Sudan University of Science and Technology

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Estimation of Some Liver Enzymes Among Sudanese Using Atorvastatin Drug

قياس مستوي انزيمات الكبد عند السودانين الذين يستخدمون عقار اتورفاستاتين

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الآيـــــــــة

بسم الله الرحمن الرحيم

قال تعالي:

((علم الإنسان ما لم يعلم))

صدق الله العظيـــم

ســـــورة العلـــــق (الآيـــة 5)

Dedication

To...

My

Mother

She is

My life

My brothers & sister

Whom my supportance

My friends:

The source of my strength

Thank you for your presence in my life

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Abstract

Background: The primary uses of atorvastatin are for the treatment of dyslipidemia and the prevention of cardiovascular disease. Decreases in cholesterol levels were dose-related and stability throughout the treatment period.

Objective: To estimation of serum AST, ALT, and ALP among Sudanese using Atorvastatin drug.

Methodology: This study was conducted in period between March to May 2017, included 80 participants 50 patients (60%) as cases and 30 indiveduals (40%) as control with age between (20-70) years. Case group included 60% female and 40% male, used different doses of drug (10, 20 and 40 mg/day) and different durations using for variable conditions such as (DM, HTN or protection for risk of CVD). Control group was matched with cases group in age and gender but not used any lipid lowering drugs. The samples were obtained after filling a proper questionnaire.

The estimation of liver enzymes was done by fully automated Cobas c311analyzer.

Results: The results showed insignificant difference in the liver enzymes activity in cases and control group as following: in cases (21.34±7.26) (15.46±12.88) (85.04±20.23) compared to control (26.03±6.52),(18.00±12.88),(81.73±36.91) by ***P***-value(0.044,0.470,0.606 for AST,ALT, and ALP respectively).No correlation between duration of drug used and concentration of AST,ALT, and ALP (***P***-value: 0.213, 0.999, and 0.701 respectively)

Conclusion: insignificant difference of AST, ALT and ALP activity between cases and control, no correlation between duration of drug use and concentration of AST, ALT and ALP.

المستـخلص

خلفية:أتورفاستاتين هو دواء لإنقاص مستوي الدهون بالبلازما للحماية من امراض القلب بانقاص مستوي الكوليسترول في الدم.

الهدف: تهدف الدراسة لقياس تراكيز انزيمات الكبد في السودانيين الذين يستخدمون دواء أتورفاستاتين.

الطريقة:أجريت هذه الدراسة التحليلية في الفترة بين مارس إلى مايو2017، شـملت 80 مشـارك 60٪ حالات و 40٪ تحكم بمعدل أعمار يتراوح بين (20–70) سنة. وكانت مجموعة الحالات بها 60٪ مـن الإناث و 40٪ من الذكور الذين يستخدمون جرعات مختلفة من أتورفاستاتين (10 و 20 و 40 ملـغ / يوم) لفترات مختلفة (1 إلى 20) سنة. لمختلف الحالات كالـ (السكري، الضغط أو كحماية من امـراض القلب). تمت مطابقة مجموعة التحكم بمجموعة الحالات في العمر والنوع ولكن لا تستخدم أتورفاستاتين.

تم الحصـول علـى العينـات بعـد ملـئ الاسـتبيان المناسـب وقيـاس انزيمـات الكبـد بواسـطة Cobasc311analyzer الآلي.

النتائج: قد اظهرت النتائج أن ليس هنالك ارتباط ذو دلالة إحصائية بين متوسط تراكيز اننزيمات الكبـد ALT,ASTوALP في الحالات(21.34±7.26) ، (15.46±12.88) ، (85.04±20.23) ، مقارنة بـالتحكم (26.03±6.52)، (18.00±12.88) ،(81.73±36.91)، بقيمـة (*P*–value) (0.044،0.606،0.470,، لكل من ALP, ALT,AST على التوالي).

عند إستحدام معامل بيرسون للإرتباط اوضحت الدراسه ايضا عدم وجود علاقه بين ذيادة فترة اسـتخدام الدواء ومتوسط تراكيز انزيمات الكبد ALP,ALT,AST قيمة*P*(0.213 و0.999و0.701)علي التوالي.

الخلاصة: خلصت الدراسة لعدم وجود تأثير علي تراكيز انزيمات الكبد ALP,ALT,AST بين مستخدمي أتورفاستاتين. لا توجد علاقة ارتباطية بين فترة المستخدم و تركيز المعاملات.

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List of Abbreviation

AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
AMI	Acute Myocardial Infraction.
BP	Blood Pressure.
CHD	Coronary Heart Diseases.
CHL OR TCHL	Cholesterol.
CRP	C - Reactive Protein.
CVD	Cardiovascular Diseases.
DM	Diabetes Mellitus.
GREACE	Greek- Atorvastatin & Coronary-Heart Diseases Evaluation
HDL	High-Density Lipoprotein.
HMG-COA	3-Hydroxy-3-Methylglutaryl-Coa.
HT OR HTN	Hypertension.
HCC	Hepatocellular Carcinoma.
IDL	Intermediate- Density Lipoprotein.
SGOT OR GOT	SerumGlutamic Oxaloacetic Transaminase
SGPT OR GPT	Serum Glutamic Pyruvic Transaminase
UNL	Upper Normal Limits

Chapter one

Introduction

1.1 Introduction:

Many studies have proved the link between coronary heart disease and cholesterol. Cholesterol lowering drugs are usually required. The most recent drug developed for this purpose is atorvastatin. The primary uses of atorvastatin are for the treatment of dyslipidemia and the prevention of cardiovascular disease. Decreases in cholesterol levels were dose-related and stability throughout the treatment period, (Nissan *et al.*, 2006).

Atorvastatin, is a calcium salt with the trade name Lipitor, is a member of the drug class known as statins. It is a synthetic lipid-lowering agent that lowers blood cholesterol. It also stabilizes plaque and prevents strokesthrough anti-inflammation and other mechanisms (Walsh *et al*., 1996). Atorvastatin is a competitive inhibitor of HMG-CoA reeducates. Unlike most others, however, it is a completely synthetic compound. HMGCoA reeducates catalyzes the reduction of 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) to mevalonate, which is the rate-limiting step in hepatic cholesterol biosynthesis. Inhibition of the enzyme decreases denovo cholesterol synthesis, increasing expression of low-density lipoprotein receptors (LDL receptors) on hepatocytes. This increases LDL uptake by hepatocytes, decreasing LDLcholesterol in blood. (Villa *et al.*, 2010)

The liver is the primary site ofdrugs action and the principal site for cholesterol synthesis and LDL clearance. Atorvastatin like other lipid lowering therapies, have been associated with biochemical abnormalities of liver function. Increases in alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were transient and dose related in severity. Hepatic lesions were reversible with discontinued treatmentand dose-related in severity and distribution. Hepatic microgranulomas and hepatocellular degeneration were seen,

hepatocellular lipofuscin deposits were also increased. Bile stasis occurred as well. Upon dose reduction, interruption or discontinuation, transaminases returned to or near pretreatment levels without sequel, (Walsh *et al.*, 1999).

1.2 Rationale

Atorvastatin considered the drugs of first choice for modifying lipid which acts as risk factors for Coronary Heart Diseases. Atorvastatin like all medications, have potential adverse effects. The most serious are liver and muscle adverse effects. Congnitive loss, neuropathy, sexual dysfunction and, pancreatic dysfunction are examples of other adverse effects.

Because of the absence of data concerning the hepatic toxicity of atorvastatin in Sudan, the present study was performed to evaluate the hepatic adverse effects of Atorvastatin by measuring the serum ALT, AST, and ALP activities in patients used atorvastatin drug.

1.3 Objectives of the study:

1.3.1 General objective:

To estimate serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) among Sudanese patients using Atorvastatin drug.

1.3.2 Specific objectives:

1. To measure and compare mean of serum levels of (AST), (ALT), and (ALP) in case and control groups.
2. To compare between mean of serum levels of (AST), (ALT), and (ALP), in patients under different doses of atorvastatin drug.
3. To correlate the levels of (AST), (ALT), and (ALP), with the duration of atorvastatin use.

Chapter two

Literature Review

2. Literature Review:

2.1.The liver:

The liver is a very large and complex organ responsible for performing vital tasks that impact all body systems. Its complex functions include metabolism of carbohydrates, lipids, proteins, and bilirubin; detoxification of harmful substances; storage of essential compounds; and excretion of substances to prevent harm. The liver is unique in the sense that it is a relatively resilient organ that can regenerate cells that have been destroyed by some short-term injury or disease or have been removed. However, if the liver is damaged repeatedly over a long period of time, it may undergo irreversible changes that permanently interfere with its essential functions. If the liver becomes completely nonfunctional for any reason, death will occur within approximately 24 hours due to hypoglycemia (Bishop *et al.*, 2010).

2.1.1. Gross anatomy:

Understanding the function and dysfunction of the liver depends on understanding its gross and microscopic structure. The liver is a large and complex organ weighing approximately 1.2 to 1.5 kg in the healthy adult. It is located beneath and attached to the diaphragm, 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. The right lobe is approximately six times larger than the left lobe. The lobes are functionally insignificant; however, 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 into the hepatic sinusoid, which is lined with hepatocytes capable of removing potentially toxic substances from the blood. From the sinusoid, blood flows to the central canal (central vein) of each lobule. It is through the central canalthat blood leaves the liver. 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 canaliculi. 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. 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.*, 2010).

2.1.2. Microscopic anatomy:

The liver is divided into microscopic units called lobules. The lobules are the functional units of the liver; they are responsible for all metabolic and excretory functions performed by the liver. Each lobule is roughly a six-sided structure with a centrally located vein (called the central vein) with portal triads at each of the corners. Each portal triad contains a hepatic artery, a portal vein, and a bile duct surrounded by connective tissue. The liver contains two major cell types:

hepatocytes and Kupffer cells. The hepatocytes, making up approximately 80% of the volume of the organ, are large cells that radiate outward from the central vein in plates to the periphery of the lobule.These cells perform the major functionsassociated with the liver and are responsible for the regenerative properties of the liver. Kupffer cells are macrophages that line the sinusoids of the liver and act as active phagocytes capable of engulfing bacteria, debris, toxins, and other substances flowing through the sinusoids (Bishop *et al*., 2010).

2.2. Biochemical functions:

2.2.1. Excretory and secretory:

One of the most important functions of the liver is the processing and excretion of endogenous and exogenous substances into the bile or urine such as the major heme waste product, bilirubin. The liver is the only organ that has the capacity to rid the body of heme waste products. Bile is made up of bile acids or salts, bile pigments, cholesterol, and other substances extracted from the blood. The body produces approximately 3 L of bile per day and excretes 1 L of what is produced. Bilirubin is the principal pigment in bile, and it is derived from the breakdown of red blood cells. Approximately 126 days after the emergence from the reticuloendothelial tissue, red blood cells are phagocytized and hemoglobin is released. Hemoglobin is degraded to heme, globin, and iron. The iron is bound by transferrin and is returned to ironstores in the liver or bone marrow for reuse. The globin is degraded to its constituent amino acids, which are reused by the body. The heme portion of hemoglobin is converted to bilirubin in 2 to 3 hours. Bilirubin is bound by albumin and transported to the liver. This form of bilirubin is referred to as unconjugated or indirect bilirubin. This form of bilirubin is insoluble in water

and cannot be removed from the body until it has been conjugated by the liver. Once at the liver cell, unconjugated bilirubin flows into the sinusoidal spaces and is released from albumin so it can be picked up by a carrier protein called ligandin. Ligandin, which is located in the hepatocyte, is responsible for transporting unconjugated bilirubin to the endoplasmic reticulum, where it may be rapidly conjugated. The conjugation (esterification) of bilirubin occurs in the presence of the enzyme uridyldiphosphate glucuronyl transferase (UDPGT), which transfers a glucuronic acid molecule to each of the two propionic acid side chains of bilirubin to form bilirubin diglucuronide, also known as conjugated bilirubin. This form of bilirubin is water soluble and is able to be secreted from the hepatocyte into the bile canaliculi. Once in the hepaticduct, it combines with secretions from the gallbladder through the cystic duct and is expelled through the common bile duct to the intestines. Intestinal bacteria (especially the bacteria in the lower portion of the intestinal tract) work on conjugated bilirubin to produce mesobilirubin, which is reduced to form mesobilirubinogen and then urobilinogen (a colorless product). Most of the urobilinogen formed (roughly 80%) is oxidized to an orange-colored product called urobilin (stercobilin) and is excreted in the feces. The urobilin or stercobilin is what gives stool its brown color. There are two things that can happen to the remaining 20% of urobilinogen formed. The majority will be absorbed by extrahepatic circulation to be recycled through the liver and reexcreted. The other very small quantity left will enter systemic circulation and will subsequently be filtered by the kidney and excreted in the urine. Approximately 200 to 300 mg of bilirubin is produced per day, and it takes a normally functioning liver to process the bilirubin and eliminate it from the body. This, as stated earlier, requires that bilirubin be conjugated. Almost all the bilirubin formed is eliminated inthe feces, and a small amount of the colorless product, urobilinogen, is excreted in the urine. The healthy adult has very low levels of total

bilirubin (0.2 to 1.0 mg/dL) in the serum, and of this amount, the majority is in the unconjugated form (Bishop *et al.*, 2010).

2.2.2. Metabolism:

The liver has extensive metabolic capacity; it is responsible for metabolizing many biological compounds including carbohydrates, lipids, and proteins... The liver is the major player in maintaining stable glucose concentrations due to its ability to store glucose as glycogen (glycogenesis) and degrade glycogen (glycogenolysis) depending on the body's needs, the liver will create glucose from no sugar carbon substrates like pyruvate, lactate, and amino acids (gluconeogenesis). Lipids are metabolized in the liver under normal circumstances when nutrition is adequate and the demand for glucose is being met... Almost all proteins are synthesized by the liver except for the immunoglobulins and adult hemoglobin. The liver plays an essential role in the development of hemoglobin in infants. One of the most important proteins synthesized by the liver is albumin. The liver is also responsible for synthesizing the positive and negative acute-phase reactants and coagulation proteins, and it also serves to store a pool of amino acids through protein degradation. The most critical aspect of protein metabolism includes transamination and deamination of amino acids. Transamination (via a transaminase) results in the exchange of an amino group on one acid with a ketone group on another acid. After transamination, deamination degrades them to produce ammonium ions that areconsumed in the synthesis of urea and urea is excreted by the kidneys. Although it would seem logical that any damage to the liver would result in a loss of synthetic and metabolic functions of the liver that is not the case. The liver must be extensively impaired before it loses its ability to

perform these essential functions (Bishop *et al*., 2010).

2.2.3. Detoxification and Drug metabolism:

The liver serves as a gatekeeper between substances absorbed by the gastrointestinal tract and those released into systemic circulation. Every substance that is absorbed in the gastrointestinal tract must first pass through the liver; this is referred to as first pass. This is an important function of the liver because it can allow important substances to reach the systemic circulation and can serve as a barrier to prevent toxic or harmful substances from reaching systemic circulation. The body has two mechanisms for detoxification of foreign materials (drugs and poisons) and metabolic products (bilirubin and ammonia). It may either bind the material reversibly so as to inactivate the compound or it may chemically modify the compound so it can be excreted. The most important mechanism is the drug-metabolizing system of the liver. This system is responsible for the detoxification of many drugs through oxidation, reduction, hydrolysis, hydroxylation, carboxylation, and demethylation. Many of these take place in the liver microsomes via the cytochromeP-450 isoenzymes (Bishop *et al.*, 2010).

2.3. Liver function alterations during disease:

2.3.1. Jaundice:

The word jaundice comes from the French word jaunt, which means “yellow,” and it is one of the oldest known pathologic conditions reported, having been described

by Hippocratic physicians. Jaundice, or icterus, is used to describe the yellow discoloration of the skin, eyes, and mucous membranes most often resulting from the retention of bilirubin; however, it may also occur due to the retention of other substances. Although the upper limit of normal for total bilirubin is 1.0 to 1.5 mg/dL, jaundice is usually not noticeable to the naked eye (known as overt jaundice) until bilirubin levels reach 3.0 to 5.0 mg/dL. Although the terms jaundice and icterus are used interchangeably, the term icterus is most commonly used in the clinical laboratory to refer to a serum or plasma sample with a yellow discoloration due to an elevated bilirubin level. Jaundice is most commonly classified based on the site of the disorder: prehepatic, hepatic, and posthepatic jaundice. This classification is important because knowing the classification of jaundice will aid health-care providers in formulating an appropriate treatment or management plan (Bishop *et al*., 2010).

2.3.2. Cirrhosis:

Cirrhosis is a clinical condition in which scar tissue replaces normal, healthy liver tissue. As the scar tissue replaces the normal liver tissue, it blocks the flow of blood through the organ and prevents the liver from functioning properly. Cirrhosis rarely causes signs and symptoms in its early stages, but as liver function deteriorates, the signs and symptoms appear, including fatigue, nausea, unintended weight loss, jaundice, bleeding from the gastrointestinal tract, intense itching, and swelling in the legs and abdomen. Although some patients with cirrhosis may have prolonged survival, they generally have a poor prognosis. Cirrhosis was the twelfth leading cause of death by disease in 2010, killing just over 31,000 people.15 In the United States, the most common cause of cirrhosis is chronic alcoholism. Other

causes of cirrhosis include chronic hepatitis B (HBV), C (HCV), and D virus (HDV) infection, autoimmune hepatitis, inherited disorders (e.g., α1-antitrypsin deficiency, Wilson disease, hemochromatosis, and galactosemia), nonalcoholic steatohepatitis, blocked bile ducts, drugs, toxins, and infections (Bishop *et al.*, 2010).

2.3.3. Tumers:

Cancers of the liver are classified as primary or metastatic. Primary liver cancer is cancer that begins in the liver cells while metastatic cancer occurs when tumors from other parts of the body spread (metastasize) to the liver. Metastatic liver cancer is much more common than primary liver cancer; 90% to 95% of all hepatic malignancies are classified as metastatic. Cancers that commonly spread to the liver include colon, lung, and breast cancer. Tumors of the liver may also be classified as benign or malignant. The common benign tumors of the liver include hepatocellular adenoma (a condition occurring almost exclusively in females of child-bearing age) and hemangiomas (masses of blood vessels with no known etiology). Malignant tumors of the liver include hepatocellular carcinoma (HCC) (also known as hepatocarcinoma, and hepatoma) and bile duct carcinoma. Of those, HCC is the most common malignant tumor of the liver. Hepatoblastoma is an uncommon hepatic malignancy of children (Bishop *et al.*, 2010).

2.4. Liver enzymes:

2.4.1. Aspartate aminotransferase (AST):

Is an enzyme belonging to the class of transferases, It is commonly referred to as a transaminase and is involved in the transfer of an amino group between aspartate and α-keto acids. The older terminology, serum glutamic oxaloacetic transaminase (SGOT, or GOT), may also be used. Pyridoxal phosphate functions as a coenzyme.

The transamination reaction is important in intermediary metabolism because of its function in the synthesis and degradation of amino acids. The ketoacids formed by the reaction are ultimately oxidized by the tricarboxylic acid cycle to provide asource of energy (Bishop *et al*., 2010).

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2.4.1.1. Tissue Source

AST is widely distributed in human tissue. The highest concentrations are found in cardiac tissue, liver, and skeletal muscle, with smaller amounts found in the kidney, pancreas, and erythrocyte (Bishop *et al*., 2010).

2.4.1.2. Diagnostic Significance

The clinical use of AST is limited mainly to the evaluation of hepatocellular disorders and skeletal muscle involvement. In AMI, AST levels begin to rise within 6 to 8 hours, peak at 24 hours, and generally return to normal within 5 days. However, because of the wide tissue distribution, AST levels are not useful in the diagnosis of AMI. AST elevations are frequently seen in pulmonaryembolism. Following congestive heart failure, AST levels also may be increased, probably reflecting liver involvement as a result of inadequate blood supply to that organ.

AST levels are highest in acute hepatocellular disorders. In viral hepatitis, levels may reach 100 times the ULN. In cirrhosis, only moderate levels approximately four times the ULN are detected. Skeletal muscle disorders, such as the muscular dystrophies, and inflammatory conditions also cause increases in AST levels (4 to 8× ULN). AST exists as two isoenzyme fractions located in the cell cytoplasm and mitochondria. The intracellular concentration of AST may be 7,000 times higher than the extracellular concentration. The cytoplasmic isoenzyme is the predominant form occurring in serum. In disorders producing cellular necrosis, the mitochondrial form may be significantly increased. Isoenzyme analysis of AST is not routinely performed in the clinical laboratory (Bishop *et al.*, 2010).

Table (2.1): Adult Reference Rang for AST. (Bishop *et al.*, 2010)

Normal rang	Unit
5 to 35	U/L

2.4.2. Alanine aminotransferase (ALT)

Is a transferase with enzymatic activity similar to that of AST, Specifically, it catalyzes the transfer of an amino group from alanine to α-ketoglutarate with the formation of glutamate and pyruvate. The older terminology was serum glutamic pyruvic transaminase (SGPT, or GPT) (Bishop *et al.*, 2010).

2.4.2.1. Tissue Source

ALT is distributed in many tissues, with comparatively high concentrations in the liver. It is considered the more liver-specific enzyme of the transferase (Bishop *etal*., 2010).

2.4.2.2.Diagnostic Significance:

Clinical applications of ALT assays are confined mainly to evaluation of hepatic disorders. Higher elevations are found in hepatocellular disorders than in extrahepatic or intrahepatic obstructive disorders. In acute inflammatory conditions of the liver, ALT elevations are frequently higher than those of AST and tend to remain elevated longer as a result of the longer half-life of ALT in serum (16 and 24 hours, respectively). Cardiac tissue contains a small amount of ALT activity, but the serum level usually remains normal in AMI unless subsequent liver damage has occurred. ALT levels have historically been compared with levels of AST to help determine the source of an elevated AST level and to detect liver involvement concurrent with myocardial (Bishop *et al* .,2010).

Table (2.2): Adult Reference Rang for ALT. (Bishop *et al*., 2010).

Normal rang	Unit
7 TO 45	U/L

2.4.3.Alkaline phosphatase

ALP belongs to a group of enzymes that catalyze the hydrolysis of various phosphomonoesters at an alkaline pH. Consequently, ALP is a nonspecific enzyme

capable of reacting with many different substrates. Specifically, ALP functions to liberate inorganic phosphate from an organic phosphate ester with the concomitant production of an alcohol (Bishop *et al*., 2010).

2.4.3.1 Tissue Source:

ALP activity is present on cell surfaces in most human tissue. The highest concentrations are found in the intestine, liver, bone, spleen, placenta, and kidney. In the liver, the enzyme is located on both sinusoidal and bile canalicular membranes; activity in bone is confined to the osteoblasts, those cells involved in the production of bone matrix. The specific location of the enzyme within this tissue accounts for the more predominant elevations in certain disorders (Bishop *et al.*, 2010).

2.4.3.2 Diagnostic Significance:

Elevations of ALP are of most diagnostic significance in the evaluation of hepatobiliary and bone disorders. In hepatobiliary disorders, elevations are more predominant in obstructive conditions than in hepatocellular disorders; in bone disorders, elevations are observed when there is involvement of osteoblasts. In biliary tract obstruction, ALP levels range from 3 to 10 times the ULN. Increases are primarily a result of increased synthesis of the enzyme induced by cholestasis. In contrast, hepatocellular disorders, such as hepatitis and cirrhosis, show only slight increases, usually less than three times the ULN. Because of the degree of overlap of ALP elevations that occurs in the various liver disorders, a single elevated ALP level is difficult to interpret. It assumes more diagnostic significance

when evaluated along with other tests of hepatic function. Elevated ALP levels may be observed in various bone disorders. Perhaps the highest elevations of ALP activity occur in Paget's disease (osteitisdeformans). Other bone disorders include osteomalacia, rickets, hyperparathyroidism, and osteogenic sarcoma. In addition, increased levels are observed in healing bone fractures and during periods of physiologic bone growth. In normal pregnancy, increased ALP activity, averaging approximately 1½ times the ULN, can be detected between weeks 16 and 20 and is two to three times the ULN during the third trimester. ALP activity increases and persists until the onset of labor. Activity then returns to normal within 3 to 6 days. Elevations also may be seen in complications of pregnancy such as hypertension, preeclampsia, and eclampsia, as well as in threatened abortion. ALP levels are significantly decreased in the inherited condition of hypophosphatasia. Subnormal activity is a result of the absence of the bone isoenzyme and results in inadequate bone calcification. ALP exists as a number of isoenzymes, which have been studied by a variety of techniques. The major isoenzymes, which are found in the serum and have been most extensively studied, are those derived from the liver, bone, intestine, and placenta (Bishop *et al.*, 2010).

Table (2.3): Adult Reference Rang for ALP. (Bishop *et al*., 2010).

Male/female	4-15 y	54-369	U/L
Male	20-50y	53-128	U/L
male	≤ 60y	56-119	U/L

Female	20-50y	42-98	U/L
female	≤60 y	53-141	U/L

2.5.Atorvastatine

Atorvastatin, marketed under the trade name Lipitor among others, is a member of the drug class known as statins, which are used primarily as a lipid-lowering agent and for prevention of events associated with cardiovascular disease.

Like all statins (atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin and simvastatin),(Sweetman *et al*., 2009).atorvastatin works by inhibiting HMG-CoA reeducates, an enzyme found in liver tissue that plays a key role in production of cholesterol in the body. Atorvastatin was first made in August 1985 at Warner-Lambert's Parke-Davis research facility in Ann Arbor,Michigan,(Winslow *et al.,* 2015).By a team led by Bruce Roth. Roth BD (2002), (Simons *et al*., 2003). The primary uses of atorvastatin is for the treatment of dyslipidemia and the prevention of cardiovascular disease as following: Hypercholesterolemia (heterozygous familial and non-familial) and mixed dyslipidemia to reduce total cholesterol, LDL, apo-B, triglycerides levels, and CRP as well as increase HDLlevels. Homozygous familial hypercholesterolemia, (Marais *et al.,* 1997) Hypertriglyceridemia, Primary dysbetalipoproteinemia & combine dhyperlipidemia , (Rossi *et al.,* 2006).Primary prevention of heart attack, stroke, and need for patients who have risk factors but have not yet developed clinically evident coronary heart disease. Secondary prevention of myocardial infarction, stroke, unstable angina, (Sever *et al.,* 2003).and revascularization in people with established coronary heart disease. Myocardial infarction and stroke prophylaxis in patients with type II diabetes, (Furie .,2012).

Adverse effects of atorvastatin include: Diabetes mellitus type 2, an uncommon

class effect of all statins, (Boyles *et al.*, 2015). Myopathy with elevation of creatine kinase and rhabdomyolysis are the most serious side effects, occurring rarely, (Macedo *et al.*, 2014).Persistent liver enzyme abnormalities occurred in 0.7% of patients. High dose atorvastatin have also been associated with worsening glycemic control, (Kostapanos *et al.*, 2010). Recent studies have shown that, high-dose statin treatment may play a plaque-stabilizing role. At high doses, statins have anti-inflammatory effects, incite reduction of the necrotic plaque core, and improve endothelial function, leading to plaque stabilization and, sometimes, plaque regression. However, there is an increased risk of statin-associated adverse effects with such high-dose statin treatment, (Rosa *et al*., 2014).

Chapter three
Materials & methods

3. Materials and methods

3.1 Study design:

Case-control study.

3.2 Study population:

The study included 80 Sudanese patients with ages between 20 to 70 years using atorvastatin drug (case) and those not use any lipid lowering drugs matched in age and gender with cases (control).

3.3 Study area/setting:

The study was conducted at (Al-Mualim Medical Center &Zenam Diabetic Center) Khartoum-Sudan.

3.4 Inclusion criteria:

Sudanese patients with ages between 20 to 70 years using atorvastatin drugs with different conditions (DM, HTN & protective for CVD,and those not use any lipid lowering drugs, who's voluntarily accepted to be enrolled in this study.

3.5 Exclusion criteria:

- Patients with ages less than 20 and over 70 years.
- Patients using lipid lowering drugs other than atorvastatin.
- Patients use any drugs or with disorders that affect (AST), (ALT), and (ALP) level.
- Patients with liver problem.

3.6 Sample size:

The study included 80 Sudanese volunteers selected by appropriated questionnaire.

Case: 50 patients using atorvastatin drug with different correlated diseases.

Control: 30 individuals matching in age and gender with cases but not used atorvastatin or lipid lowering drugs.

3.7 Sampling type and procedure:

3.7.1 Sample type: Venous blood sample

3.7.2 Samples collection

2.5 ml venous blood sample was obtained from each participant using standard vein puncture technique, blood specimens were collected in plain blood container, allowed to clot at room temperature for 30 minute and centrifuged at 3000 rpm for 5minutes to obtain the serum. Then serum was separated in penal and preserved at-20C until sample was analyzed.

3.8 Data Collection:

Data was collected by filing questionnaire by investigator during each time of blood samples collect.

3.8.1 Tools:

For collection and examination: questionnaire, permit, plain blood container, syringe, gloves, Sterile alcohol preps, Tourniquet, Sterile gauze pads, paper adhesive tape, centrifuge and full automated chemistry analyzer Cobas C311.

3.8.2 Study variables:

- Ages and sex.
- Atorvastatin dose.
- Duration of atorvastatin use.
- Correlated drugs used and associated diseases.

3.9 Data management and analysis:

Data was analyzed and tabulated using the Statistical Package for Social Sciences (SPSS), program version 21. T test, a crosstabs and correlation was performed with differences categorical data. P-value $\leq$ 0.05considered significant.

3.10 Ethical Considerations:

Informed consent was provided to participant and ethical approval obtained from

Sudan Universityof science and Technology research committee.

All participants gave an oral consent.

3.11 Cobas c 311 analyzer:

Information about cobas c 311 (appendix-4), web sidewww.roche.com/diagnostic.

3.12 Method of estimation:

Method used for estimation of all parameter, reagent handling, Storage and stability, calibration, quality control & calculation of result automatedcobas c311analyzer are shown in the appendices.

3.12.1Method of estimation of AST

Homogeneous enzymatic colorimetric assay (appendix-2).

3.12.2 Method of estimation of ALT

Enzymatic colorimetric assay (appendix-3).

3.12.3 Method of estimation of ALP

Enzymatic colorimetric assay (appendix-4).

3.13 Calibration:

Calibrators: **S1**: H2O, S2: Calibrator f.a.s.

Calibration mode: Linear

Calibration frequency:

2-point calibration after reagent lot change and as required following quality control procedures.

3.14 Quality control

For quality control, use control materials as listed in the "Order information" section. Other suitable control material can be used in addition. Serum/plasma for

quality control use undiluted serum control material as listed above. Other suitable control material can be used in addition.

3.15 Calculation:

Roche/Hitachi cobas c systems automatically calculate the analyst's concentration of each sample.

Chapter four

Results

4. Results

In this case-control study, 80 volunteers involved for measurement of AST, ALT, and ALP concentration in patients used atorvastatin and compare with those not use atorvastatin.

[Figure 4-1]Show percentage of gender distribution among study groups was (60%) female and (40%) male.

[Figure 4-2] Show percentage of the different dose among cases 40(mg) 16%,20 (mg) 74%, and 10(mg) 10%.

[Table 4-1]Show percentage and Frequency of different correlated condition such as DM [diabetics: frequency (43)(86%), non-diabetics: frequency (7)(14%)] and Hypertension (HTN) [Hypertension: frequency (33) (66%), Non Hypertension frequency (17)(34%)].

[Table 4-2] the means of biochemical's measured (AST, ALT, and ALP) among study population shows within normal range and there is no different between cases and control as following: for

AST (case 21.34±7.26, and control 26.03±6.52,***P***-value 0.044), ALT (case 15.46±12.88, and control 18.00±12.88, ***P***-value0.470), and

For ALP (case 85.04±20.23, and control 81.73±36.91, P-value 0.606),

[Figure 4-3, 4-4,and 4-5: shows no significant effect in AST, ALT, and ALP concentration when compare with duration use of drug (***P***-value: 0.213, 0.999, and 0.701 respectively).

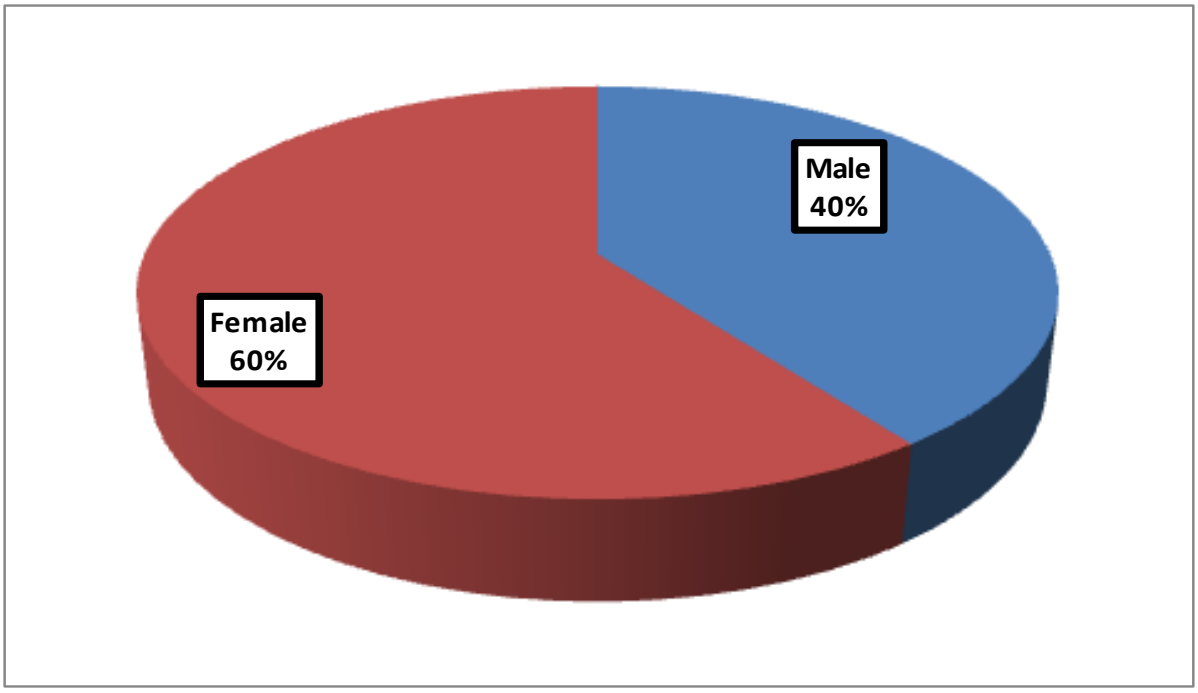

Figure (4-1): percentage of gender distribution among the study group.

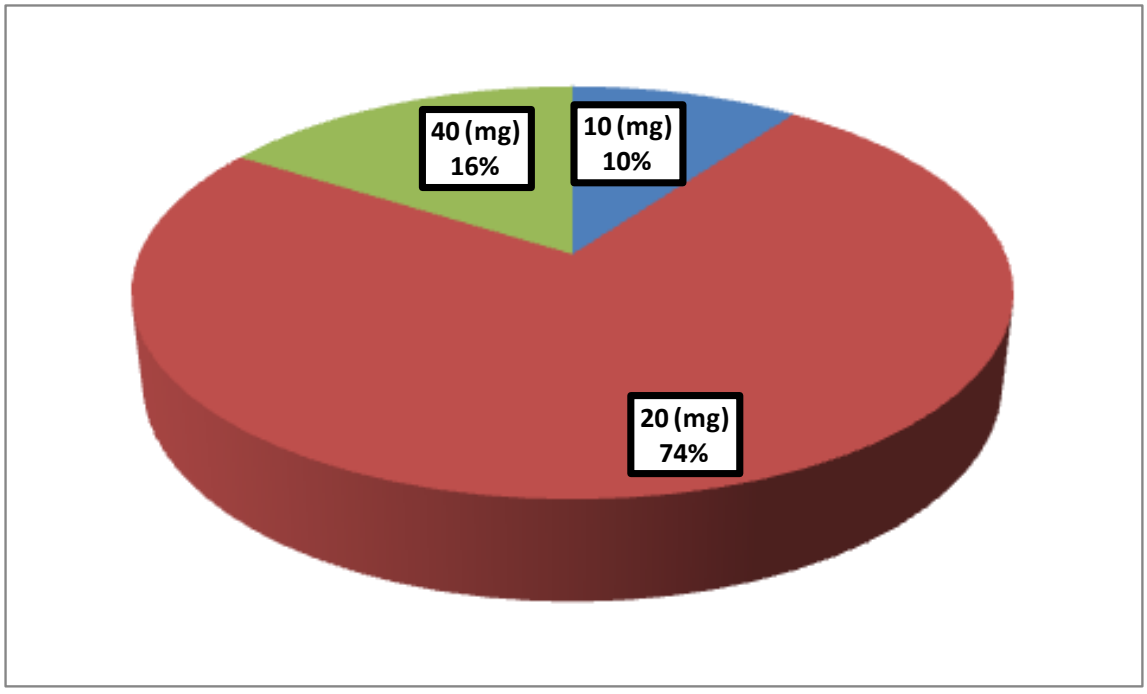

Figure (4-2): percentage of the different drug dose of Atorvastatin among case group study.

Table (4-1): percentage and Frequency of conditions among the case study group.

Variables	Frequency	Percentage (%)
D.M		
Yes	43	86.0
No	7	14.0
HTN		
Yes	33	66.0
No	17	34.0
Total	50	100.0

Table (4-2): Comparison of biochemical measured among the study group

Parameters	Case (Mean±SD)	Control (Mean±SD)	*P-value*
AST (IU/L)	21.34±7.26	26.03±6.52	0.044
ALT (IU/L)	15.46±12.88	18.00±12.88	0.470
ALP (IU/L)	85.04±20.23	81.73±36.91	0.606

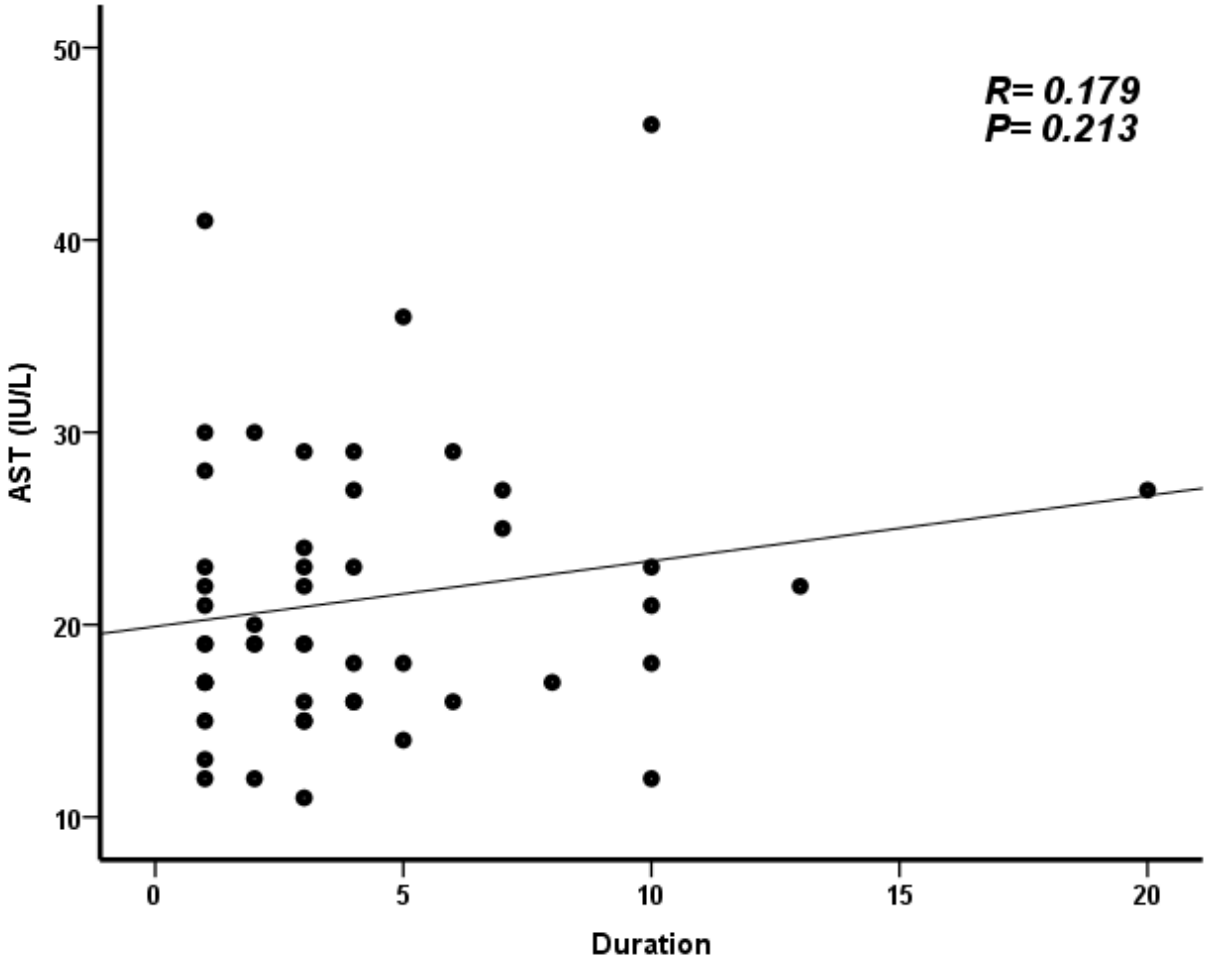

Figure (4-3): Correlation between AST concentration and duration of Atorvastatinuse.

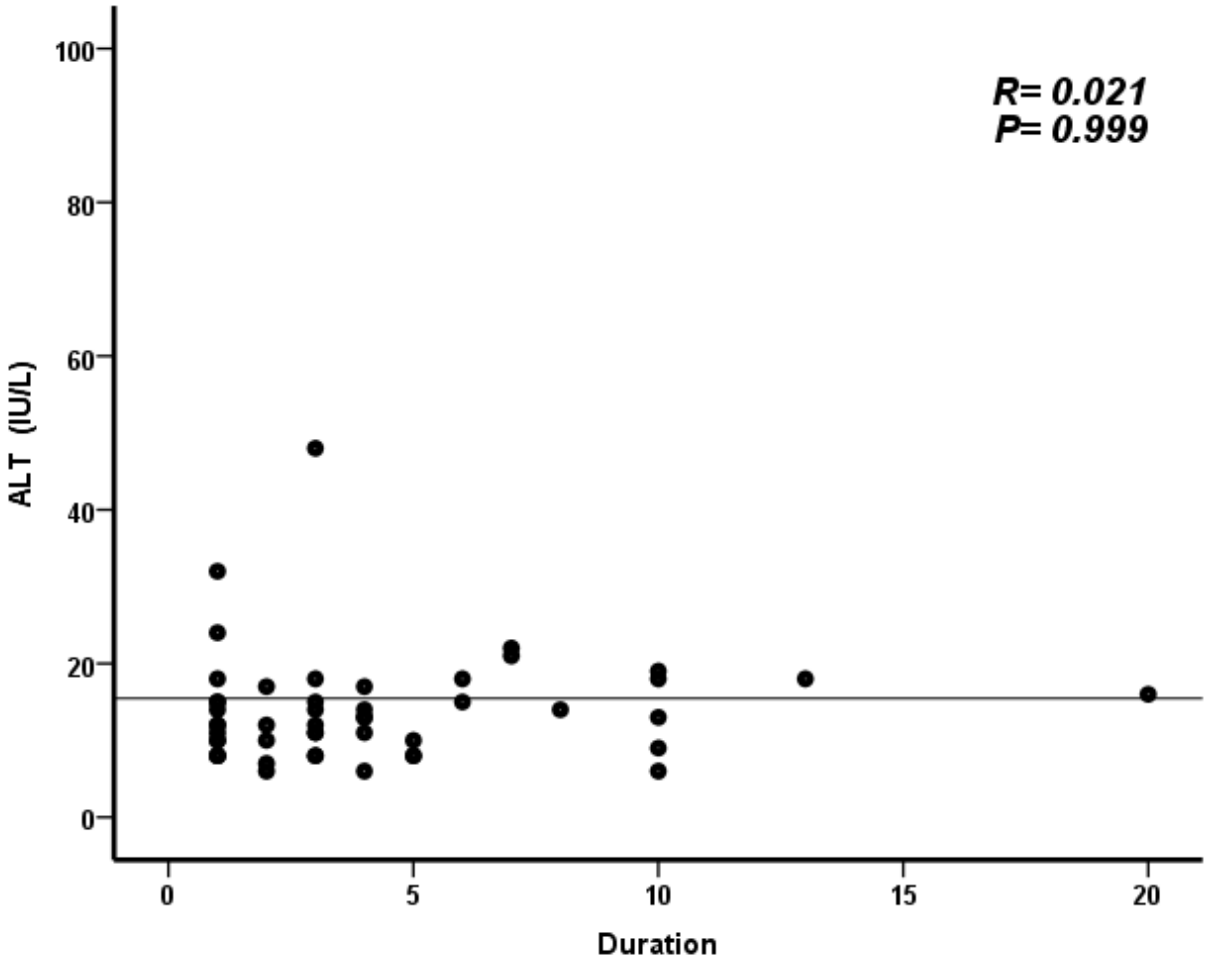

Figure (4-4): Correlation between ALT concentration and duration of Atorvastatin use

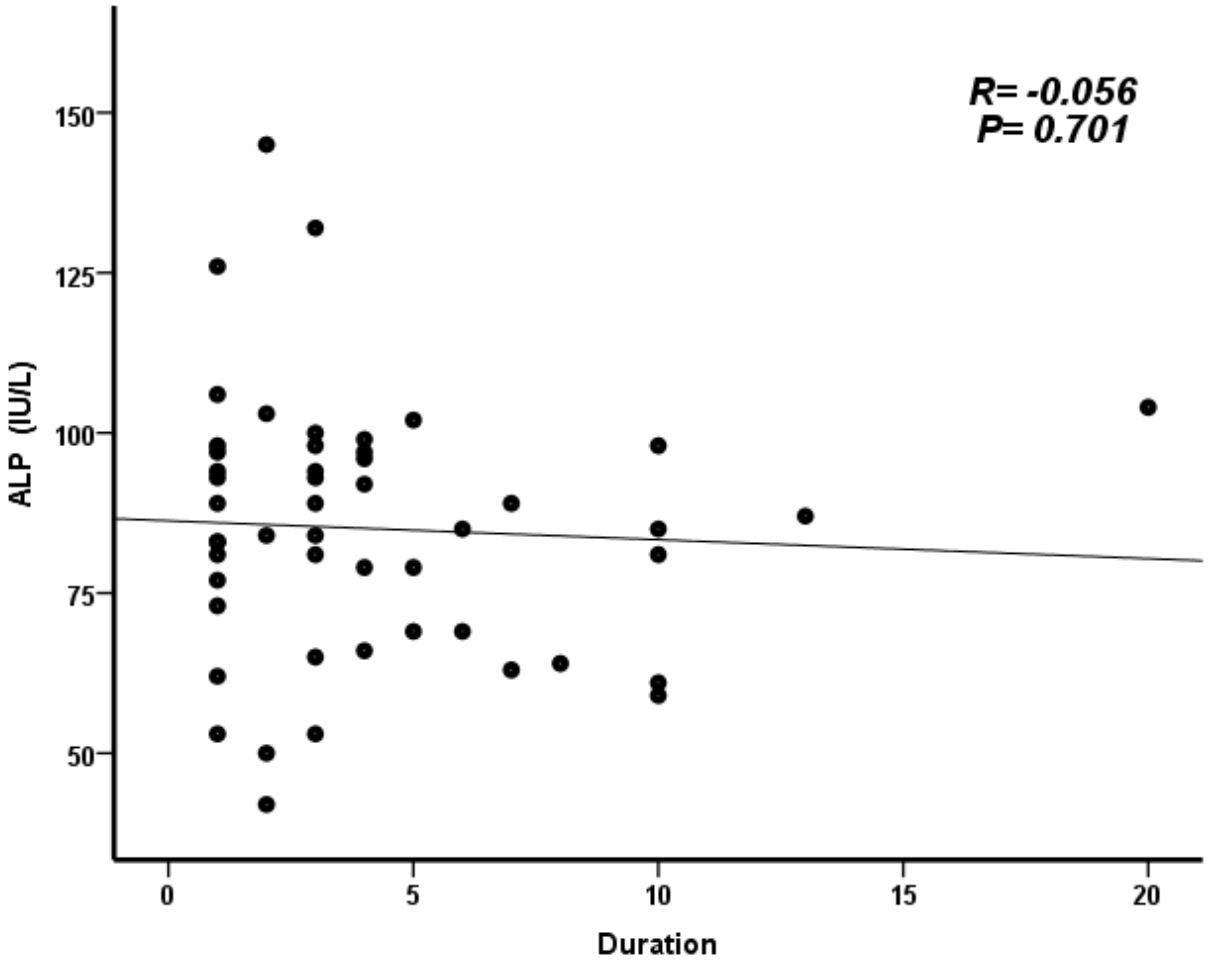

Figure (4-5): Correlation between ALP concentration and duration of Atorvastatin use

Chapter five

Discussion, Conclusion & Recommendation

5.1 Discussion

Atorvastatin is hypolipidemic drug that mainly reduce TCho& LDL by inhibiting HMG-CoA reeducates, an enzyme plays a key role in production of cholesterol in the body, (Sweetman *et al.*, 2009).Atorvastatin like other lipid lowering therapies, have been associated with biochemical abnormalities of liver function(Walsh *et al.*, 1999).

Our results in accordance with earlier studied showed that atorvastatin use has been associated with elevations in serum alanine aminotransferase (ALT) levels. Such elevations are not clinically significant.Serum aminotransferases generally resolve (Thapar *et al.*, 2013). It was found that in 1% of patients receiving low to intermediate atorvastatin dose (10 - 40 mg daily) and in 2% - 3% of patients on high dose therapy (80 mg/daily) ALT elevation occurs, (Cohen *et al.*, 2006). In the present study shows there is no significant effect on liver aminotransferases and atorvastatin use by (*P*-value 0.470) for ALT, for AST (*P*-value 0.044) and for ALP (*P*-value 0.606).The previous study shown the Patterns of liver abnormalities seen with atorvastatin include: asymptomatic elevations of ALT: usually transient and mild (ALT <3 × ULN),hepatitis: with ALT >3 × ULN and clinical symptoms of liver disease, autoantibody-associated liver injury(Russo *et al.*, 2009).The mechanisms behind these adverse effects are unclear, but a few possibilities have been suggested. It has been noted that atorvastatin can induce a transient acute phase response on initiation, especially at high doses and this may represent a transient chemical hepatitis due to disturbance of the Cholesterol-bile acid pathways, (Wierzbicki *et al.*, 2003).Moreover, it was speculated that the increased transaminases serum activity reflects alterations to the hepatocellular membrane (e.g., enhanced permeability) that permit leakage of these intracellular proteins, (Tolman, 2002).

Therefore, it was shown that Atorvastatin use affected the liver, with elevation of

transaminases (ALT being more specific to liver insult), and a complete normalization upon stopping the drug, suggesting no structural injury, a condition usually identified as “transaminitis”, which is asymptomatic, dose-related and reversible, (Tolman, 2002).elevations in AST levels higher than those of ALT and not dropping to normal levels after discontinuing the drug. But in the study under discussion shows no correlation between the levels of AST, ALT and ALP and duration of drug use by (*P*-value 0.231, 0.999, 0.701respectively).

5.2 Conclusion

In conclusion this study shows that is in significant difference in AST, ALT and ALP activity between cases and controls .Insignificant correlation between different doses of atorvastatin drug and concentration of AST, ALT and ALP.

There is no correlation between duration of drug use and concentration of AST, ALT and ALP.

5.3 Recommendation:

1. Although the recent study did not detected significant change in liver enzymes, patients under treatment with Atorvastatin should be monitored.
2. Further studies should be done to cover more dose and longer duration.
3. Atorvastatin can use by persons with family history of hyperlipidemia for protection.

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Appendices

Appendix I

Sudan University of Science and Technology
College of Graduate studies
Department of Clinical Chemistry

Estimation of serum aspartateaminotranseferase, alanine aminotransferase, alkaline phosphatase, total cholesterol and LDL among Sudanese using Atorvastatin drug

Questionnaire NO ( )

A-General Information:

-Name:……

- Age: ( ) years. -Gender: Male ( ), Female ( )

B- Inclusion Criteria and Exclusion Criteria:

- Use atorvastatin: Yes ( ) No ( ) Dose: ……..... / Duration: ……....

- DM: Yes ( ) No ( )

- Hypertension: Yes ( ) No ( )

- Liver problem: Yes ( ) No ( )

D- Investigation results:

-Serum AST result:.....IU/L

-Serum ALT result:.....IU/L

-Serum ALP result:.....U/L

-Date: ……/……/……. **Signature:**

……..

Appendix II

The clinical chemistry analyzer cobas c 311

5th generation of routine and dedicated chemistry experiences

cobas® 4000 analyzer series **5th generation**

MODULAR® ANALYTICS 4th generation

COBAS INTEGRA 3rd generation

Hitachi **900** series 2nd generation

Hitachi **700** series 1st generation

Appendix III