatic synthesis or reduced secretion by the liver. In presence of insulin resistance, hormone-sensitive triglyceride activity is uninhibited resulting in increased fat mobilization. This resulted in increased levels of free fatty acids circulating in the serum then entering the liver and forms a large amount triglyceride deposition in the liver. This triglyceride deposition contributes to hepatocyte degeneration and fatty liver disease (19). The elevation of the transaminase enzymes is related to liver cell damages. The rupture of a plasma membrane due to high concentrations of the metabolites, mitochondrial damage and the inactivation of the regulatory metabolic enzymes results in hepatic cell injury (59). The elevation of these serum liver enzymes may be due to leakage of them to serum from that of damaged hepatocytes.

CHAPTER SEVEN: CONCLUSION

Our study found that there was high prevalence of one or more elevated liver enzymes in diabetic patients in our study area than many earlier studies. As elevation of liver marker enzymes are indicator of liver dysfunction; this result might be indicates liver impairment among diabetic patients. This elevation of liver enzymes among diabetic patients might be due to diabetic induced liver injuries and/or due to other liver diseases.

Serum ALT activity the most elevated serum liver enzyme followed by elevated serum ALP activity. Elevated serum ALT was more frequently found among DM patients resides in urban, live with DM for more than eleven years, treated with both insulin and OHA, those who had high WHR and hypertension.

Elevation of serum AST activity was significantly associated with duration of DM for more than eleven years, having high WHR and FBS greater than 300 mg/dl. Living with DM for more than fifteen years, being overweight, high WHR and hypertension were significant predictors of elevation of serum ALP activity in diabetic patients. Elevation of serum GGT activity in diabetic patients was positively and significantly associated with female gender, following both insulin and OHA treatment, and having high WHR.

CHAPTER EIGHT: RECOMMENDATION

Routine assessment of serum liver enzymes might be beneficial for diabetic patients to control and follow up liver dysfunction. It might be better if public health policy makers, service providers and other stakeholders pay attention for the liver function impairments among diabetic patients. In addition, the comprehensive researches that include a larger sample size, physical examination, other liver function tests, liver histology and imaging are important for further clarification of causative factors and mechanisms of elevation of liver enzymes and liver damage in diabetic patients.

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ANNEX I: INFORMATION SHEETS FOR PARTICIPANT

Title of the research: Assessment of serum liver enzymes and associated factors among diabetic patients at Ambo General Hospital and Ambo University Referral Hospital.

Study design: Inistitutional-based cross-sectional study.

Name of researcher: Kebede Tessema (MSc candidate)

Name of Advisors: Mr. Sintayehu Asaye (BSc, MSc)

Mr. Shiferaw Bekele (MSc, Assi.prof)

Name of the organization: Jimma University, Institute of Health, Faculty of Health Science, School of Medical Laboratory Science.

Introduction: This information sheet is prepared to explain about the research that you are asked to participate. Please read the following statements and ask any unclear points before you agree to participate. If you agree to be included in this study, I would like to ask you to sign on a document to show your agreement, and give clinical specimen

Aim of the study: The aim of this research to assess serum liver enzymes and associated factors among diabetic patients attending AGH and AURH, Ambo, Ethiopia from July 20 to October 20, 2020. This study will provide evidence-based information on the elevation of liver enzymes and associated risk factors among diabetic patients. The findings of this study may help you to get evidence-based information on the functional status of your liver that is important to take preventive and curative action. Additionally, it may be used for health care policymakers to develop strategies to reduce morbidity and mortality of diabetic patients from diabetes-associated liver disease.

Procedure: If you are willingness to participate in this study after the investigator or a nurse give you verbal and/or written information about the study, you will sign the consent form. The information will be collected using structured questionnaires and the height, weight, waist and hip circumference will be measured by the assigned nurse. If you are fit for the study 5 ml of

blood samples will also be collected for laboratory examination of fasting blood glucose, and liver enzymes (ALT, AST, ALP, and GGT).

Risks: The risk of participating is minimal and not different from routine procedures. It may be taking some time and little pain during venous blood collection. The amount of blood (5ml) taken will not affect your health.

Benefits: If you participate in this study you will help us to assess serum liver enzymes and associated factors among diabetic patients. If a medical and laboratory examination reveals any abnormalities, your doctor will be notified. There would not be any direct payment for participating in this study.

Confidentiality: The information you will provide us will be confidential except your doctor and principal investigator. There will be no information that identifies you. The findings of the study will be generalized and will not reflect any particular individual. The questionnaires will be coded and kept unnamed

Rights: You have the full right to participate or not participate in this study. If you decide to participate, you have the right to withdraw from the study at any time and this will not label you for any loss of benefits which you otherwise are entitled. You do not have to answer any question that you do not want to answer.

Contact Address: If you have any question or any enquires any time regarding the study or the procedures, please contact any of the following addresses:

Kebede Tessema: Mobile no: 0921462312

E-mail: **kebede072@gmail.com**

ANNEX II. CONSENT FORMS **(in English version)**

I, the undersigned, confirm that, as I give consent to participate in the study, it is with a clear understanding of the objectives and conditions of the study and with recognition of my right to withdraw from the study if I change idea. I have been given the necessary information about the research. I have also been assured that I can withdraw my consent at any time without penalty or loss of benefits. The proposal has been explained to me in the language I understand.

I----------do hereby give consent to Dr/Mr./Mrs/Miss----------to include me in the proposed research.

Patient code _____ signature_____ date_____

Name of data collector _____ signature_____ date_____

INFORMATION SHEETS FOR PARTICIPANTS (Afaan Oromo Version)

Unkaa Odeeffannoo Hirmaataa

Mata Duree Qorannichaa: Inzaayimoota tiruu dhigaa keessatti argamanii fi dhimmoota ittin wal-qabatu wal'aanamtoota dhibee sukkaraa Hospitaala walii-galaa Ambootti fi Hospitaala Riferaala Universitii Amboo yaalamanii Qorachuu ta'a.

Qorataa: Obbo Kabbadaa Tasammaa

Gorsitoota: Obbo Sintaayyoo Asaayyee

Obbo Shifarraa Baqalaa

Maqaa Dhaabataa: Yuuniversittii Jimmatti, Instituyittii Faayyaa, Fakaltii Saayinsii Fayyaa, Mana Barumsaa Saayinsii Laaboraatorii Meedikaalaa.

Seensa: Unkaan odeeffannoo kun kan qophaa'e waa'ee qorannoo isin keessatti hirmaachuuf deemtan kan ibsuu dha. Qoranicha kessatti hirmaachuun dura odeeffannoo armaan gadii sirnaan dubbisaa, waan siniif hin gallee gaafadhaa. Hirmaachuuf yoo fedhii qabaatan walii galtee keessan mallattoo keessanin mirkanneessaa.

Kaayyoo: kaayyoon qorannichaa Inzaayimoota tiruu dhiigaa keessatti argamanii fi dhimmoota ittin wal-qabatan wal'aanamtoota dhibee sukkaaraa Hospitaala walii-galaa Ambootti fi Hospitaala Riferaala Universitii Ambootti yaalaman irratti ji'a Adoolessa guyyaa 20 hanga Onkololeessa guyyaa 20, bara 2020 tti qorachuu dha. Qorannoon kun wal'aanamtoota dhibee sukkaaraa keessatti olka'iinsa inzaayimoota tiruu fi sababoota ittin wal-qabatan odeeffannoo qabatamaa kan kennu dha. Kun immoo fayyummaa tiruu keessanii hanga tokko kan ittin beektanii fi faayyummaa tiruu kessaani eegachuuf illee isin gargaara. Dabalataanis firiin qorannoo kanaa Eegumsii Fayyaa Hawaasaa, dhukkuba fi du'aatii sababa dhibee sukkaaraa fi rakkoo tiruun walqabatuun dhufu xiqqeessuuf karoora akka baasuu ni gargara.

Adeemsa: Qorannoo kana keessatti hirmaachuuf fedhii yoo qabattan walii galtee keessaa mallatoon mirkaneessitu. Sana boodaa odeeffannoon barbaachiisu gaafilee qindaa'aniin sassabama. Ulfaatinni qaamaa, hojjaa, marsaan mudhii fi Mo'oo kessaan ogeessa Narsiin ni safarama. Ulagaa qorannoo yoo guuttan dhiigni 5 ml qorannoo sukkaaraa fi inzaayimoota tiruu kanneen akka AST, ALT, ALP, fi GGT tif ni kennitu.

Rakkoolee muudachuu danda'an: Rakkoolee adeemsa qorannoo kana kessatti muudachuu dand'aan baayyee xiqqaa fi kan yeroo qorannoo yaalaa caaluu miti. Isaan kuniis yeroo xiqqoo fi dhukkubbii xiqqoo yeroo dhiigaa kennitan isinitti dhaga'amuu dha. Dhiigaa 5ml keennuun fayyaa keessan irratti rakkoo homaatuu hin qabu.

Faayidaa: Qorannoo kana irratti hirmaachuu keessaniif faayidaan akka dhunfatti argachuu dandeessan xiqqoo ta'uu danda'a. Haa ta' u malee, dhibee tiruu fi wantoota dhibicha

hammeessan dursitanii beekuuf sin gargaara. Qorannoon meedikaalaa fi Laaboraatoorii keessan rakkoo faayyummaa kan agarsiisu yoo ta'e karaa ogeessa isin wal'aanuu isin beeksisna.

Iccitii Eeguu: Odeeffannoo keessan ogeessa isin wal'aanuu fi ogeessota qorannoo irratti hirmaataniin ala qaamni biroof hin dabarfamu. Maqaan keessaan odeeffannoo funaanamu irratti hin guutamu. Firiin qorannoo kanaas yoo ibsamu akka walii galaatti malee akka nama dhuunfatti miti.

Mirga: Qorannoo kana keessatti hirmaachuu fi hirmaachuu dhiisuuf mirga qabdu. Hirmaachuuf yoo murteessitaniis yoo yaada keessan geeddartan yeroo kamitu qorannichaa keessaa bahu mirga qabdu. Sababa qorannichaa irratti hin hirmaanneef yaala argachuu qabdan irratti dhiibbaa isin irra ga'u tokko hin jiru.

Teessoo: Gaaffii ykn yaada yoo qabaattan yeroo barbaaddan teessoo armaan gadii fayyadamaa.

Kabbadaa Tasammaa: Yuuniversitti Jimmaatti, Institutii Fayyaa, Mana barnoota laaboraatorii meedikaalaa.

Lakk. Moobayilaa: 0921462312

E-mail: kebade072@gmail.com

Jimmaa, Itoophiyaa

Guca Walii Galtee (Afan Oromo Version)

Ani armaan gaditti mallatoo kiyyaan kanaan mirkaneessu, odeeffannoo armaan olitti barreeffame dubbisee kaayyoo fi haala qoranichaa hubadheera. Yeroo kamittuu yaada koo yoon jijjire adabii fi taajajila dhabuu tokko malee qorannicha keessaa bahuu akkan danda'u naaf himameera. Qorannichi afaan ani hubadhuun naaf ibsameera.

Ani_____Dr./Adde/Obbo_____n qorannoo yaadame keessatti hirmaachuuf waliigaleera.

Koodii hirmaataa _____ mallattoo_____ guyyaa_____

Maqaa nama ragaa funaanuu _____ mallattoo_____ guyyaa_____

INFORMATION SHEETS FOR PARTICIPANTS (Amharic version)

ለጥናቱ መረጃና ተሳታፊነት መግለጫ ቅጽ

የጥናቱ ርዕስ፡ የጉባት እንዛይሞችና ተያያዥ ችግሮች በሱካር ሕመምታሞች ዉፅጥ መጥናት ይሆናል

ዋና ተመራማሪ ፡ ከበደ ተሰማ (MSc candidate)

አማካሪዎች፡1. አቶ ሲንታዮ አሳየ (BSc, MSc)

2 አቶ ሺፈራዉ በቀለ (MSc, Assi. prof)

የድርጅቱ ስም: ጅማ ዩኒቨርሲቲ

መግብያ፡ ይህ መግለጫ ቅጽ የተዘጋጀዉ ሰለምተሳተፉበት ጥናት የምያብራራ ነዉ። እባኮት በጥናቱ ዉስጥ ከመሳተፋቹ በፍት ይህንን ቅጽ በጥሞና አንብባቹ ያልገባቹትን መጠየቅ ትችላላቹዉ። ጥናቱ ዉስጥ መሳተፍ የምትፈልጉ ከሆነ በፍርማቹ አረጋጊጡት።

የጥናቱ ላላማ፡ የጥናቱ ላላማ በሱካር ታካምዎች ደም ዉስጥ የምገኙ የጉበት ኢንዛይሞችና ተያያዥ ችግሮችን ማጥናት ይሆናል። ጥናቱ በሱካር ታካምዎች ደም ዉስጥ የምገኙ የጉበት ኢንዛይሞች ከፍታና ተያያዥ ችግሮችን የምገልፅ ተጨባጭ ማስረጃ ይሰጣል።

የአሰራሩ ሂደት፡ በጥናቱ ዉስጥ ለመሳተፍ ከተስማማቹ ቡኋላ በፍርማቹዉ ታረጋግጣላቹዉ። ቀትሎም ለጥናቱ የምያስፈሊጉ ማስራጃዎች በጹሁፍ ጥያቄዎች ይሰበሰባል። የጥናቱን አላማ የምታሚሉት ከሆነ ቁመት፤ ክብደት፣ ወገብና የወገብ ዙሪያ በጤና ባለሙያ ይለካል። በተጨማሪም 5 ml ደም ለላቦራቶሪ ጥናት ትሰጣላችዉ።

በጥናቱ ሊከሰቱ የሚችሉ ተያያዥ ችግሮች፡ በጥናቱ ግዜ ልከሰቱ የምችሉ ችጊሮች በጣም ጥቅትና ከሕክምናዉ ጊዜ የማይበልጥ ነዉ። እነኚም በጥናቱ የሚወስድባቹዉ ጊዜና ደም ስትሰጡት መጠነኛ ሕመም ናቸዉ። 5 ml ደም በመስጠት ጤናቹ ላይ ምንም ችግር አደርስም።

ጥቅም፡ በጥናቱ ዉስጥ በመሳተፋቹዉ በገንዘብ መልክ የምታገኙት ጥቅም የለም። ነገርግን ይህ ጥናት የጉበት በሽታና የጉበት በሽታን የምያባብስ ነገሮችን አስቀዲማቹ እንድታቁት ይረዳቸዋል። የላብራቶሪ ዉጤታቹ የጤና ቺግር የምያሳይ ከሆኔ በሓክማቹዉ በኩል እናሳዉቃለን።

የጥናቱ መረጃዎች ሚስጥራዊነት ፡በጥናቱ ዉስጥ የተሰበሰቡ ማናቸውም መረጃዎች ሚስጥራዊነታቸው የተጠበቀ ይሆናል።

ከጥናቱ ስለመውጣትና ስለማቋረጥ ፡ ይህ ጥናት በፈቃደኝነት ላይ የተመሰረተ እንደመሆኑ መጠን በማንኛውም ወቅት በፈቃድዎ ከጥናቱ መዉጣት ይችላሉ። ከጥናቱ ቢወጡም እንኳን የተለመደውን የህክምና እርዳታ በጤና ተቋሙ ውስጥ በማንኛውም ጊዜ የማግኘት መብት አለዎት።

አድራሻ፡ ከጥናቱ ጋር በተያያዘ ማናቸውም ጥያቄ ቢኖርዎት በሚከተለው አድራሻ ጥያቄዎን ማቅረብ ይችላሉ። ከበደ ተሰማ(ስልክ:0921462312)

እመል: kebade072@gmail.com

ጅማ ዩኒቨርሲቲ፣

ጅማ፣ ኢትዮጵያ፡

Consent form (Amharic version)

ስለ ስምምነቱ ማረጋገጫ ፊርማ

እኔ ስሜ ከታች የተገለፀዉ የጥናቱ ተሳታፊ ለመሆን ስወስን የጥናቱን አላማዎች አሰራሮችና ቅድመሁኔታዎች በግልጽ በመረዳትና ከጥናቱ ተሳታፊነት ፈቃደኛነቴን በማንኛዉም ደረጃ የማንሳት መብቴን በማረጋጥ ነዉ።

እኔ ---------- በጥናቱ ተሳታፊ መሆኔን በፊርማዬ እያረጋገጥሁ ይህንን ስወስን በጥናቱ ሳቢያ ሊከሰቱ የሚችሉ አደጋዎች በሚገባ የተረዳሁ ና ከጥናቱ በማንኛዉም ደረጃ እራሴን ለመሰረዝ ብወስን ተገቢ የሆኑ ህክምናዎችና እገዛዎች ሁሉ እነደማይነፍጉኝ በማመን ነዉ። እነዚህ መረጃዎች ሁሉ በሚገባ በምረዳዉ ቋንቋ የተገለጸልኝ መሆኑን በፊርማዬ አረጋግጣለሁ ።

የተሳታፊው መለያ ኮድ ---------- ፊርማ---------- ቀን----------

የተመራማሪዉ ሙሉ ስም ---------- ፊርማ-------- ቀን ----------

ANNEX III: QUESTIONNAIRES (ENGLISH VERSION)

Jimma University, Institute of Health, Faculty of Health Science, School of Medical Laboratory Science

Instruction: This questionnaire is prepared to assess the serum liver enzymes and associated factors in diabetic patients. You are kindly requested to answer part I and Part II as much as possible by filling the blank space and encircling one appropriate choice from the given alternatives.

Participant Card No/ID_____

Part I. Socio-demographic characteristics

SN	Questions	Choices	Remark
1.	Gender	1. Male 2. Female	
2.	Age	_____ (year)	
3.	Place of residence	1. Urban 2. Rural	
4.	Marital status	1. Single 2. Married 3. Divorced 4. Widowed /Widower	
5.	Educational level	1. Illiterate 2. Read and Write 3. Primary school 4. Secondary school 5. College/ University	

6.	Religion	1. Muslim 2. Orthodox 3. Protestant 4. Catholic 5. Others, specify_____	
7.	Ethnicity	1. Oromo 2. Amhara 3. Gurage 4. Tigrie 5. Other, specify _____	
8.	Occupational status	1. Farmer 2. Merchant 3. Government 4. private employed 5. Unemployed 6. House wife	
9.	Physical activities	1. Never 2. Sometimes 3. Regular	

Part II: Clinical data

10.	Type of diabetes	1. T1DM 2. T1DM	
11.	Duration of diabetes	_____(in year)	
12.	Anti-diabetic medication	1. Oral hypoglycemic agents	

		2. Injection (Insulin) 3. Oral and injection	

Part III: Anthropometrics and clinical measurements

13. Height (m)_____

14. Weight (Kg)_____ BMI (Kg/m2)_____

15. Waist circumference (cm) _____

16. Hip circumference (cm) _____

17. Blood pressure

a. Systolic blood pressure (mmHg)_____

b. Diastolic blood Pressure (mmHg)_____

Part IV: Laboratory measurements

18. FBS (mg/dl)_____

19. ALT (IU/L)_____

20. AST (IU/L)_____

21. ALP (IU/L)_____

22. GGT (IU/L)_____

This is the end of the questionnaires.

Thank for your participation!

Name of data collector_____ sign_____ date _____

QUESTIONNAIRES (Afaan Oromoo)

Yuuniversitii Jimmaatti, Instituutii Fayyaa, Faakaaltii Saayinsii Fayyaa, Mana Barumsa Saayinsii Laaboraatoorii Meedikaalaa.

Qajeelfama: Uunkaan kun gaafilee qorannoo fi qorannoo waliin hidhata qabanii dha. Gaafilee kutaa I fi kutaa II hamma danda'ameetti filannoo deebii ta'u tokkotti maruun akka deebistan kabajaan isin gaafanna.

Tartiiba galmee hirmaataa_____ koodii _____

Kutaa I: Ibsa Eenyummaa

Lakk.	Gaafilee	Filannoo	Yaada
1.	Saala	1. Dhiira 2. Dhalaa	
2.	Umrii (Waggaan)		
3.	Iddoo jireenya	1. Magaalaa 2. Baadiyyaa	
4.	Haala gaa'ilaa	1. Kan hin fuune/heerumne 2. Kan fuudhe/heerumte 3. Kan wal hiikan 4. Gursummaa	
5.	Sadarkaa barnoota	1. Kan hin baranne 2. Dubbisuu fi barreessuu 3. Sadarkaa tokkoffaa 4. Sadarkaa lammaffaa 5. Kollajjii/ Yuuniversiitii	

6.	Amantaa	1. Musliima 2. Ortoodooksii 3. Piroteestaantii 4. kaatoolikii 5. Kan biraa _____	
7.	Sabummaa	1. Oromoo 2. Amaaraa 3. Guraagee 4. Tigree 5. Kan biraa----------	
8.	Haala hojii	1. Qotee bulaa 2. Daldaalaa 3. Hojjetaa Mootummaa/dhuunfaa 4. Haadha manaa 5. Kan biraa (ibsi) _____	

Kutaa II: Odeeffannoo wal'aansaa

9.	Garee dukkuba sukkaaraa	1. T1DM 2. T1DM	
10.	Erga dhukkubbichi isin qabee hagami ?	1. Waggaa 5 gadi 2. Waggaa 5-10 3. Waggaa 11-20 4. Waggaa 20 ol	
11.	Gosa qoricha fudhachaa turan	1. Kan liqimfamu 2. Kan lilmoon kennamu 3. Kan liqimfamu fi lilmoon	

		kennamu	

Kutaa III: Safara qaama fi dhiibbaa dhiigaa

12. Dheerina (m)_____

13. Ulfaatina (Kg)_____ BMI (Kg/m2)_____

14. Marsaa Mudhii (cm) _____

15. Marsaa Mo'oo (cm) _____

16. Dhiibbaa dhiigaa

    a. Systolic blood pressure (mmHg)_____

    b. Diastolic blood Pressure (mmHg)_____

Kutaa IV: Qorannoo Laaboraatorii

18. FBS (mg/dl)_____

19. ALT (IU/L)_____

20. AST (IU/L)_____

21. ALP (IU/L)_____

22. GGT (IU/L)_____

Xummurame!

Hirmaannaa keessaniif galatoomaa!

Maqaa nama odeeffannoo funaanuu_____ mallattoo_____guyyaa_____

AMHARIC VERSION QUESTIONNAIRES

ጅማ ዩኒቨርሲቲ፤ ጠና እንስቲትዩት፤ ጠና ሳይንስ ፋካልቲ፤ ሜድካል ላቦራቶሪ ትምርተ ክፍል

መመሪያ፤ ይህ ጥያቄዎች ከጥናቱ አላማ ጋር የተያያዙ ናቸዉ። እርሰዎም ትክክለኛዉን መልስ እንድሰጡን በትህትና እንጠይቆታለን። በተቸላ መጠን በጥንቃቄ ባዶ ቦታዎችን በመሙላት ወይም ከተሰጡት አማራጮች ዉስጥ ተገቢ የሆኑትን መልሶች ይምራጡ ካልሆኔ ጥያቄ ስነበብለዎት ትክክለኛዉን መልስ ይንገሩ።

የተሳታፊ ተራ ቁጥር _____

የተሳታፊ መለያ ኮድ_____ አድራሻ_____ፍርማ_____ ቀን_____

ክፍል 1.የማህበራዊና ስን-ህዝብ ባህሪያት

ተ.ቁ	ጥያቄዎች	አመራጮች	አስተያየት
1.	ፆታ	1. ወንድ 2. ሴት	
2.	ዕድሜ	በዓመት	
3.	አድራሻ	1. ከተማ 2. ገጠር	
4.	የጋቢቻ ሁኔታ	1. ያላገባ/ች 2. ያገባ/ች	
5.	የትምህርት ደረጃ	1. መደበኛ ትምህርት ያልተማረ/ች 2. መምበብና መጻፍ 3. አንደኛ ደረጃ 4. ሁለተኛ ደረጃ 5. ኮለጅ/ዩኒቨርሲቲ	
6.	ሀይማኖት	1. ሙስሊም 2. ኦርቶዶክስ 3. ፕሮቴስታንት 4. ካቶልክ 5. ለላ ከሆኔ ይጠቀሱ----------	
7.	ብሔር	1. ኦሮሞ 2. አማራ 3. ጉራጌ 4. ትግሬ 5. ለላ ከሆኔ ይጠቀሱ----------	
8.	ሥራ	1. አርሶአደር 2. ነጋዴ 3. መንግስት ሰራተኛ 4. የግል ሰራተኛ 5. ያልተቀጠረ	

		6. የቤት እመቤት	

ክፍል 2. የህክምና መስረጀ

9.	የሱካር በሽታ አይነት	1. T1DM 2. T1DM	
10.	የሱካር በሽታ ከያዘዎት ምንያህል ግዜ ይሆናል	1. ከ 5 ዓመት በታች 2. 5-10 ዓመት 3. 11-20 ዓመት 4. ከ 20 በላይ	
11.	የምወስዱት መዳንት	1. የሚዋት 2. በማርፌ 3. የሚዋትና በማርፌ	
12.	Statin የምባል መዳኒት ተጠቅሟል	1. አዎ 2. አይደላም	

ክፍል 3. የሰዎነት ልኬትና የደም ግፍት ልኬት

13. ቁመት (ሜ.)
14. ክብደት (ክ.ግ)
15. Waist circumference (cm) _____
16. Hip circumference (cm) _____
17. የደም ግፍት ልከት

    ሲስቶልክ የደም ግፍት (mmHg)_____

    ዳይስቶሊክ የደም ግፍት (mmHg)_____

ክፍል 4. የላቦራቶሪ ዉጠት

23. FBS (mg/dl)_____
24. ALT (IU/L)_____
25. AST (IU/L)_____
26. ALP (IU/L)_____
27. GGT (IU/L)_____
28. HBsAg _____
29. HCV-Ab_____

ስለትብብሩ እናመሰግናለን!

Name of data collector_____ sign_____ date _____

ANNEX IV: LABORATORY PRINCIPLES AND PROCEDURES

Cobas c311 chemistry analyzer

The Roche Diagnostic cobas C311 analyzer is automated, software-controlled analyzer for clinical chemistry analysis. It is designed for both quantitative and qualitative in vitro determinations using a large variety of tests for analysis. The cobas C311 analyzer performs photometric assays and ion selective electrode measurements and uses serum/plasma. This instrument has been designed to perform spectrophotometric measurement at 8 wavelengths: 340nm, 405nm, 450nm, 510nm, 546nm, 578nm, 630nm, 670nm, 700nm (optional). The sample disk provides 40 sample tube positions and the reagent disk provides 40 reagent bottle positions. The reaction disk holds 8 segments (80) cuvettes. This analyzer needs small sample volume (3µl-45µl) and reagent volume (30µl -450µl).

Its sophisticated software allows us to program and permanently store in the memory of your PC almost unlimited number of tests and up to 41 test profiles, calibrators and controls. The analyzer can perform end-point or equilibrium method (one or two reagents, monochromaticor dichromatic), fixed time reaction (namely, first-order kinetic method or initial rate method) and kinetic mode method (namely, zero-order kinetic or continuous-monitoring method). The analyzer provides two calibration methods: linear calibration and nonlinear calibration.

General working procedures

1. Reconstitute Activator (W2) if it has reached its 7-day expiration by warming to room temperature and reconstituting with 12 ml D.I. water and let set for 30 minutes.
2. Following the maintenance log perform the daily check
3. Perform hands on Daily Maintenance (Place analyzer in Maintenance Mode using Maintenance).
4. Place Sysclean (W1) and Activator (W2) in W1 and W2 slots on sample disk
5. Perform Daily Pipe (Push button on Maintenance log.
6. Record photometer Check Values after Daily pipe.
7. Backup and clear information every morning.
8. Check the Preventative Action box on the Maintenance Screen

9. Reagent preparing
10. Calibration and QC select
11. Remove check from Preventative Action box on the Maintenance Screen
12. Load samples in the appropriate slots on the sample disk
13. Hit Global Start and Start
14. Review Calibrations and QC
15. . Place primary tubes or pour off tubes in patient positions on the outer row with the barcodes facing out.
16. To run a STAT sample while the instrument is running, press the Pause/Scan button, then pause and wait for the green light or simply wait for the green light and load the sample in the STAT positions on the inner row with the barcode facing inward.
17. Directions to print out selected reports when interface is down
18. Retransmit the tests to the host

Sop for Glucose determination

Glucose is the central energy source of the cells in the organism. The glucose catabolism takes place via the glycolysis as the first step, followed by the citric acid cycle and oxidative phosphorylation. Glucose regulations become executive the diagnosis and course control of carbohydrate metabolism illness like the diabetes mellitus, neonatal hypoglycemia, and idiopathic hypoglycemia and with insulinomas.

Principle

D-glucose + H_2O+ O_2 $\xrightarrow{\text{Glucose Oxidase}}$ H_2O_2 + D-Gluconate

H_2O_2 + 4-AAP + phenol $\xrightarrow{\text{peroxidase(POD)}}$ Quinoneimine dye + H_2O_2

Glucose is oxidized by glucose oxidase to gluconate and hydrogen peroxide. Phenol + 4-AAP + hydrogen peroxide, in the presence of peroxidase, produce a quinoneimine dye that is measured at 500nm. The absorbance at 500nm is proportional to the concentration of glucose in the sample.

Reagents and storage

Glucose Oxidase (microbial) 12,000 u/l, Peroxidase (horseradish) > 1,000u/l, 4-AAP >0.3mM, Phenol 4mM, Buffer, pH 7.4 ± 0.1, non-reactive stabilizers, preservative. The reagent should be stored refrigerated at 2-8°C. The reagent is stable until the indicated expiration date when stored as directed

Specimen Collection and Storage

1. Non-hemolyzed serum or heparinized plasma is recommended
2. Serum must be separated from the clot promptly since the rate of glucose decrease is approximately 7% per hour in whole blood.
3. Glucose in serum is stable for twenty-four hours when stored refrigerated (2-8°C).

Interferences

1. Grossly lipemic samples may cause falsely elevated glucose values.

2. Bilirubin to the level of 20 mg/dl and Hemoglobin to a level of 500 mg/dl have both been found to exhibit negligible interference (<3%) in this assay

Calibrator: S1: H2O S2: Calibrator for Automated Systems (CFAS)

Control: PreciControl ClinChem Multi 1(PCC1) and PreciControl ClinChem Multi 2 (PCC2)

SOP for ALT determination

Summary: The enzyme alanine aminotransferase (ALT) has been widely reported as present in a variety of tissues. The major source of ALT is the liver, which has led to the measurement of ALT activity for the diagnosis of hepatic diseases. Elevated serum ALT is found in hepatitis, cirrhosis, obstructive jaundice, carcinoma of the liver, and chronic alcohol abuse. ALT is only slightly elevated in patients who have an uncomplicated myocardial infarction.

Although both serum aspartate aminotransferase (AST) and ALT become elevated whenever disease processes affect liver cell integrity, ALT is the more liver-specific enzyme. Moreover, elevations of ALT activity persist longer than elevations of AST activity.

In patients with vitamin B6 deficiency, serum aminotransferase activity may be decreased. The apparent reduction in aminotransferase activity may be related to decreased pyridoxal phosphate, the prosthetic group for aminotransferases, resulting in an increase in the ratio of apoenzyme to holoenzyme.

Test Principle: This assay follows the recommendations of the IFCC, but was optimized for performance and stability.

Serum ALT activity is measured by an enzymatic rate method. In the reaction, ALT catalyzes the reversible transamination of L-alanine and α-ketoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of NADH to NAD. The catalytic activity of alanine aminotransferase is determined by measurements of the rate of NADH oxidation in reaction. The system monitors the rate of change in absorbance at 340 nm over a fixed time interval. The rate of change in absorbance is directly proportional to the ALT activity in the sample.

L- Alanine + α- Ketoglutarate $\xrightarrow{\text{ALT}}$ Pyruvate + L- Glutamate

Pyruvate + NADH + H^+ $\xrightarrow{\text{LDH}}$ L-Lactate + NAD^+ + H_2O

Reagents, storage

R1 TRIS buffer: 224 mmol/L, pH 7.3 (37 °C); L-alanine: 1120 mmol/L; albumin (bovine): 0.25 %; LDH (microorganisms): ≥ 45 µkat/L; stabilizers; preservative

R2 2-Oxoglutarate: 94 mmol/L; NADH: ≥ 1.7 mmol/L; additives; preservative

R1 is in position B and R2 is in position C.

The reagents are ready to use. Store the reagents at 2-8°C. The reagent is stable until the expiration date appearing on the label when stored as directed.

Specimen Collection and Storage

Plasma: Li-heparin and K2-EDTA plasma. Serum

Stability: 3 days at 15-25 °C

7 days at 2-8 °C

> 7 days at (-60) - (-80) °C

Calibrator: S1: H2O S2: Calibrator for Automated Systems (CFAS)

Control: PreciControl ClinChem Multi 1(PCC1) and PreciControl ClinChem Multi 2 (PCC2)

Calibration mode: Linear

Calibration frequency: 2-point calibration, after reagent lot change, as required following quality control procedures

Quality Control: The validity of the reaction should be monitored using control sera with known normal and abnormal ALT (SGPT) values. These controls should be run at least with every shift in which ALT (SGPT) assays are performed. It is recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Measuring range: 5-700 U/L

Lower limits of measurement: Lower detection limit of the test is 5 U/L

Reference range: Males up to 41 U/L; Females up to 33 U/L

Calculated values: A factor of 1.85 is used for the conversion from 25 °C to 37 °C.

SOP for AST determination

Summary: The enzyme aspartate aminotransferase (AST) is widely distributed in tissue, principally hepatic, cardiac, muscle, and kidney. Elevated serum levels are found in diseases involving these tissues. Hepatobiliary diseases, such as cirrhosis, metastatic carcinoma, and viral hepatitis also increase serum AST levels. Following myocardial infarction, serum AST is elevated and reaches a peak 2 days after onset.

In patients undergoing renal dialysis or those with vitamin B6 deficiency, serum AST may be decreased. The apparent reduction in AST may be related to decreased pyridoxal phosphate, the prosthetic group for AST, resulting in an increase in the ratio of apoenzyme to holoenzyme.

2 isoenzymes of AST have been detected, cytoplasmic and mitochondrial. Only the cytoplasmic isoenzyme occurs in normal serum, while the mitochondrial, together with the cytoplasmic isoenzyme, has been detected in the serum of patients with coronary and hepatobiliary disease.

Principle: This assay follows the recommendations of the IFCC, but was optimized for performance and stability

Serum AST activity will be also measured by an enzymatic rate method on Cobas c311analyzer in which AST catalyzes the transfer of the amino group from L-aspartate to α-Ketoglutarate to yield oxalacetate and L-glutamate. The oxalacetate undergoes reduction with simultaneous oxidation of NADH to NAD in the malate dehydrogenase (MDH) catalyzed indicator reaction. The resulting rate of decrease in absorbance at 340nm is directly proportional to the AST activity.

L- Aspartate + α- Ketoglutarate $\xrightarrow{\text{AST}}$ Oxaloacetate + L-Glutamate

Oxaloacetate + NADH + H^+ $\xrightarrow{\text{MDH}}$ L-Malate + NAD^+ + H_2O

Reagents, storage

R1 TRIS buffer: 264 mmol/L, pH 7.8 (37 °C); L-aspartate: 792 mmol/L; MDH (microorganism): ≥ 24 µkat/L; LDH (microorganisms): ≥ 48 µkat/L; albumin (bovine): 0.25 %; preservative

R2 NADH: ≥ 1.7 mmol/L; 2-oxoglutarate: 94 mmol/L; preservative

R1 is in position A and R2 is in position B and C.

Store the reagents at 2-8 °C, On-board in use and refrigerated on the analyzer the reagent can stay for 12 weeks

Specimen Collection and Storage

1. Non-hemolyzed serum is recommended. Red cells contain AST which can give falsely elevated results.
2. Plasma: Li-heparin and K2-EDTA plasma
   Stability: 4 days at 20-25 °C; 7 days at 4-8 °C; 3 months at -20 °C.

Calibrator: S1: H2O S2: Calibrator for Automated Systems (CFAS)

Calibration mode: Linear

Calibration frequency: 2-point calibration, after reagent lot change and as required following quality control procedures

Control: PreciControl ClinChem Multi 1(PCC1) and PreciControl ClinChem Multi 2 (PCC2)

Quality Control: The validity of the reaction should be monitored using control sera with known normal and abnormal ALT (SGPT) values. These controls should be run at least with every shift in which ALT (SGPT) assays are performed. It is recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Measuring range: 5-700 U/L

Lower limits of measurement: Lower detection limit of the test is 5 U/L

Reference range: Males: up to 40 U/L; Females: up to 32 U/L

Sop for ALP determination

Summary: Alkaline phosphatase in serum consists of four structural genotypes: the liver-bone-kidney type, the intestinal type, the placental type and the variant from the germ cells. It occurs in osteoblasts, hepatocytes, leukocytes, the kidneys, spleen, placenta, prostate and the small intestine. The liver-bone-kidney type is particularly important.

A rise in the alkaline phosphatase occurs with all forms of cholestasis, particularly with obstructive jaundice. It is also elevated in diseases of the skeletal system, such as Paget's disease, hyperparathyroidism, rickets and osteomalacia, as well as with fractures and malignant tumors. A considerable rise in the alkaline phosphatase activity is sometimes seen in children and juveniles. It is caused by increased osteoblast activity following accelerated bone growth.

This assay follows the recommendations of the IFCC, but was optimized for performance and stability.

Test Principle: Serum ALP activity will be determined by measuring the rate of hydrolysis of ρ-Nitrophenyl phosphate to ρ-nitrophenol and inorganic phosphate. The rate at which the ρ-Nitrophenyl is hydrolyzed, measured at 405 nm, is directly proportional to the ALP activity.

Reagents, preparation, storage

After combining R1 and R2 as directed the reagent contains: AMP Buffer (pH 10.45), ρ-NPP ≥16mM, Magnesium ions ≥1.0mM, activators and preservatives. The reagents are ready to use. Store reagent set at 2-8°C. The reagents are stable until the expiration date if stored as directed. Protect from direct light and avoid microbial contamination. NOTE: The R2 reagent is temperature sensitive and can be affected by prolonged exposure to room temperature. Return reagent to 2-8°C as soon as possible after use.

Specimen Collection and Storage

1. Use non-hemolyzed serum (plasma should not be used since anticoagulant agents inhibit alkaline phosphatase activity).
2. Serum samples should be stored at 2-8°C and run within two days.
3. All specimen of human origin should be considered potentially infectious.

Calibrator: S1: H2O S2: Calibrator for Automated Systems (CFAS)

Control: PreciControl ClinChem Multi 1(PCC1) and PreciControl ClinChem Multi 2 (PCC2)

Sop for GGT determination

GGT measurements are used in the diagnosis and treatment of liver diseases such as alcoholic cirrhosis, and primary and secondary tumors. Elevated GGT levels appear earlier and are more pronounced than those of other liver enzymes, in cases of obstructive jaundice and metastatic neoplasms.

Principle: Serum GGT activity will be determined by modified kinetic method in which GGT catalyzes the transfer of the glutamyl group from gamma-glutamyl-3-carboxy-4-nitroanilide to glycylglycine and 5-amino-2-nitrobenzoate. The change in absorbance at 410/480 nm is due to the formation of 5-amino-2-nitrobenzoate and is directly proportional to the GGT activity in the sample.

Specimen Collection and Storage

1. Use serum only. GGT activity is inhibited by most anticoagulants.
2. It is recommended that specimen collection be carried out in accordance with sops.
3. Serum GGT is reported stable in serum for up to seven days when stored at 2-25°C, up to one month when stored at 4°C, and up to one year at (-20°C) and protected from evaporation.
4. All specimens and controls should be handled in accordance with good laboratory practices using appropriate precautions.

Stability: 7 days at 15-25^{o}C, 7 days at 2-8^{o}C, 1 year at -20^{o}C ±5°C.

Centrifuge samples containing precipitates before performing the assay. Universal precautions

REAGENTS / MATERIALS:

γ-Glutamyltransferase ver.2 Standardized against Szasz, 400 tests – the reagent cassette is labeled as GGT-2. R1 is in position B and R2 is in position C. Ready to use.

R1 - TRIS: 492 mmol/L, pH 8.25; glycylglycine: 492 mmol/L; preservative; additive

R2 - L-γ-glutamyl-3-carboxy-4-nitroanilide: 22.5 mmol/L; acetate: 10 mmol/L, pH 4.5; stabilizer; preservative

Diluent NaCl 9%, 50 mL – the diluent cassette is labeled as NACL.

Storage: γ-Glutamyltransferase ver.2 Standardized against Szasz - Store at 2-8°C.

Diluent NaCl 9% - Store at 2-8°C.

Stability: γ-Glutamyltransferase ver.2 Standardized against Szasz - Unopened at 2-8°C - up to the stated expiration date. On-board in use and refrigerated on the c311 – 12 weeks.

Diluent NaCl 9% - Unopened at 2-8°C - up to the stated expiration date. On-board in use and refrigerated on the c311– 12 weeks.

Calibrator: S1: H2O S2: Calibrator for Automated Systems (CFAS)

Control: PreciControl ClinChem Multi 1(PCC1) and PreciControl ClinChem Multi 2 (PCC2)

Quality Control

The validity of the reaction should be monitored by the use of control serums with known normal and abnormal GGT values. These controls should be run at least with every working shift in which GGT assays are performed.

Sop for Hepatitis B Surface Antigen Test

Introduction: Hepatitis B virus (HBV) is the most common cause of persistent viremia and the most important cause of chronic liver disease and hepatocellular carcinoma. HBV is a hepatotropic DNA virus. The core of the virus contains a DNA polymerase, the core antigen (HBcAg) and the e antigen (HBeAg). The core of HBV is enclosed in a coat that contains lipid, carbohydrate and protein including an antigen termed hepatitis B surface antigen (HBsAg).

HBsAg is the first marker to appear in the blood in acute hepatitis B, detectable 1 week to 2 months after exposure and 2 weeks to 2 months before the onset of symptoms. Three weeks after the onset of acute hepatitis almost half of the patients will still be positive for HBsAg. In the chronic carrier state, HBsAg persists for long periods (6-12 months) with no sero-conversion to the corresponding antibodies. Therefore, screening for HBsAg is highly desirable for all donors, pregnant women and people in high-risk groups.

The OnSite HBsAg Rapid Test detects HBsAg in serum or plasma in 15 minutes and can be performed by untrained or minimally skilled personnel without the use of laboratory equipment.

Principle: The OnSite HBsAg Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing mouse anti-HBsAg antibody conjugated with colloidal gold (HBsAg Ab conjugates) and a control antibody conjugated with colloidal gold, and 2) a nitrocellulose membrane strip containing a test line (T line) and a control line (C line). The T line is pre-coated with non-conjugated HBsAg antibody, and the C line is pre-coated with a control line antibody.

When an adequate volume of test specimen is dispensed into the sample well of the cassette, the specimen migrates by capillary action across the test cassette. HBsAg, if present in the specimen, will bind to the HBsAg Ab conjugates. The immunocomplex is then captured on the membrane by the pre-coated non-conjugated HBsAg antibody forming a burgundy colored T line, indicating a HBsAg positive test result. Absence of the T line suggests a negative result.

Reagents and Materials

1. Individually sealed foil pouches containing: one cassette device, one desiccant

2. Plastic droppers

3. One package insert (instruction for use)

4 Positive and negative Control may be required

Warnings and Precautions

For in Vitro Diagnostic Use

1. The package insert must be read completely before performing the test. Failure to follow the insert may lead to inaccurate test results.
2. Do not open the sealed pouch unless ready to conduct the assay.
3. Do not use expired devices.
4. Bring all reagents to room temperature (15-30°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolyzed blood specimens for testing.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
9. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
10. Handle the negative and positive controls in the same manner as patient specimens.
11. The test result should be read 15 minutes after a specimen is applied to the sample well or sample pad of the device. Reading the result after 20 minutes may give erroneous results.
12. Do not perform the test in a room with strong air flow, i.e. electric fan or strong air conditioning.

Reagent Preparation and Storage Instructions

All reagents are ready to use as supplied. Store unused test devices unopened at 2-30°C. If stored at 2-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit to temperatures over 30°C.

Specimen Collection and Handling

Plasma

A. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®) by venipuncture.
B. Separate the plasma by centrifugation.
C. Carefully withdraw the plasma into a new pre-labeled tube.

Serum

a) Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by venipuncture.
b) Allow the blood to clot.
c) Separate the serum by centrifugation.
d) Carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2-8°C, if not tested immediatel. Specimens can be stored at 2-8°C for up to 5 days. The specimens should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference with result interpretation.

Assay Procedure

1. Bring the specimen and test components to room temperature if refrigerated or frozen. Once thawed, mix the specimen well prior to assay.
2. When ready to test, open the pouch at the notch and remove the device. Place the test device on a clean, flat surface.

3. Be sure to label the device with the specimen ID number.
4. Fill the plastic dropper with the specimen. Holding the dropper vertically, dispense 2 drops (about 60-90 μL) of specimen into the sample well making sure that there are no air bubbles.
5. Result can be read in 15 minutes. Positive results may be visible in as soon as 1 minute. Do not read result after 20 minutes. To avoid confusion, discard the test device after interpreting the result.

Quality Control

A. **Internal Control**: This test contains a built-in control feature, the C line. The C line develops after adding the specimen. If the C line does not develop, review the entire procedure and repeat the test with a new device.
B. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to ensure the proper performance of the assay.

Interpretation of Assay Result

A. **Negative Result**: If only the C line is developed, the test indicates that the level ofHBsAg in the specimen is undetectable (lower than 1 ng/mL). The result is negative or non-reactive.
B. **Positive Result**: If both the C and the T lines are developed, the test indicates that the specimen contains HBsAg at a level equal to or higher than 1 ng/mL. The result is positive or reactive.
C. **Invalid**: If no C line is developed, the assay is invalid regardless of color development on the T line as indicated below. Repeat the assay with a new device.

Performance Characteristics of Onsite HBsAg Rapid Test

Relative Sensitivity: 100%, Relative Specificity: 100%, Overall Agreement: 100% when compared with a commercial HBsAg ELISA kit.

Limitations of Test

1. The Onsite HBsAg Rapid Test is limited to the qualitative detection of HBsAg in human serum or plasma. The intensity of the test line does not have a linear correlation with the HBsAg titer in the specimen.
2. A non-reactive test result does not preclude the possibility of exposure to or infection with HBV.
3. A non-reactive result can occur if the quantity of HBsAg present in the specimen is below the detection limits of the assay (lower than 1 ng/ml) or the HBsAg that is detected was not present during the stage of disease in which a sample is collected.
4. If the symptoms persist when the result from OnSite HBsAg Rapid Test is non-reactive, it is recommended to re-sample the patient a few days later or to test with an alternative test method.
5. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

Sop for HCV Antibody Test by HCV Rapid Test Cassette

Introduction: Hepatitis C Virus (HCV) is a small, enveloped, positive-sense, single-stranded RNA virus. HCV is now known to be the major cause of parenterally transmitted non-A, non-B hepatitis. Antibody to HCV is found in over 80% of patients with well documented non-A, non-B hepatitis.

Conventional methods fail to isolate the virus in cell culture or visualize it by electron microscope. Cloning the viral genome has made it possible to develop serologic assays that use recombinant antigens. Compared to the first generation HCV EIAs using single recombinant antigen, multiple antigens using recombinant protein and/or synthetic peptides have been added in new serologic tests to avoid nonspecific cross-reactivity and to increase the sensitivity of the HCV antibody tests.

The HCV Rapid Test Cassette (Serum/Plasma) is a rapid test to qualitatively detect the presence of antibody to HCV in a serum or plasma specimen. The test utilizes colloid gold conjugate and recombinant HCV proteins to selectively detect antibody to HCV in serum or plasma. The recombinant HCV proteins used in the test kit are encoded by the genes for both structural (nucleocapsid) and non-structural proteins.

Principle: The HCV Rapid Test Cassette (Serum/Plasma) is a qualitative, membrane based immunoassay for the detection of antibody to HCV in serum or plasma. The membrane is pre-coated with recombinant HCV antigen on the test line region of the cassette. During testing, the serum or plasma specimen reacts with recombinant HCV antigen conjugated colloid gold. The mixture migrates upward on the membrane chromatographically by capillary action to react with recombinant HCV antigen on the membrane and generate a colored line. Presence of this colored line indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear at the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

Reagents: The test cassette contains recombinant HCV antigen conjugated colloid gold and HCV antigen coated on the membrane.

Precaution

1. For professional in vitro diagnostic use only. Do not use after expiration date.
2. Do not eat, drink or smoke in the area where the specimens or kits are handled.
3. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens.
4. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
5. Humidity and temperature can adversely affect results.

Storage and Stability: The kit can be stored at room temperature or refrigerated (2-30°C). The test cassette is stable through the expiration date printed on the sealed pouch. The test cassette must remain in the sealed pouch until use. Do not freeze.

Specimen Collection and Preparation

- The HCV Rapid Test Cassette (Serum/Plasma) can be performed using either serum or plasma.
- Separate the serum or plasma from blood as soon as possible to avoid hemolysis. Only clear, non-hemolyzed specimens can be used.
- Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with federal regulations for transportation of etiologic agents.

Procedures

1. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour.
2. Place the test cassette on a clean and level surface. Hold the dropper vertically and transfer 1 drop of serum or plasma (approximately 25 µL) to the specimen well of the test cassette, then add 2 drops of buffer (approximately 80 µL) and start the timer. Avoid trapping air bubbles in the specimen well. See the illustration below.
3. Wait for the colored line(s) to appear. The test result should be read at 10 minutes. Do not interpret the result after 20 minutes

Interpretation of Results

Positive: Two distinct colored lines appear. One color line should be in the control region (C) and another color line should be in the test region (T).

Negative: One color line appears in the control region (C). No apparent red or pink line appears in the test region (T).

Invalid: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

Quality Control: Internal procedural controls are included in the test. A color line appearing in the control region (C) is an internal valid procedural control. It confirms sufficient specimen volume and correct procedural technique.

Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

Performance Characteristics

Relative sensitivity of the HCV Rapid Test Cassette is 99.1%, and the relative specificity is 99.6% when compared with commercial HCV ELISA test.

Limitations

- The HCV Rapid Test Cassette (Serum/Plasma) is for in vitro diagnostic use only. This test should be used for the detection of antibodies to HCV in serum or plasma specimen.
- The HCV Rapid Test Cassette (Serum/Plasma) will only indicate the presence of antibodies to HCV in the specimen and should not be used as the sole criteria for the diagnosis of Hepatitis C viral infection.
- As with all diagnostic tests, all results must be considered with other clinical information available to the physician.
- If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is recommended. A negative result at any time does not preclude the possibility of Hepatitis C Virus infection.

ANNEX V: DECLARATION

I, the undersigned, declare that this M.Sc. research paper is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

Name: Kebede Tessema (MSc candidate) Sign_____ Date of submission_____

This Research has been submitted with our approval as advisors.

1. Mr. Sintayehu Asaye (BSc, MSc) Sign_____ Date_____
2. Mr. Shiferaw Bekele (MSc, Assi. Prof) Sign_____ Date_____

Approval of Assessor

Name _____Sign_____ Date_____

Name _____Sign_____ Date_____

Head of Department

Name _____Sign_____ Date_____