

**بسم الله الرحمن الرحيم**

**Sudan University of Science and Technology**

**College of Graduate Studies**

**Assessments of liver enzymes (AST, ALT and ALP) activity  
in Sudanese patients with type 2 diabetes mellitus**

**تقييم نشاط انزيمات الكبد الالانين ترانساميناس , الاسبارتات ترانساميناس و الفوسفاتيز  
القاعدي لدى السودانين المصابين بمرض السكري من النوع الثاني**

**A dissertation submitted in partial fulfillment for the requirements  
of M.Sc degree in Clinical Chemistry**

**By:**

**Rania Mirghani Ahmed**

**B.Sc (Clinical Chemistry)**

**University of Science and Technology (2002)**

**Supervisor:**

**Dr. Khalda Mirghani Hamza**

## الآية

قال تعالى :

بسم الله الرحمن الرحيم

{ يَا أَيُّهَا الَّذِينَ آمَنُوا إِذَا قِيلَ لَكُمْ تَفَسَّحُوا فِي الْمَجَالِسِ فَافْسَحُوا  
يَفْسَحِ اللَّهُ لَكُمْ <sup>ط</sup> وَإِذَا قِيلَ انشُرُوا فَانْشُرُوا يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا  
مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ <sup>ج</sup> وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ }

صدق الله العظيم

سورة المجادلة

الآية (11)

*Dedication*

*A lot of thanks for my God*

*To My father,*

*To My mother,*

*To My beloved Nephew,*

*To My Husband*

*To all My Family*

*And My supervisor*

## **Acknowledgement**

All praise to “ALLAH”, the lord of the world, the Almighty, with whose gracious help it was possible to accomplish this work may peace and blessing be upon Mohammed the last of the Messengers.

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## **List of Abbreviations**

<b>ALP</b>	<b>alkaline phosphatase</b>
<b>ALT</b>	<b>alanine aminotransferase</b>
<b>AST</b>	<b>aspartate aminotransferase</b>
<b>BMI</b>	<b>body mass index</b>
<b>DM</b>	<b>diabetes mellitus</b>
<b>GGT</b>	<b>gamma glutamil transferees</b>
<b>LDH</b>	<b>lactate dehydrogenase</b>
<b>MDH</b>	<b>malate dehydrogenase</b>
<b>NADH</b>	<b>nicotinamide adenine (coenzyme)</b>

## **Abstract**

This is a case control study carried out to assess the activity of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase (AST, ALT and ALP) liver enzymes. The study was conducted in Omdurman Teaching Hospital from August to October 2014. A hundred type 2 diabetic patients and forty apparently healthy individuals were included in the study. Age of selected group ranged between 30–60 years, both males and females were included. Five ml of venous blood was collected from each participant in heparinized containers. Automatic analyzer Mindray (ACCENT-200) was used for determination of AST, ALT and ALP liver enzymes activity.

Duration of diabetes ranged between 5 and 20 years. ALT and ALP significantly increased in patients compared to control, AST insignificantly ( $P$ -value = 0.1). The diabetic group had no significant difference in three liver enzymes (AST, ALT and ALP) in males compared to females.

According to the disease duration and AST, ALT the correlation was significantly increased with weak positive correlation, ( $P$ -value = 0.04 and 0.02) ( $r$  = 0.2 and 0.2) respectively, ALP showed insignificant differences, ( $P$ -value = 0.2)( $r$ =0.1) .

It is concluded that diabetes mellitus is one of the causes of increased plasma level of AST, ALT and ALP liver enzymes, this fact should be taken in consideration, and liver function test should be performed regularly to diabetic patients.

## مستخلص الدراسة

هذه دراسة الحالات المراقبة لتقييم نشاط انزيمات الكبد الالانين ترانساميناس , الاسبارتات ترانساميناس و الفوسفاتيز القاعدي . تمت الدراسة في مستشفى ام درمان التعليمي في الفترة ما بين اغسطس الى اكتوبر 2014. مئمن مرضى السكري من النوع الثاني و اربعون مشاركون اصحاء تم اختيارهم للدراسة . تتراوح اعمار مجموعة الدراسة بين 30 الى 60 عاما . شملت الدراسة الذكور و الاناث معا . اخذت 5 مل من عينة الدم من كل مشارك وضعت في انبوب اختبار محتوية على الهيبيرين .جهاز مندرى اكسنت 200 تم استخدامه لقياس نشاط الانزيمات الالانين ترانساميناس , الاسبارتات ترانساميناس و الفوسفاتيز القاعدي .

فتره الاصابة بمرض السكري تتراوح بين خمسة الى عشرون عاماً . الالانين ترانساميناس و الفوسفاتيز القاعدي انزيمات الكبد اظهرت ارتفاعا ذات دلالة احصائية عند المرضى مقارنة بمجموعة الضبط. الاسبارتات ترانساميناس لم يظهر فروقات ذات دلالة احصائية بالمقارنة مع مجموعة الضبط . لا توجد فروقات ذات دلالة احصائية في الانزيمات الثلاثة عند الذكور مقارنة بالاناث.

وفق زمن الاصابة بالمرض الاسبارتات ترانساميناس و الالانين ترانساميناس اظهرت علاقه موجبه ضعيفه ذات دلالة احصائية (r = 0.2 and 0.2) (P.value = 0.04 and 0.02) على التوالي. الفوسفاتيز القاعدي لم يظهر فروقات ذات دلالة احصائية (r = 0.1) (P.value = 0.2) .

خلاصة الدراسة اوضحت ان مرضى السكري من النوع الثاني أحد أسباب ارتفاع في انزيمات الكبد الالانين ترانساميناس, الاسبارتات ترانساميناس والفوسفاتيز القاعدي, لذلك يجب قياس هذه الانزيمات بانتظام خاصه عند مرضى السكري من النوع الثاني.

# ***CHAPTER ONE***

## ***Introduction and Literature Review***

# **CHAPTER ONE**

## **1. Introduction and Literature Review**

### **1.1 Introductions**

Diabetes mellitus is one of health problem worldwide uncontrolled blood glucose level Lead to diabetic complication such as nephropathy, retinopathy, neuropathy and arteriosclerosis. Liver enzymes are also affected by complications of diabetes mellitus, and changes in their activates may lead to metabolic disorders.

Type 2 diabetes is associated with a many of liver disorders including elevated liver enzymes, fatty liver disease, cirrhosis, hepatocellular carcinoma, and acute liver failure. Liver plays a major role in the regulation of carbohydrate homeostasis. Hepatocellular glycogen accumulation leads to hepatomegaly and liver enzyme abnormalities in poorly controlled diabetic patients. In hyperglycemic condition, there will be an intracellular glycogen accumulation in the hepatocytes due to increased glycogen synthesis, causing mild to moderately elevated aminotransferases, with or without mild elevations of ALP. All these biochemical disturbances associated with hepatomegaly are found to be reversible with good glycemic control (Elmahi and Abdrabo, 2014).

Liver can be affected by steatosis or accumulation of fat, a condition known as non-alcoholic fatty liver disease (NAFLD). It is a well-recognized complication of diabetes with frequency of 40–70%. The most common clinical finding is hepatomegaly, with normal or only mildly elevated transaminases. These changes are not reversible with sustained glucose control (Elmahi and Abdrabo, 2014).

According to previous effects of high concentration of blood glucose on liver, this study was conducted to assess liver enzyme which may reflect complications of type 2 diabetes mellitus.

## **1.2 Literature Review**

### **1.2.1 Diabetes Mellitus**

Diabetes mellitus is a clinical syndrome associated with an abnormally high plasma glucose concentration, either when fasting or after ingestion of carbohydrate, and is often accompanied by the presence of glucose in the urine, from which the name of the condition is derived. It may result from diminished insulin production by the  $\beta$ -cells of the langerhans, but this is not always the case and hence diabetes is of heterogeneous origin. It occurs with differing degrees of severity and in its severest form causes ketoacidosis, coma and ultimately death if not treated. At the other extreme, it may be present without clinical symptoms. However, for most diabetes patients, the long term sequelae are the greatest problem and include nephropathy, retinopathy, neuropathy and arteriosclerosis (Gowenlock, 1988).

#### **1.2.1.1 Type of diabetes mellitus**

**Type 1 diabetes** is characterized by inappropriate hyperglycemia primarily a result of pancreatic islet-cell destruction and a tendency to ketoacidosis. Type 1 diabetes mellitus is a result of cellular-mediated autoimmune destruction of the cells of the pancreas, causing an absolute deficiency of insulin secretion. Upper limit of 110 mg/dL on the fasting plasma glucose is designated as the upper limit of normal blood glucose (Bishop *et al.*, 2001).

**Type 2 diabetes mellitus** Is characterized by hyperglycemia as a result of an individual's resistance to insulin with an insulin secretory defect. This resistance results in a relative, not an absolute, insulin deficiency. Type 2 constitutes the majority of the diabetes cases. Most patients in this type are obese or have an increased percentage of body fat distribution in the abdominal region. This type of

diabetes often goes undiagnosed for many years and is associated with a strong genetic predisposition, with patients at increased risk with an increase in age, obesity, and lack of physical exercise. Characteristics usually include adult onset of the disease and milder symptoms than in type 1, with ketoacidosis seldom occurring. However, these patients are more likely to go into a hyperosmolar coma and are at an increased risk of developing macrovascular and microvascular complications (Bishop *et al* ., 2001) .

**Other specific types of diabetes** are associated with certain conditions (secondary), including genetic defects of cell function or insulin action, pancreatic disease, diseases of endocrine origin, drug- or chemical-induced insulin receptor abnormalities, and certain genetic syndromes. The characteristics and prognosis of this form of diabetes depend on the primary disorder. Maturity-onset diabetes of youth (MODY) is a rare form of diabetes that is inherited in an autosomal dominant fashion (Bishop *et al* ., 2001) .

**Gestational diabetes mellitus (GDM)** is “any degree of glucose intolerance with onset or first recognition during pregnancy.”<sup>4</sup> Causes of GDM include metabolic and hormonal changes. Patients with GDM frequently return to normal postpartum. However, this disease is associated with increased perinatal complications and an increased risk for development of diabetes in later years. Infants born to mothers with diabetes are at increased risk for respiratory distress syndrome, hypocalcemia, and hyperbilirubinemia. Fetal insulin secretion is stimulated in the neonate of a mother with diabetes. However, when the infant is born and the umbilical cord is severed, the infant’s oversupply of glucose is abruptly terminated, causing severe hypoglycemia (Bishop *et al* ., 2001) .

### **1.2.1.2 Causes of type 2 diabetes mellitus:**

Type 2 diabetes is characterized by decline in insulin action due to the resistance of tissue cells to the action of insulin. The problem is intensified by the inability of the beta cells of the pancreas to produce enough insulin to counteract the resistance. Thus, type 2 diabetes is a disorder of both insulin resistance and relative deficiency of insulin. Insulin resistance syndrome, also known as metabolic syndrome and syndrome X, affects the metabolism of many nutrients, including glucose, triglycerides, and high-density lipoprotein (HDL) cholesterol. Individuals who are diagnosed with metabolic syndrome may show abdominal obesity and high blood Pressure. Such individuals are at increased risk for cardiovascular disease. The etiology of type 2 diabetes is complex and multifaceted. There is evidence to show that there is an association of obesity with the development of type 2 diabetes. Other factors, such as family history of type 2 diabetes and lack of physical activity, have also been associated with the disorder. Previous diagnosis of gestational diabetes is a risk factor for type 2 diabetes, as are increasing age, hypertension, and dyslipidemia. Increased risk for developing the disease is also associated with membership in certain racial and ethnic groups, such as African-Americans, Hispanic-Americans, Native Americans, Asian-Americans, and Pacific Islanders (Arneson *et al* ., 2007) .

### **1.2.1.3 Signs and symptoms**

The classic symptoms of untreated diabetes are weight loss, polyuria (frequent urination), polydipsia (increased thirst), and polyphagia (increased hunger). Symptoms may develop rapidly (weeks or months) in type 1 diabetes, while they usually develop much more slowly and may be subtle or absent in type 2 diabetes. Several other signs and symptoms can mark the onset of diabetes, although they are not specific to the disease. In addition to the known ones above, they include



blurry vision, headache, fatigue, slow healing of cuts, and itchy skin. Prolonged high blood glucose can cause glucose absorption in the lens of the eye, which leads to changes in its shape, resulting in vision changes. A number of skin rashes that can occur in diabetes are collectively known as diabetic dermadromes (Polonsky, 2012) .

#### **1.2.1.4 Complications**

All forms of diabetes increase the risk of long-term complications. These typically develop after many years (10–20), but may be the first symptom in those who have otherwise not received a diagnosis before that time. The major long-term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease and about 75% of deaths in diabetics are due to coronary artery disease. Other "macrovascular" diseases are stroke, and peripheral vascular disease. The primary microvascular complications of diabetes include damage to the eyes, kidneys, and nerves. Damage to the eyes, known as diabetic retinopathy, is caused by damage to the blood vessels in the retina of the eye, and can result in gradual vision loss and potentially blindness. Damage to the kidneys, known as diabetic nephropathy, can lead to tissue scarring, urine protein loss, and eventually chronic kidney disease, sometimes requiring dialysis or kidney transplant. Damage to the nerves of the body, known as diabetic neuropathy, is the most common complication of diabetes. The symptoms can include numbness, tingling, pain, and altered pain sensation, which can lead to damage to the skin. Diabetes-related foot problems (such as diabetic foot ulcers) may occur, and can be difficult to treat, occasionally requiring amputation. Additionally, proximal diabetic neuropathy causes painful muscle wasting and weakness. There is a link between cognitive deficit and diabetes. Compared to those without diabetes, those with the disease have a 1.2 to 1.5-fold greater rate of decline in cognitive function (Polonsks , 2012)

**Acute Complications:** Hypoglycaemia, hypo, is a blood glucose level (BGL) less than 4mmol and is most common in people who use insulin or are taking insulin stimulating tablets. It can lead to a medical emergency if not treated promptly. Therefore, people at risk of hypos need to be aware of the signs and symptoms and the treatment .

**Hyperglycaemia:** Acute hyperglycaemia is blood glucose levels (BGLs) above 15mmol/L over a short period with very high blood glucose levels are of medical emergency.

**Chronic Complications:** High blood glucose level over a long period of time will cause damage to the large blood vessels in the heart, brain and feet and smaller blood vessels supplying eyes, kidneys and nerves. However, it is just as important to maintain a healthy blood pressure and cholesterol level to reduce risk of complications (Park , 2011) .

**Vascular disease:** Over time blood vessel diseases can affect the heart, brain and feet. These are known consecutively as cardiovascular, cerebrovascular and peripheral vascular disease.

**Kidneys:** The kidneys act as a filter for the blood and help to excrete waste products. High blood glucose levels and blood pressure increase the risk of long term damage to the kidneys. Kidney function is measured by blood and urine tests which are done at diagnosis and at least every 12 months thereafter ( Park , 2011) .

**Eyes:** High blood glucose levels over time can damage the tiny blood vessels at the back of the eye. These damaged vessels can cause loss of vision if they bleed or become blocked. This is referred to as diabetic retinopathy. Symptoms may not occur until damage is well advanced, so regular screening is vital. Early detection

of eye damage can often be treated successfully by laser treatment. Patients with diabetes are also at greater risk of developing cataracts and glaucoma (raised pressure in the eye). Eyes should be checked by an optometrist or eye specialist at diagnosis and every one to two years depending on existing problems (Park , 2011) .

**Nerves:** Nerve damage is referred to as neuropathy. There are two types of nerve damage associated with diabetes: Peripheral neuropathy – abnormal sensations, for example, numbness, burning, pins and needles or pain in the feet, legs, and arm. Autonomic neuropathy – damage to the nerves that control the intestinal tract, bladder and genitals causing delayed stomach emptying, diarrhoea, constipation, bladder problems and erectile dysfunction. Screening for autonomic neuropathy will be a part of complications assessment (Park , 2011) .

**Liver:** Liver function can be affected by a condition known as Non Alcoholic Fatty Liver Disease (NAFLD). This is associated with insulin resistance and increased waist measurement, both of which are risk factors for type 2 diabetes (Park , 2011) .

### **1.2.1.5 Insulin hormone :**

Insulin is the principal hormone affecting blood glucose levels, and an understanding of its action is an important prerequisite to the study of diabetes mellitus. Insulin is the small protein synthesized in the beta cells of the islets of langerhans of the pancreas. It acts through membrane receptor and its main target tissues are liver, muscle and adipose tissue. The overall effect of insulin is to promote cellular uptake and storage of metabolic. It should be noted that glucose cannot enter the cells of most body tissues in the absence of insulin (GAW *et al* ., 1999) .

### **1.2.3 Liver anatomy and physiology :**

The liver is a large and complex organ weighting approximately 1.2–1.5 kg in the healthy adult. It is located beneath and is attached to the diaphragm, it is protected by the lower rib cage, and is held in place by ligamentous attachments. Despite the functional complexity of the liver, it is relatively simple in structure. It is divided unequally into two lobes by the falciform ligament, with the right lobe being approximately six times larger than the left lobe. There is no known functional difference between the lobes, and communication flows freely between all areas of the liver. Unlike most organs, which have a single blood supply, the liver is an extremely vascular organ that receives its blood supply from two sources: the hepatic artery and the portal vein. The hepatic artery, a branch of the aorta, supplies oxygen-rich blood from the heart to the liver and is responsible for providing approximately 25% of the total blood supply to the liver. The portal vein supplies nutrient-rich blood (collected as food is digested) from the digestive tract, and it is responsible for providing approximately 75% of the total blood supply to the liver. The two blood supplies eventually merge and flow into the sinusoids, which course between individual hepatocytes. Approximately 1,500 ml of blood passes through the liver per minute. The liver is drained by a collecting system of veins that empties into the hepatic veins and ultimately into the inferior vena cava. The excretory system of the liver begins at the bile canaliculated. The bile canaliculi are small spaces between the hepatocytes that form intrahepatic ducts, where excretory products of the cell can drain. The intrahepatic ducts join to form the right and left hepatic ducts, which drain the secretions from the liver (Bishop *et al .*, 2001).

The right and left hepatic ducts merge to form the common hepatic duct, which is eventually joined with the cystic duct of the gallbladder to form the common bile

duct. Combined digestive secretions are then expelled into the duodenum (Bishop *et al.*., 2001) .

### **1.2.3.1 Tests for liver function**

The clinical laboratories offer several tests for assessment of liver function. The enzymes alkaline phosphatase, ALT, AST, GGT, and 5'-nucleotidase are helpful in the assessment of the proper functioning and inflammatory status of the liver. since the liver is the site for metabolism of carbohydrate, protein, and lipids, as well as for the synthesis of many proteins, the conjugation of bilirubin, and detoxification of drugs and other substances, the liver may be assessed by measurement of total and direct bilirubin, total protein and albumin, cholesterol and triglycerides, and urea and ammonia. In this case, increased levels of enzymes and bilirubin and lowered protein are correlated with liver diseases. The extent of the increased alkaline phosphatase and the presence of high level in both total and direct bilirubin help to specify this liver disorder as obstructive jaundice (Arneson, *et al.*., 2007) .

### **1.2.3.2 Pathophysiology of liver enzymes**

Enzymes analysis is used to aid in diagnosis and treatment of diseases. In particular, enzymes that are synthesized within cellular organelles and carry out their functions within cells and are released into body fluids when those cells are damaged. Thus, an increase in enzyme activity when compared to the reference range can indicate pathological changes in certain types of cells and tissues. Enzymes activity levels in body fluids can reflect leakage from cells due to cellular injury, or changes in enzyme production rate or actual enzyme induction due to metabolic or genetic states or proliferation of neoplasm's. Some enzymes are present in the plasma due to tissue necrosis or inflammation and increase at such a

slow rate that they are not useful for early detection or treatment of the disease. Other enzymes rapidly decline in circulation because of inactivation or metabolism. The clinical utility of enzyme activity in relationship to specific tissue pathology and clinical signs is enhanced when the enzyme activity quickly rises following the onset of the disorder and remains elevated for an adequate time frame, particularly when other clinical signs and symptoms are not sufficient to provide diagnosis. Damage to tissue can release different types of enzymes based on their location. For example, mild inflammation of the liver reversibly increases the permeability of the cell membrane and releases cytoplasmic enzymes such as lactate dehydrogenase (LD), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), while cellular death (necrosis) will release mitochondrial sources of ALT and AST. Distributions of these enzymes within specific types of hepatic tissues varies. ALP and gamma glutamyl transaminase (GGT) are more concentrated in the biliary ducts or tissues of the small ducts (canaliculi), while AST, ALT, and LD are present mainly in structural (parenchymal) hepatic cells. Multiple forms of enzymes, called isoenzymes, are distributed in several different tissue types. For example, ALP is found in hepatobiliary tissues but also present in all cytoplasmic membranes of all cells of the body, especially in osteoblasts (bone forming cells), intestinal mucosa, placenta, and renal tubules (Arneson, *et al.* , 2007) .

#### **1.2.4 Enzymes**

Liver enzymes play an important role in the assessment of liver function because injury to the liver resulting in cytolysis or necrosis will cause the release of enzymes into circulation. Enzymes also play an important role in differentiating hepatocellular (functional) from obstructive (mechanical) liver diseases, which is an important clinical distinction because failure to identify an obstruction will

result in liver failure if the obstruction is not rapidly treated. Although many enzymes have been identified as useful in the assessment of liver functions, the most clinically useful enzymes are aminotranferases (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]), the phosphates (alkaline phosphatase [ALP] and 5-neucleotidase), -glutamyl transferees (GGT), and lactate dehydrogenase (Bishop *et al* ., 2001) .

#### **1.2.4.1 Aminotransferases**

The most two common aminotranferases assessed in clinical laboratories are AST (formerly referred to as serum glutamic-oxaloacetic transaminase [SGOT]) and ALT (formerly referred to as serum glutamic-pyruvic transaminase [SGPT]). The aminotranferases are responsible for catalyzing the conversion of aspartate and alanine to oxaloacetate and pyruvate, respectively. In the absence of acute necrosis or ischemia of other organs, these enzymes are most useful in the detection of hepatocellular (functional) damage to the liver. ALT is present mainly in the liver (lesser amounts in skeletal muscle and kidney), whereas AST is widely distributed in equal amounts in the heart, skeletal muscle, and liver, making ALT a more “liver-specific” marker than AST. Regardless, activity of both transaminases rises rapidly in almost all liver diseases and may remain elevated for up to 2–6 weeks. The highest levels of AST and ALT are found in acute conditions such as viral hepatitis, drug- and toxin-induced liver necrosis, and hepatic ischemia (Bishop *et al.*, 2001).

The increase in ALT activity is usually greater than that for AST. Only moderate increases are found in less severe conditions. AST and ALT are found to be normal or only mildly elevated in cases of obstructive liver damage. Because AST and ALT are present in other tissues beside the liver, elevations in these enzymes may

be a result of other organ dysfunction or failure such as acute myocardial infarction, renal infarction, progressive muscular dystrophy, and those conditions that result in secondary liver disease such as infectious mononucleosis, diabetic ketoacidosis, and hyperthyroidism. It is often helpful to conduct serial determinations of aminotransferases when following the course of a patient with acute or chronic hepatitis, and caution should be used in interpreting abnormal levels because serum transaminases may actually decrease in some patients with severe acute hepatitis, owing to the exhaustive release of hepatocellular enzymes (Bishop *et al.*, 2001) .

Transaminases are widely distributed throughout the body AST is found primarily in the heart, liver, skeletal muscle, and kidney. ALT is found primarily in the liver and kidney. ALT is exclusively cytoplasmic; both mitochondrial and cytoplasmic forms of AST are found in cells. These are genetically distinct is enzymes with a dimeric structure composed of two identical polypeptide subunits of about 400 amino acid residues. About 5% to 10% of the AST activity in serum from healthy individuals is of mitochondrial origin (Burtis *et al.* , 2008) .

**Clinical Significance of AST and ALT:** Liver diseases are the most important cause of increased transaminase activity in serum. In most types of liver diseases, ALT activity is higher than that of AST. Exceptions may be seen in alcoholic hepatitis, hepatic cirrhosis, and liver neoplasia. In viral hepatitis and other forms of liver diseases associated with acute hepatic necrosis, serum AST and ALT concentrations are elevated even before the clinical signs and symptoms of disease (such as jaundice) appear. Activities for both enzymes may reach values as high as 100 times the upper reference limit, although ten fold to forty fold elevations are most frequently encountered. Peak values of aminotransferase activity occur between the seventh and twelfth days. Activities then gradually decrease, reaching



normal activities by the third to fifth week if recovery is uneventful. Peak activities bear no relationship to prognosis and may fall with worsening of the patient's condition (Burtis *et al.*, 2008) .

Persistence of increased ALT for more than 6 months after an episode of acute hepatitis is used to diagnose chronic hepatitis. Most patients with chronic hepatitis have maximum ALT less than seven times the upper reference limit. ALT may be persistently normal in 15% to 50% of patients with chronic hepatitis C. In patients with acute hepatitis C, ALT should be measured periodically over the following 1 to 2 years to determine if its activity returns to normal. In acetaminophen-induced hepatic injury, the aminotransferase peak is more than 85 times the upper reference limit in 90% of cases, a value rarely seen with acute viral hepatitis. Furthermore, AST and ALT activities typically rises early and fall rapidly. Other than viral and alcoholic hepatitis, nonalcoholic steatohepatitis is the most common cause of aminotransferase elevation (Burtis *et al.* , 2008).

Increased aminotransferase concentrations have been observed in extrahepatic cholestasis, with activities tending to be higher the more chronic the obstruction. The aminotransferase activities observed in cirrhosis vary with the status of the cirrhotic processes and range from the upper reference limit to four to five times higher, with an AST/ALT ratio greater than 1. The ratio's elevation can reflect the grade of fibrosis in these patients. This appears to be attributable to a reduction of ALT production in a damaged liver. Twofold to fivefold elevations of both enzymes occur in patients with primary or metastatic carcinoma of the liver, with AST usually being higher than ALT, but activities are often normal in the early stages of malignant infiltration of the liver. Slight or moderate elevations of both AST and ALT activities have been observed after administration of various medications. Although serum activities of both AST and ALT become elevated

whenever disease processes affect liver cell integrity, ALT is the more liver-specific enzyme. Serum elevations of ALT activity are rarely observed in conditions other than parenchymal liver disease. Moreover, elevations of ALT activity persist longer than do those of AST activity. After acute myocardial infarction, increased AST activity appears in serum. AST activity also increases in progressive muscular dystrophy and dermatomyositis, reaching concentrations up to eight times the upper reference limit. They are usually within the reference interval in other types of muscle diseases, especially in those of neurogenic origin. Slight to moderate AST elevations are noted in hemolytic disease. Several studies have described AST linked to immunoglobulins, or macro-AST ( Burtis *et al* ., 2008) .

Reference Range ,AST = 5 to 30 U/L (37°C ) ,ALT = 6 to 37 U/L (37°C ) (Bishop *et al* ., 2001) .

#### **1.2.4.2 Phosphatases: Alkaline Phosphatase**

The ALP family of enzymes is zinc metalloenzymes that are widely distributed in all tissues; however, highest activity is seen in the liver, bone, intestine, kidney, and placenta. The clinical utility of ALP lies in its ability to differentiate hepatobiliary disease from estrogenic bone disease. In the liver, the enzyme is localized into the microvilli of the bile canaliculi, and therefore it serves as a great marker of extrahepatic biliary obstruction, such as a stone in the common bile duct, or in intrahepatic cholestasis, such as drug cholestasis or primary biliary cirrhosis. ALP is found in very high concentrations in cases of extrahepatic obstruction with only slight to moderate increases seen in those with hepatocellular disorders such as hepatitis and cirrhosis. Because bone is also a source of ALP, it may be elevated in bone-related disorders such as Paget's disease, bony metastases, diseases

associated with an increase in osteoblastic activity, and rapid bone growth during puberty. ALP is also found elevated in pregnancy due to its release from the placenta, where it may remain elevated up to several weeks post delivery. As a result, interpretation of ALP concentrations is difficult because enzyme activity of ALP can increase in the absence of liver damage (Bishop *et al* ., 2001) .

ALP activity is present in most organs of the body and is especially associated with membranes and cell surfaces located in the mucosa of the small intestine and proximal convoluted tubules of the kidney, in bone (osteoblasts), liver, and Placenta. Although the exact metabolic function of the enzyme is not understood, it appears that ALP is associated with lipid transport in the intestine and with the calcification process in bone. ALP exists in multiple forms, some of which are true isoenzymes, encoded at separate genetic loci. The bone, liver, and kidney ALP forms share a common primary structure coded for by the same genetic locus, but they differ in carbohydrate content (Burtis *et al* ., 2008) .

**Clinical Significance:** Elevations in serum ALP activity is commonly originated from the liver and bone. Consequently, serum ALP measurements in the investigation of hepatobiliary disease and bone diseases associated with increased osteoblastic activity are recommended (Burtis *et al* ., 2008) .

**Hepatobiliary Disease:** The response of the liver to any form of biliary tree obstruction induces the synthesis of ALP by hepatocytes. Some of the newly formed enzyme enters the circulation to increase the enzyme activity in serum. The elevation tends to be more notable (greater than three fold) in extrahepatic obstruction than in intrahepatic obstruction and is greater the more complete the obstruction. Serum enzyme activities may reach 10 to 12 times the upper reference limit and usually return to normal on surgical removal of the obstruction. A similar

increase is seen in patients with advanced primary liver cancer or widespread secondary hepatic metastases. Liver diseases that principally affect parenchymal cells, such as infectious hepatitis, typically show only moderately (less than three fold) increased or even normal serum ALP activities. Increases may also be seen as a consequence of a reaction to drug therapy. Intestinal ALP isoenzyme, an asialoglycoprotein normally cleared by the hepatic asialoglycoprotein receptors, is often elevated in patients with liver cirrhosis (Burtis *et al.* , 2008) .

An increase of up to two to three times the upper reference limit is observed in women in the third trimester of pregnancy, with the additional enzyme being of placental origin. There are also reports of a benign familial elevation in serum ALP activity because of increased concentrations of intestinal ALP. Transient, benign increases in serum ALP may be observed in infants and children, with changes often more than 10 times the upper reference limit. Increases in both the liver and the bone form are seen. These changes seem to reflect a reduction in the removal of ALP from blood caused by transient modifications of enzyme glycosylation (Burtis, *et al.* , 2008) .

Reference Range ,ALP = 30 to 90 U/L (30°C ) (Bishop *et al.* , 2001) .

### **1.2.5 Previous study**

Type 2 diabetes patients have been reported to be associated with higher incidence of abnormal liver function tests (LFT) compared to the individuals without diabetes, elevated ALT being the most common abnormal enzyme. A cross sectional study from Iran ,Meybodi *et al.*, (2008) demonstrated a rise of ALT and AST of type 2 diabetic patients. In UK a cohort study, Gonem *et al.*, (2007) showed that ALT and ALP were elevated. According to a study in Sudan, Idris *et al.*, (2011) the means of ALT, AST were reported to be significantly higher among

diabetic patient compared to control. Among the Iran diabetic population, Meybodi *et al.*, (2008) although raised ALT was seen with increasing age. . The risk of higher ALT was significantly lower with longer duration of diabetes. Erbey *et al.*,(2000) who reported that the prevalence of elevated ALT levels among the U.S. type 2 diabetics and this prevalence was higher among obese (BMI > 25kg/m<sup>2</sup>) than non-obese diabetics .In Sudan, Idris *et al.*, (2011) high serum alanine aminotransferase level is greater among type 2 diabetic patient, overweight or obese and men. The elevated ALT levels were significantly related to a BMI of > 25 kg / m<sup>2</sup> among the type 2 diabetics (p=0.0103) . In a study conducted in India, Prashanth *et al.*, (2009) positive correlation was also reported between glycaemic control (FBS and PPBS) and duration of diabetes mellitus with the ALT levels. Han Ni *et al.*,( 2012) raised ALT and AST are more common among the diabetes patients .

### **1.3 Rationale :**

Uncontrolled plasma glucose level of diabetic patients may cause many complications with progression of the disease . The liver is a vital metabolic organ may also be affected with diabetes mellitus previous studies revealed significant correlation between liver enzymes AST, ALT and ALP and type 2 diabetes mellitus Odewabi *et al* (2013), was Observed ALT and ALP of the patients with type 2 DM are significantly elevated relative to control subjects . Few studies regarding this problem was conducted in Sudan ,so this study was carried to assess liver enzyme activity among Sudanese diabetic patients and to find out the most affected enzyme , so as to include it in routine liver function test to diabetes patients .

## **1.4 Objectives**

### **1.4.1 General Objectives:**

Assessments of plasma levels of (AST, ALT and ALP) liver enzymes in Sudanese patients with type 2 diabetes mellitus.

### **1.4.2 Specific Objectives:**

1. To measure the activity of aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and alkaline phosphatase (ALP) liver enzymes in type 2 diabetic patients and control.
2. To compare between the levels of three liver enzymes according to gender.
3. To correlate between the levels of three liver enzymes activity and duration of the diseases in type 2 diabetic patients.

***CHAPTER TWO***  
***Materials and Methods***



## **CHAPTER TWO**

### **2. Materials and Methods:**

#### **2.1 Materials:**

**2.1.1 Study design:** This is case control study conducted from August to October 2014, in Omdurman Teaching Hospital.

#### **2.1.2 Study population:**

The study included 100 patients already diagnosed to have type 2 diabetes mellitus and 40 apparently healthy subjects selected as control.

#### **Inclusion Criteria:**

Type 2 diabetic patients with age ranged between 30-60 years, both males and females were included.

#### **Exclusion criteria:**

Types 2 diabetic patients with other diseases which may affect activity of liver enzymes under study .

#### **2.1.3 Ethical consideration:**

Subjects who voluntarily accepted to participate in the study were included.

#### **2.1.4 Sample collection:**

Five ml of venous blood was collected from each subject in heparinized containers.

Blood sample was centrifuged for 3 minutes at 3000 rpm to obtain clear plasma.

### 2.1.5 Equipments:

Automatic analyser (mindray ) ACCENT-200, centrifuge, heparinized containers, disposable syringes, fluoride oxalate containers, tourniquets .

### Requirements :

70% alcohol, cotton .

### 2.1.6 Reagents:

1) Reagents of aspartate aminotransferase:

Package:

1-reagent                 $2 \times 35$  ml

2- Reagent               $1 \times 17.5$  ml

The reagent when stored at 2-8°C are stable up to expiry date printed on the package. Stability on board of the analyser at 2-10°C: ACCENT-200 expired 10 weeks after use .

### Concentrations in the test

Tris (pH 7.8)	80 mmol/l
L-aspartate	240 mmol/l
MDH	$>10 \mu\text{kat/l}$
LDH	$>20 \mu\text{kat/l}$
2-oxoglutarate	15 mmol/l
NADH	0.18 mmol/l
Sodium hydroxide	$< 1 \%$

## 2) Reagents of alanine aminotransferase :

### Package:

1-reagent                 $2 \times 35 \text{ ml}$

2-reagent                 $1 \times 17.5 \text{ ml}$

The reagent when stored at 2-8°C are stable up to expiry date printed on the package. Stability on board or the analyzer at 2-10°C: ACCENT-200 expired 10 weeks after use .

Protect from light and avoid contamination!

### Concentrations in the test:

Tris (pH 7.5)                 $100 \text{ mmol/l}$

L-alanine                       $500 \text{ mmol/l}$

LDH                             $>36.7 \text{ } \mu\text{kat/l}$

2-oxoglutarate                 $15 \text{ mmol/l}$

NADH                            $0.18 \text{ mmol/l}$

## 3) Reagent of alkline phosphatase :

### Package

1-reagent                 $1 \times 30 \text{ ml}$

2-reagent                 $1 \times 8 \text{ ml}$

The reagent when stored at 2-8°C are stable up to expiry date printed on the package. Stability on board of the analyser at 2-10°C: ACCENT-200 expired 6 weeks after use .

### **Concentrations in the test:**

2-amino-2methyl-1-propanol(AMP)	350 mmol/l
Mg <sup>2+</sup>	2.0 mmol/l
Zn <sup>2+</sup>	1.0 mmol/l
HEDTA	2.0 mmol/l
p-nitrophenylphosphate	16.0 mmol/l

### **2.1.7 Quality control:**

For internal quality control used the CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) with each batch of samples.

For the calibration of automatic analyser systems the CORMAY MULTICALIBRATOR LEVEL 1 (cat. No 5-174; 5-176 ) or LEVEL 2 (Cat. No 5-175; 5-177).

## **2.2 Methods:**

### **2.2.1 (1).Estimation of aspartate aminotransferase activity using automatic analyser (Mindray ) ACCENT-200:**

#### **Principle of the method:**

Optimized, modified method according to International Federation of Clinical Chemistry (IFCC), without pyridoxal phosphate.

L-aspartate + 2-oxoglutarate  $\xrightleftharpoons{\text{ASAT}}$  oxalacetate + L-glutamate

Oxalacetate + NADH + H<sup>+</sup>  $\xrightleftharpoons{\text{MDH}}$  malate + NAD<sup>+</sup>

The rate of absorbance changing at  $\lambda = 340$  nm directly proportional to aspartate aminotransferase activity.

### Procedure:

Using automatic analyser (mindray )ACCENT-200: kinetic method

1-Reagent and 2-Reagent were ready to use.

For reagent blank deionized water was recommended.

Heparinized plasma free from hemolysis was collected and placed in automatic analyser for determination the enzyme activity (AST)U/L was measured at primary wave 340 nm and with secondary wave 450 nm.

Reference values:

Serum /plasma	37°C	
female	Up to 31 U/L	Up to 0.518 $\mu\text{kat/l}$
Male	Up to 37 U/L	Up to 0.618 $\mu\text{kat/l}$

### 2.2.2 (2).Estimation of alanine aminotransferase activity using automatic analyser( Mindray ) ACCENT-200:

#### Principle of the method:

Optimized, modified method according to International Federation of Clinical Chemistry (IFCC), without pyridoxal phosphate.

L-alanine + 2-oxoglutarate  $\xrightleftharpoons{\text{ALAT}}$  pyruvate + L-glutamate

Pyruvate + NADH+H<sup>+</sup>  $\xrightleftharpoons{\text{LDH}}$  Lactate + NAD<sup>+</sup>

The rate of absorbance changing at  $\lambda=340$  nm is directly proportional to alanine aminotransferase activity.

### Procedure:

Using automatic analyser (mindray ) ACCENT-200: kinetic method

1-Reagent and 2-Reagent were ready to use.

For reagent blank deionized water was recommended.

Heparinized plasma free from hemolysis was collected and placed in automatic analyser for determination the enzyme activity (ALT)U/L was measured at primary wave 340 nm and with secondary wave 450 nm.

Reference values:

Serum /plasma	37°C	
Female	Up to 31 U/L	Up to 0.517 $\mu\text{kat/l}$
Male	Up to 41 U/L	Up to 0.683 $\mu\text{kat/l}$

### 2.2.3 (3). Estimation of alkaline phosphatase activity using automatic analyser (Mindray ) ACCENT-200:

#### Principle of the method:

Kinetic method recommended by International Federation of Clinical Chemistry (IFCC).

2-amino-2-methyl-1-propanol + p-nitrophenylophosphate + H<sub>2</sub>O  $\xrightarrow{\text{ALP}}$

4-nitrophenol + 2-amino-2-methyl-1-propanol phosphate

The rate of 4-nitrophenol formation is directly proportional to the ALP activity.

### Procedure:

Using automatic analyzer (mindray ) ACCENT-200: kinetic method

1-Reagent and 2-Reagent are ready to use.

For reagent blank deionized water was recommended.

Heparinized plasma free from hemolysis was collected and placed in automatic analyzer for determination the enzyme activity (ALP)U/L was measured at primary wave 405 nm and with secondary wave 670 nm.

Reference values:

gender	Age	U/L(37°C)	μkat/l (37°C)
female	20-50 years	42-98	0.71-1.67
	≥60 years	53-141	0.90-2.40
male	20-50 years	53-128	0.90-2.18
	≥60 years	56-119	0.95-2.02

### 2.3 Data analysis:

Data were analyzed used SPSS computer program (version,20) , independent T test and correlation test were used to obtain mean value± SD and P. value at (0.05) is considered significant, and strength of the correlations.

## ***CHAPTER THREE***

### ***Result***



## CHAPTER THREE

### RESULT

#### 3. Result:

A total of 100 patients already diagnosed as type 2 diabetes mellitus of which 50 were females and 50 were males and 40 apparently healthy non diabetic individuals were selected for this study. Aged range from (30 – 60 years) and duration of diseases from (5 - 20 years) . The samples were obtained from Omdurman Teaching Hospital.

Table (3.1): Showed distribution of study group according to gender, 50 were females, and 50 were males

Most common age group ranged between 51-60 years, followed by age group 41-50 years, as shown in table (3.2).

According to Table (3.3): Most longest duration of the disease ranged between 5-10 years, followed by duration of 11-15 years, and least frequent group, with disease duration between 16-20 years.

Table (3.4): showed a significant difference of ALT and ALP in patients compared to control (P. value = 0.004 and 0.008) respectively.

Comparison between three levels of liver enzymes (AST, ALT and ALP) was observed between males and females in table (3.5): Insignificant difference in the three liver enzymes .

Figure (3.1): correlation between duration of the disease and AST enzyme showed significant increase (P. value = 0.04), with weak positive correlation ( $r = 0.2$ ) .

ALT enzyme increased significantly (P. value = 0.02), when correlated with duration of the disease and, with weak positive correlation ( $r = 0.2$ ) figure (3.2) .

Figure (3.3): showed correlation between duration of the disease and ALP enzyme. Which insignificant difference (P. value = 0.2).

**Table (3.1): Distribution of study group according to gender :**

<b>Gender</b>	<b>no. of patients</b>	<b>Control</b>
Female	50	20
Male	50	20
Total	100	40

**Table (3.2): Distribution of study group according to age:**

<b>Age group of patients(years)</b>	<b>no. of patients</b>	<b>Percentage%</b>
30__40	15	15
41__50	41	41
51__60	44	44
Total	100	100

**Table (3.3): Distribution of study group according to duration of the diseases( years)**

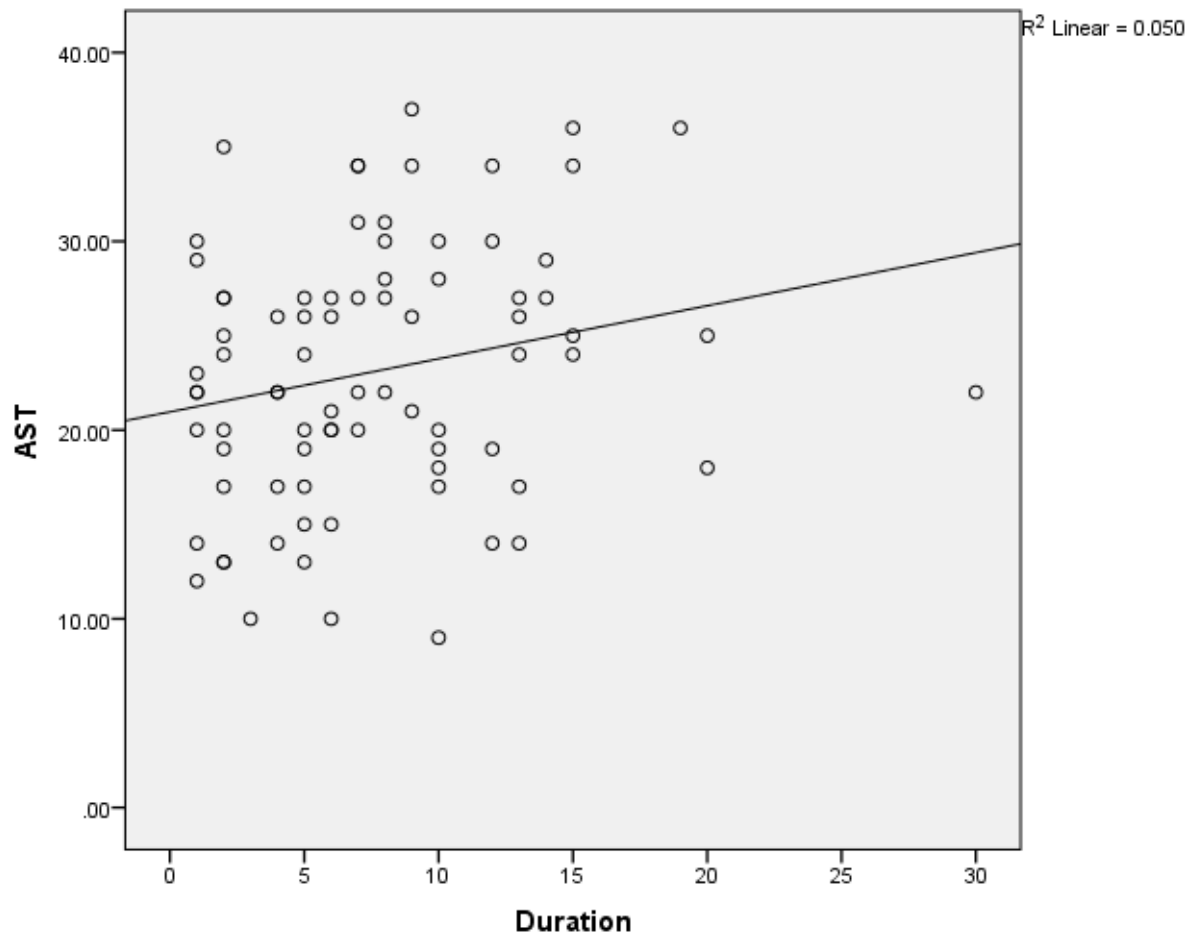
<b>Duration of DM(years)</b>	<b>no. of patients</b>	<b>Percentage %</b>
5 — 10	43	43
11—15	41	41
16 — 20	16	16
Total	100	100

**Table (3.4): Comparison between three levels of liver enzymes (AST, ALT and ALP) of patients and control:**

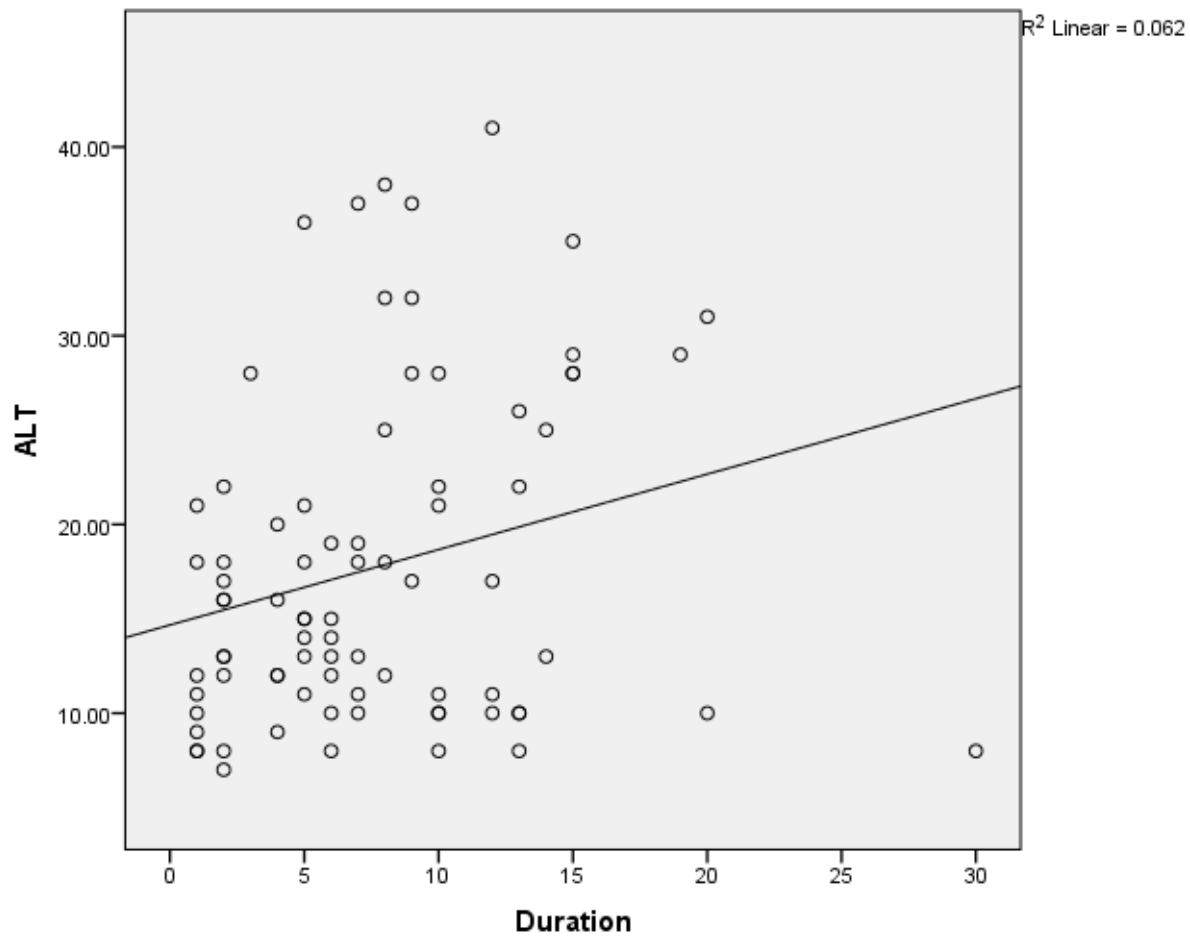
<b>Variable</b>	<b>Study group</b>	<b>Mean <math>\pm</math>SD</b>	<b>P. value</b>
AST (U/L)	Case	23.1 $\pm$ 8.6	0.1
	Control	21.4 $\pm$ 6.1	
ALT (U/L)	Case	17.7 $\pm$ 8.7	0.004
	Control	13.7 $\pm$ 5.8	
ALP (U/L)	Case	87.3 $\pm$ 26.3	0.008
	Control	74.8 $\pm$ 18.6	

**Table (3.5): comparison between three levels of liver enzymes (AST, ALT and ALP) of patients (male compared to female):**

Variable	Gender	Mean $\pm$ SD	P. value
AST (U/L)	Male	24.4 $\pm$ 5.8	0.2
	Female	22.4 $\pm$ 7.1	
ALT (U/L)	Male	20.3 $\pm$ 9.9	0.07
	Female	16.5 $\pm$ 7.9	
ALP (U/L)	Male	89.4 $\pm$ 27.1	0.6
	Female	86.3 $\pm$ 26.0	

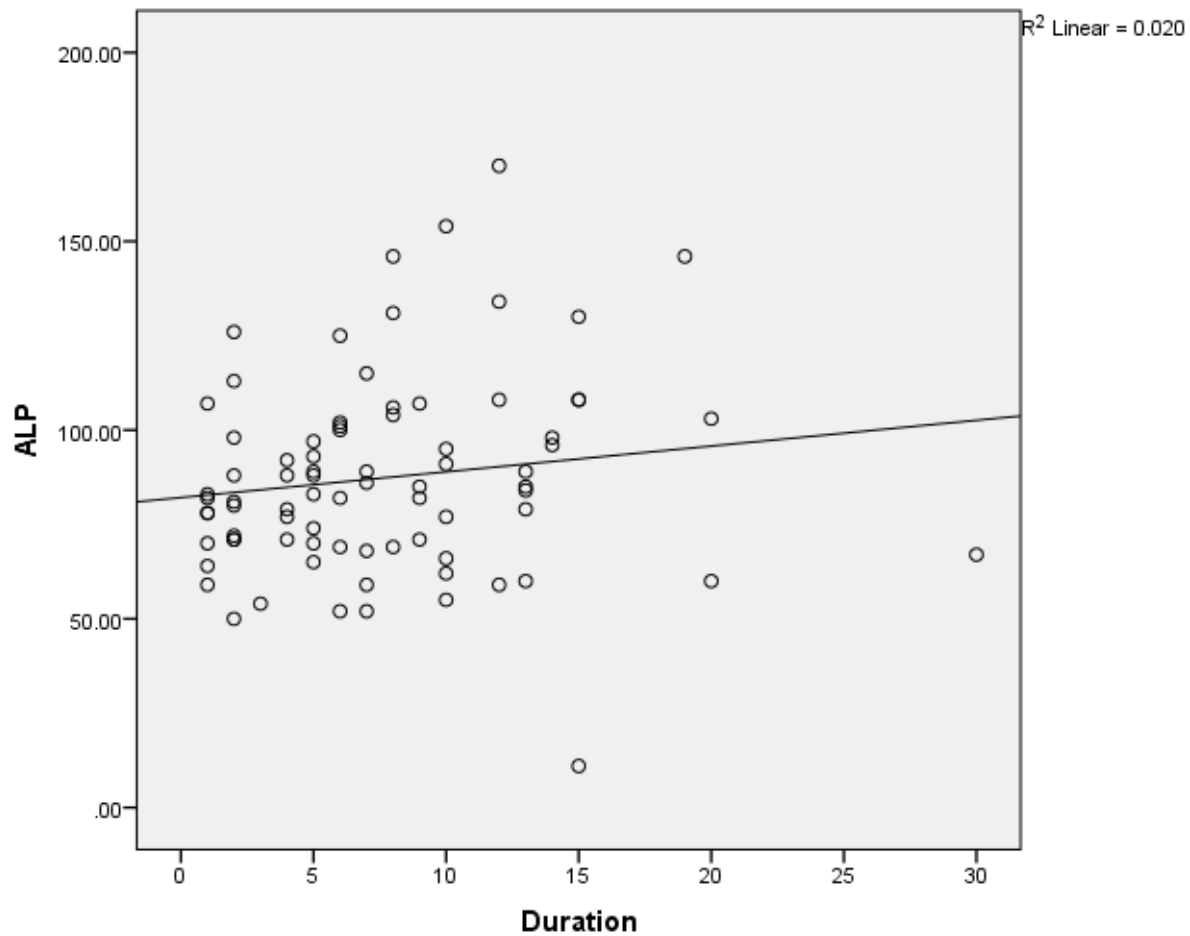


**Figure (3.1): showed correlation between duration of the disease and AST enzyme. ( $r=0.2$ )(P. value=0.04)**



**Figure (3.2):** showed correlation between duration of the disease and ALT enzyme. ( $r=0.2$ )( $P.value=0.02$ )





**Figure (3.3):** showed correlation between duration of the disease and ALP enzyme. ( $r=0.1$ )(P.value=0.2)

## ***CHAPTER FOUR***

### ***Discussion, Conclusion, and Recommendation***

## CHAPTER FOUR

### 4. Discussion, Conclusion, and Recommendation:

#### 4.1 Discussion:

The study aimed to assess activity of AST, ALT and ALP liver enzymes was assess in type 2 diabetic patients from Omdurman Teaching Hospital, 140 individuals were included, hundred diabetic patients (50 were males and 50 of females patients) compared to 40 apparently healthy non diabetic individuals.

AST activity insignificant difference in patients compared to control and gender, this results agreed with Han NI *et al* ., (2012) who found that ALT and AST increased among diabetic patients with higher BMI, and disagreed with Elmahi and abdrbo, (2014), who found the means of ALT, AST, ALP, were within normal range in diabetic patients.

Significant increased of ALT and ALP enzyme activity when compared to control, but insignificant difference when compared to gender. This results agreed with Odewabi *et al* ., (2013) who observed that the mean value of ALT and ALP of the patients with type 2 diabetes mellitus on treatment and newly diagnosed are significantly elevated compared to control subjects. Also agreed with the results of Idris *et al* ., (2011), who concluded that the mean of ALT and AST were increased and more frequently had elevated ALT. In addition, Foster *et al* (2014), reported similar findings.

The correlation between duration of the disease and AST, ALT enzyme showed weak positive correlation and it is significant which agreed with Elmahi and Abdrbo, (2014), only ALT was found to be significantly positively correlated with duration of diabetes, p.value (0.000).

Correlation between duration of the disease and ALP enzyme showed insignificant difference, this agreed with Odewabi *et al* ., (2013) who reported that overall T2DM in both categories (type 2 DM on treatment and the newly diagnosed ) of patients predisposes to elevated levels of ALT and ALP but only ALT may be a useful biomarker for monitoring hepatic complication in T2DM without underlying hepatitis.

According to the results of the present study which explained the association between diabetes and liver injury . Liver plays a major role in the regulation of carbohydrate homeostasis. Hepatocellular glycogen accumulation leads to hepatomegaly and liver enzyme abnormalities in poorly controlled diabetes patients. In hyperglycemic states, there will be intracellular glycogen accumulation in the hepatocytes due to increased glycogen synthesis, causing typical biochemical findings of mild to moderately elevated aminotransferases, normal liver synthetic function, with or without mild elevations of alkaline phosphatase. All these biochemical disturbances and hepatomegaly are found to be reversible with good glycemic control. Secondly, liver can be affected by steatosis or accumulation of fat, a condition known as non-alcoholic fatty liver disease (NAFLD). It is a well-recognized complication of diabetes with frequency of 40–70%. Associated obesity is a confounding variable for fatty liver. Increased transport of fatty acids to the liver, enhanced hepatic fat synthesis as well as decreased oxidation or removal of fat from the liver lead to fat accumulation in the liver. The steatosis is either microvesicular or macrovesicular and is found to progress to fibrosis and cirrhosis. The most common clinical finding is hepatomegaly, with normal or only mildly elevated transaminases. These changes are not reversible with sustained glucose control . Nonalcoholic fatty liver disease (NAFLD) is the main cause of chronic liver disease associated with diabetes and

obesity. Without treatment, compensated steatosis in NAFLD will eventually lead to decompensated steatosis with necroinflammation and fibrosis, i.e stage of non-alcoholic steatohepatitis (NASH). NASH is a leading cause of end-stage liver disease and also a contributor of cardiovascular disease in type 2 diabetes mellitus.

Serum amino transferases such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) indicate the concentration of hepatic intracellular enzymes that have leaked into the circulation. These are the markers for hepatocellular injury and are used as primary screening, Han Ni *et al* ,.(2012) .

## 4.2 Conclusion:

- AST increased insignificantly in patients compared to control and in males compared to females.
- ALT and ALP significantly increased in patients compared to control, but insignificantly increased in males compared to females.
- Correlation between duration of the disease was observed with significant difference in AST and ALT, with weak positive correlation, but insignificant difference between ALP was observed .

### **4.3 Recommendation**

From the results of this study it is recommended that:

- Blood glucose level in diabetic patients should be controlled with regular check up.
- BMI should be measured since obesity has a role in liver function.
- Assessment of GGT activity is recommend since this enzyme affects functions of the liver.

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## ***APPENDICES***

## **Appendices :**

### **Appendix (1)**

**Questionnaires :**

**Date :**

**No. :**

**Name :**

**Age :**

**Gender :**

**Duration of disease :**

**Other diseases :**

**Treatment :**

**Refer to doctor :**

**Other :**

**Results :**

Enzyme	Result
AST	
ALT	
ALP	

## Appendix (2)

اقرار بالمشاركة :

موافقة للاشتراك في البحث العلمي

انا الطالبة رانيا ميرغني احمد سعيد

انت مدعو<sup>(ة)</sup> للمشاركة ببحث علمي في جامعة السودان للعلوم و التكنولوجيا بعنوان

تقيم نشاط انزيمات الكبد الالنين ترانساميناس , الاسبارتات ترانساميناس و الفوسفاتيز القاعدي لدى السودانين

المصابين بمرض السكري من النوع الثاني

بامكانك طلب استفسار أو معلومات اضافيه عن هذه الدراسه . وفي حال وافقت على المشاركة في هذه

الدراسة سيبقى اسمك طي الكتمان .

موافقة الباحث :

لقد شرحت بالتفصيل للمشارك في البحث العلمي لانزيمات الكبد ولقد اجبت على كل الاسئلة بوضوح على

خير ما استطيع .وسوف اعلم المشارك باي تغييرات في مجريات هذا البحث او تاثيره السلبيه او فوائده في

حال حصولها اثناء البحث .

موافقة المشارك :

لقد قرأت استمارة القبول هذه وفهمت مضمونها . تمت الاجابه على اسئلتي جميعها وبناء عليه فانني حرا

مختارا اجيز اجراء هذا البحث ووافق على الاشتراك فيه .

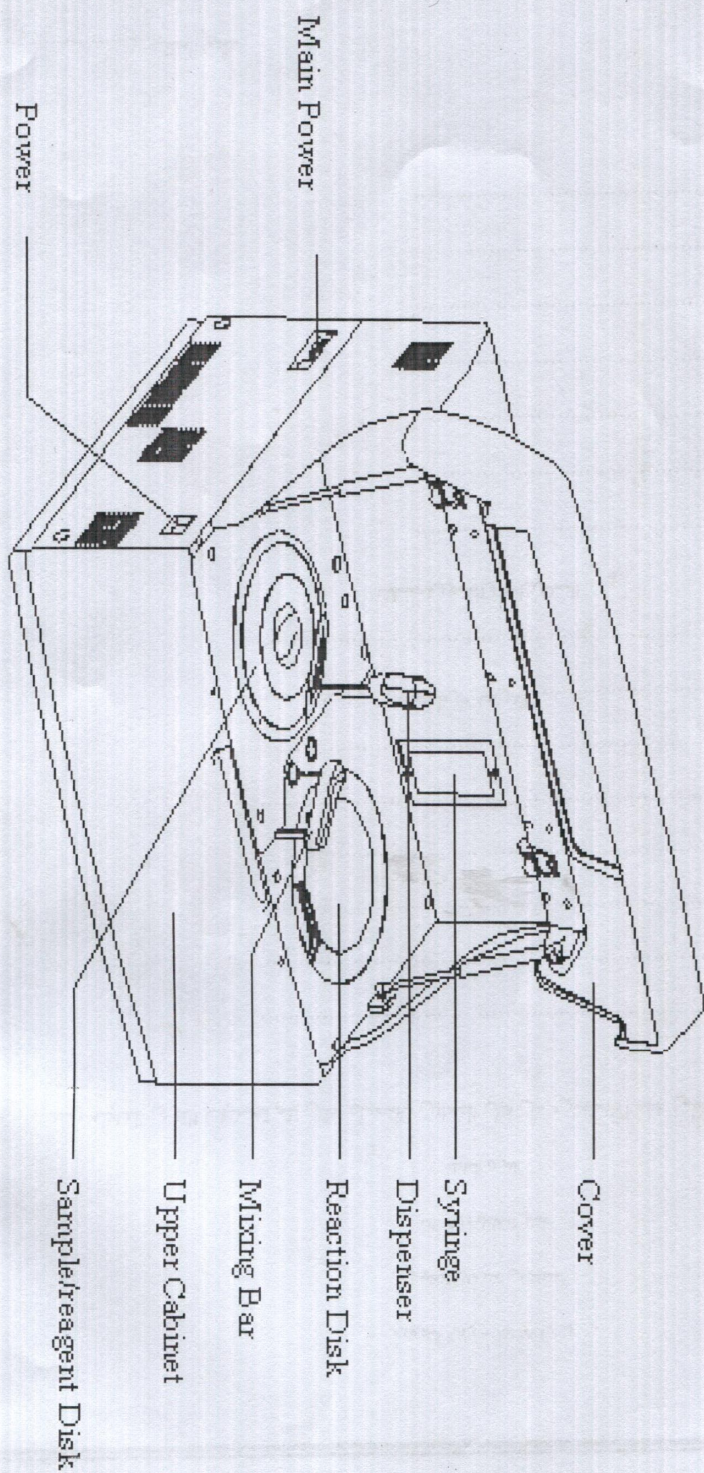
الامضاء :

التاريخ :

### Appendix (3)

- ISE Unit(optional)
- Sample/Reagent Disk
- Mixer
- Reaction Disk
- Photometric System

Figure 1-1 Analyzing unit

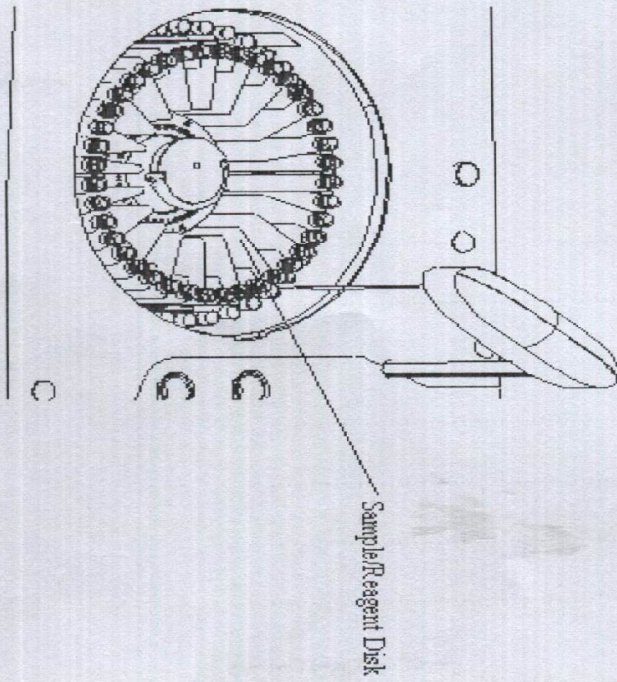




### 1.1.1.1 Sample/Reagent Disk

The sample/reagent disk holds sample tubes and reagent bottles.

Figure 1-2 Sample/Reagent disk



The disk is composed of two circles – sample disk on the outer circle and reagent disk on the inner circle.

The sample disk provides 40 sample tube positions and the reagent disk provides 40 reagent bottle positions. On the reagent disk, No. 37 and 38 are for cleaning solution and urine diluent of the ISE module, No. 39 is for detergent and No. 40 is for distilled water.



## Appendix (4)

Cat. No 7-214

**II GEN**  
**ACCENT 200**  
**ACCENT 200**

### ACCENT-200 ASAT

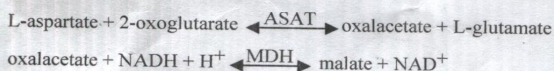
#### DIAGNOSTIC KIT FOR DETERMINATION OF ASPARTATE AMINOTRANSFERASE ACTIVITY

##### INTRODUCTION

Aspartate aminotransferase (ASAT, AST, GOT) is an enzyme participated in amino acids metabolism. ASAT is found in all tissues but particularly high level of ASAT is observed in heart muscle, skeletal muscle, liver and kidney. This is why elevated ASAT serum level is marker of myocardial infarction and kidney, liver or skeletal muscle injury.

##### METHOD PRINCIPLE

Optimized, modified method according to International Federation of Clinical Chemistry (IFCC), without pyridoxal phosphate.



The rate of absorbance changing at  $\lambda=340$  nm is directly proportional to aspartate aminotransferase activity.

##### REAGENTS

###### Package

1-Reagent

2 x 35 ml

2-Reagent

1 x 17.5 ml

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. Stability on board of the analyser at 2-10°C: ACCENT-200 – 10 weeks, ACCENT-200 II GEN – 10 weeks. Protect from light and avoid contamination!

##### Concentrations in the test

Tris (pH 7.8)	80 mmol/l
L-aspartate	240 mmol/l
MDH	> 10 µkat/l
LDH	> 20 µkat/l
2-oxoglutarate	15 mmol/l
NADH	0.18 mmol/l
sodium hydroxide	< 1 %

##### Warnings and notes

- Product for in vitro diagnostic use only.
- The reagents contain (< 0.1%) sodium azide as a preservative. Avoid contact with skin and mucous membranes.
- 1-Reagent is classified as an irritant!

**Ingredients:** Contains sodium hydroxide.

**Xi – Irritating.**

**R 36/38:** Irritating to eyes and skin.

**S 26-28-45:** In case of contact with eyes, rinse immediately with plenty of water and see medical advice.

After contact with skin, wash immediately with plenty of water. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

##### SPECIMEN

Serum, heparinized or EDTA plasma free from hemolysis.

Do not use heparine ammonium salt.

Hemolysis should be avoided, since ASAT activity in erythrocytes is 10 times higher than in normal serum.

Do not freeze the samples. ASAT activity remains stable in specimen up to 1 day at 15-25°C or up to 4 days at 2-8°C.

ACCENT-200 ASAT page 1



Nevertheless it is recommended to perform the assay with freshly collected samples!

##### PROCEDURE

These reagents may be used in automatic analysers ACCENT-200 and ACCENT-200 II GEN.

1-Reagent and 2-Reagent are ready to use.

For reagent blank deionized water is recommended.

##### APPLICATION for ACCENT-200 and ACCENT-200 II GEN

###### Parameters

Test Name	ASAT	R1	200
Test No	16	R2	50
Full Name	ASAT	Sample Volume	10
Reference No	16	R1 Blank	
Analy. Type	Kinetic	Mixed Reag. Blank	
Pri. Wave.	340 nm	Concentration	5 1000
Secun. Wave.	450 nm	Linearity Limit	0.2
Trend	Descending	Substrate Limit	
Reac. Time	4 15	Factor	
Incuba. Time	10	<input type="checkbox"/> Prozone check	
Unit	U/l	q1 <input type="checkbox"/> q2 <input type="checkbox"/> q3 <input type="checkbox"/> q4 <input type="checkbox"/>	
Precision	Integer	PC <input type="checkbox"/> Abs <input type="checkbox"/>	

##### Calibration Rule

Rule	One-point Linear
Sensitivity	1
Replicates	3
Interval (day)	70
Difference Limit	0
SD	0
Blank Response	0 50000
Error Limit	0
Coefficient	0

##### REFERENCE VALUES <sup>6</sup>

serum / plasma	37°C	
female	up to 31 U/l	up to 0.518 µkat/l
male	up to 37 U/l	up to 0.618 µkat/l

It is recommended for each laboratory to establish its own reference ranges for local population.

##### QUALITY CONTROL

For internal quality control it is recommended to use the CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) with each batch of samples.

For the calibration of automatic analysers systems the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) or LEVEL 2 (Cat. No 5-175; 5-177) is recommended.

The calibration curve should be prepared every 10 weeks (ACCENT-200, ACCENT-200 II GEN), with change of reagent lot number or as required e.g. quality control findings outside the specified range.

##### PERFORMANCE CHARACTERISTICS

These metrological characteristics have been obtained using automatic analyser ACCENT-200. Results may vary if a different instrument or a manual procedure is used.

- Sensitivity:** 5 U/l (0.084 µkat/l).
- Linearity:** up to 1000 U/l (16.7 µkat/l).



**II GEN**  
**ACCENT 200**  
**ACCENT 200**

## ACCENT-200 ALAT

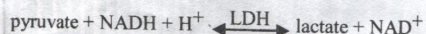
### DIAGNOSTIC KIT FOR DETERMINATION OF ALANINE AMINOTRANSFERASE ACTIVITY

#### INTRODUCTION

Alanine aminotransferase (ALAT, ALT, GPT) is an enzyme participated in amino acids metabolism. ALAT is present in all tissues but the highest level is found in liver and kidney cells. Damage of hepatocytes or kidney cells causes significant release of ALAT into the circulation. Measurement of ALT activity in serum is valuable in the diagnosis of liver diseases: jaundice, mononucleosis or hepatic cirrhosis.

#### METHOD PRINCIPLE

Optimized, modified method according to International Federation of Clinical Chemistry (IFCC), without pyridoxal phosphate.



The rate of absorbance changing at  $\lambda=340$  nm is directly proportional to alanine aminotransferase activity.

#### REAGENTS

##### Package

1-Reagent	2 x 35 ml
2-Reagent	1 x 17.5 ml

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. Stability on board of the analyser at 2-10°C: ACCENT-200 – 10 weeks, ACCENT-200 II GEN – 10 weeks. Protect from light and avoid contamination!

#### Concentrations in the test

Tris (pH 7.5)	100 mmol/l
L-alanine	500 mmol/l
LDH	> 36.7 $\mu\text{kat/l}$
2-oxoglutarate	15 mmol/l
NADH	0.18 mmol/l

#### Warnings and notes

- Product for in vitro diagnostic use only.
- The reagents contain (< 0.1%) sodium azide as a preservative. Avoid contact with skin and mucous membranes.

#### SPECIMEN

Serum, heparinized or EDTA plasma free from hemolysis. Hemolysis should be avoided, since ALAT activity in erythrocytes is 3 to 5 times higher than in normal serum. Do not freeze the samples. ALAT activity remains stable in specimen up to 3 days at 15-25°C or up to 7 days at 2-8°C. Nevertheless it is recommended to perform the assay with freshly collected samples!

#### PROCEDURE

These reagents may be used in automatic analysers ACCENT-200 and ACCENT-200 II GEN.

1-Reagent and 2-Reagent are ready to use.  
For reagent blank deionized water is recommended.



#### APPLICATION for ACCENT-200 and ACCENT-200 II GEN

##### Parameters

Test Name	ALAT	R1	200
Test No	15	R2	50
Full Name	ALAT	Sample Volume	10
Reference No	15	R1 Blank	
Analy. Type	Kinetic	Mixed Reag. Blank	
Pri. Wave.	340 nm	Concentration	4.5 1000
Secon. Wave.	450 nm	Linearity Limit	0.2
Trend	Descending	Substrate Limit	
Reac. Time	4 15	Factor	
Incuba. Time	10	<input type="checkbox"/> Prozone check	
Unit	U/l	q1 <input type="checkbox"/> q2 <input type="checkbox"/> q3 <input type="checkbox"/> q4 <input type="checkbox"/>	
Precision	Integer	PC <input type="checkbox"/> Abs <input type="checkbox"/>	

#### Calibration Rule

Rule	One-point Linear
Sensitivity	1
Replicates	3
Interval (day)	70
Difference Limit	0
SD	0
Blank Response	0 50000
Error Limit	0
Coefficient	0

#### REFERENCE VALUES <sup>6</sup>

serum / plasma	37°C	
women	up to 31 U/l	up to 0.517 $\mu\text{kat/l}$
men	up to 41 U/l	up to 0.683 $\mu\text{kat/l}$

It is recommended for each laboratory to establish its own reference ranges for local population.

#### QUALITY CONTROL

For internal quality control it is recommended to use the CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) with each batch of samples.

For the calibration of automatic analysers systems the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) or LEVEL 2 (Cat. No 5-175; 5-177) is recommended.

The calibration curve should be prepared every 10 weeks (ACCENT-200, ACCENT-200 II GEN), with change of reagent lot number or as required e.g. quality control findings outside the specified range.

#### PERFORMANCE CHARACTERISTICS

These metrological characteristics have been obtained using automatic analyser ACCENT-200. Results may vary if a different instrument or a manual procedure is used.

- Sensitivity:** 4.5 U/l (0.075  $\mu\text{kat/l}$ ).
- Linearity:** up to 1000 U/l (16.7  $\mu\text{kat/l}$ ).
- Specificity / Interferences**  
Haemoglobin up to 0.16 g/dl, ascorbate up to 62 mg/l, bilirubin up to 20 mg/dl and triglycerides up to 1000 mg/dl do not interfere with the test.



# ACCENT 200 II GEN

## ACCENT 200



### ACCENT-200 ALP

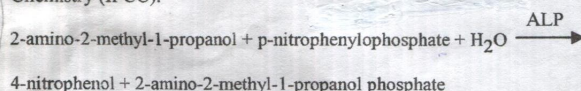
#### DIAGNOSTIC KIT FOR DETERMINATION OF ALKALINE PHOSPHATASE ACTIVITY

##### INTRODUCTION

Alkaline phosphatase (ALP) is actually a group of isoenzymes that hydrolyse monophosphate esters in alkaline medium. Optimum pH for these ALP isoforms activities is about 9-10. Alkaline phosphatase level is the highest in liver, bone, intestine, kidney and placenta. Measurement of ALP isoenzymes is useful in diagnosis of these organs diseases.

##### METHOD PRINCIPLE

Kinetic method recommended by International Federation of Clinical Chemistry (IFCC).



The rate of 4-nitrophenol formation is directly proportional to the ALP activity.

##### REAGENTS

###### Package

1-Reagent	1 x 30 ml
2-Reagent	1 x 8 ml

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. Stability on board of the analyser at 2-10°C: ACCENT-200 - 6 weeks, ACCENT-200 II GEN - 6 weeks. Protect from light and avoid contamination!

##### Concentrations in the test

2-amino-2-methyl-1-propanol (AMP)	350 mmol/l
Mg <sup>2+</sup>	2.0 mmol/l
Zn <sup>2+</sup>	1.0 mmol/l
HEDTA	2.0 mmol/l
p-nitrophenylphosphate	16.0 mmol/l

##### Warnings and notes

- Product for in vitro diagnostic use only.
- The reagents contain sodium azide (< 0.1%) as a preservative. Avoid contact with skin and mucous membranes.
- During the reaction p-nitrophenol is produced. Do not swallow or inhale, avoid contact with skin.

##### SPECIMEN

Serum, heparinized plasma free from hemolysis.

Do not use EDTA, citrate and oxalate as anticoagulants because of ALP activity inhibition!

ALP activity remains stable in specimen up to 4 hours at 15-25°C. Freezing of sample causes a loss of enzyme activity. Frozen specimens should be thawed and kept at room temperature for 18 to 24 hours before measurement to achieve full enzyme reactivation. Nevertheless it is recommended to perform the assay with freshly collected samples!

##### PROCEDURE

These reagents may be used in automatic analysers ACCENT-200 and ACCENT-200 II GEN.

1-Reagent and 2-Reagent are ready to use.

For reagent blank deionized water is recommended.

ACCENT-200 ALP page 1

#### APPLICATION for ACCENT-200 and ACCENT-200 II GEN

##### Parameters

Test Name	ALP	R1	200
Test No	18	R2	50
Full Name	Alkaline phosphatase	Sample Volume	5
Reference No	18	R1 Blank	
Analy. Type	Kinetic	Mixed Reag. Blank	
Pri. Wave.	405 nm	Concentration	28 700
Secon. Wave.	670 nm	Linearity Limit	0.2
Trend	Ascending	Substrate Limit	
Reac. Time	2 13	Factor	
Incuba. Time	10	<input type="checkbox"/> Prozone check	
Unit	U/l	q1 <input type="checkbox"/> q2 <input type="checkbox"/> q3 <input type="checkbox"/> q4 <input type="checkbox"/>	
Precision	Integer	PC <input type="checkbox"/> Abs <input type="checkbox"/>	

##### Calibration Rule

Rule	One Point Linear
Sensitivity	1
Replicates	3
Interval (day)	10
Difference Limit	0
SD	0
Blank Response	0. 50000
Error Limit	0
Coefficient	0

##### REFERENCE VALUES<sup>9,10</sup>

gender	age	U/l (37°C)	μkat/l (37°C)
female	1 - 30 days	48 - 406	0.80 - 6.77
	31 days - 1 year	124 - 341	2.07 - 5.68
	1 year - 3 years	108 - 317	1.80 - 5.28
	4 - 15 years	54 - 369	0.91 - 6.23
	20 - 50 years	42 - 98	0.71 - 1.67
	≥ 60 years	53 - 141	0.90 - 2.40
male	1 - 30 days	75 - 316	1.25 - 5.27
	31 days - 1 year	82 - 383	1.37 - 6.38
	1 year - 3 years	104 - 345	1.73 - 5.75
	4 - 15 years	54 - 369	0.91 - 6.23
	20 - 50 years	53 - 128	0.90 - 2.18
	≥ 60 years	56 - 119	0.95 - 2.02

It is recommended for each laboratory to establish its own reference ranges for local population.

##### QUALITY CONTROL

For internal quality control it is recommended to use the CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) with each batch of samples.

For the calibration of automatic analysers systems the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) or LEVEL 2 (Cat. No 5-175; 5-177) is recommended.

The calibration curve should be prepared every 10 days (ACCENT-200, ACCENT-200 II GEN), with change of reagent lot number or as required e.g. quality control findings outside the specified range.