

1.1.Introduction

The liver is the largest internal organ of the human body. It is a functionally complex organ that plays a critical biochemical role in the metabolism, digestion, detoxification, and elimination of substances from the body.

One of the liver diseases is cirrhosis that 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. Although some patients with cirrhosis may have prolonged survival, they generally have a poor prognosis (Bishop *et al.*, 2010).

Cholinesterase belongs to a family of enzymes that catalyze the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid; it has two types: Acetylcholinesterase, also known as RBC or erythrocyte cholinesterase found in blood on RBCs cell membrane in neuromuscular junctions, and in neural synapses; and Pseudocholinesterase, also known as plasma cholinesterase produced by the liver (Wang *et al.*, 2005) (Chandok *et al.*, 2010).

1.2 Rationale

Plasma Cholinesterase is synthesized mainly in hepatocytes and is released into the blood (Vincent, 1974).

Plasma cholinesterase activity is reduced in liver dysfunction due to reduced synthesis. This is in contrast to other serum enzymes associated with the clinical assessment of liver function whose activities increase as a result of enhanced release from their cellular sources following cell membrane damage. (Zhou *et al.*, 2003)

This study was aimed to know the significant value of serum cholinesterase as liver function test among Sudanese cirrhotic patients.

1.3. objectives

1.3.1. General objectives

To assess the value of serum cholinesterase as liver function test among Sudanese cirrhotic patients.

1.3.2. Specific objectives

To assess the values of cholinesterase enzyme.

To determine the values of PT and albumin level.

To comparison between PT, albumin level and cholinesterase activity.

To assess synthetic power of the liver.

2. Literature Review

2.1 The liver

The liver is the largest internal organ in the body. In the healthy adult, it weighs about 3 pounds. The liver is wedge-shaped, with the top part wider than the bottom. It is located right below the diaphragm and occupies the entire upper right quadrant of the abdomen. The liver performs over 500 vital functions. Damage to the liver can impair these and many other processes (Bishop *et al.*, 2010).

2.1.1 Liver functions

Digestion

The liver plays an active role in the process of digestion through the production of bile. Bile is a mixture of water, bile salts, cholesterol, and the pigment bilirubin. Hepatocytes in the liver produce bile, which then passes through the bile ducts to be stored in the gallbladder. When food containing fats reaches the duodenum, the cells of the duodenum release the hormone cholecystokinin to stimulate the gallbladder to release bile. Bile travels through the bile ducts and is released into the duodenum where it emulsifies large masses of fat. The emulsification of fats by bile turns the large clumps of fat into smaller pieces that have more surface area and are therefore easier for the body to digest.

Bilirubin present in bile is a product of the liver's digestion of worn out red blood cells. Kupffer cells in the liver catch and destroy old, worn out red blood cells and pass their components on to hepatocytes. Hepatocytes metabolize hemoglobin, the red oxygen-carrying pigment of red blood cells, into the components heme and globin. Globin protein is further broken down and used as an energy source for the body. The iron-containing heme group cannot be recycled by the body and is converted into the pigment bilirubin and added to bile to be excreted from the body. Bilirubin gives bile its distinctive greenish color. Intestinal bacteria further

convert bilirubin into the brown pigment stercobilin, which gives feces their brown color (Geschwind*et al.*,2004).

Metabolism

The hepatocytes of the liver are tasked with many of the important metabolic jobs that support the cells of the body. Because all of the blood leaving the digestive system passes through the hepatic portal vein, the liver is responsible for metabolizing carbohydrate, lipids, and proteins into biologically useful materials.

Our digestive system breaks down carbohydrates into the monosaccharide glucose, which cells use as a primary energy source. Blood entering the liver through the hepatic portal vein is extremely rich in glucose from digested food. Hepatocytes absorb much of this glucose and store it as the macromolecule glycogen, a branched polysaccharide that allows the hepatocytes to pack away large amounts of glucose and quickly release glucose between meals. The absorption and release of glucose by the hepatocytes helps to maintain homeostasis and protects the rest of the body from dangerous spikes and drops in the blood glucose level. (See more about glucose in the body.)

Fatty acids in the blood passing through the liver are absorbed by hepatocytes and metabolized to produce energy in the form of ATP. Glycerol, another lipid component, is converted into glucose by hepatocytes through the process of gluconeogenesis. Hepatocytes can also produce lipids like cholesterol, phospholipids, and lipoproteins that are used by other cells throughout the body. Much of the cholesterol produced by hepatocytes gets excreted from the body as a component of bile.

Dietary proteins are broken down into their component amino acids by the digestive system before being passed on to the hepatic portal vein. Amino acids entering the liver require metabolic processing before they can be used as an energy source. Hepatocytes first remove the amine groups of the amino acids and

convert them into ammonia and eventually urea. Urea is less toxic than ammonia and can be excreted in urine as a waste product of digestion. The remaining parts of the amino acids can be broken down into ATP or converted into new glucose molecules through the process of gluconeogenesis (A.burtis*etal.*,2008).

Detoxification

As blood from the digestive organs passes through the hepatic portal circulation, the hepatocytes of the liver monitor the contents of the blood and remove many potentially toxic substances before they can reach the rest of the body. Enzymes in hepatocytes metabolize many of these toxins such as alcohol and drugs into their inactive metabolites. And in order to keep hormone levels within homeostatic limits, the liver also metabolizes and removes from circulation hormones produced by the body's own glands(Bishop*etal.*,2010).

Storage

The liver provides storage of many essential nutrients, vitamins, and minerals obtained from blood passing through the hepatic portal system. Glucose is transported into hepatocytes under the influence of the hormone insulin and stored as the polysaccharide glycogen. Hepatocytes also absorb and store fatty acids from digested triglycerides. The storage of these nutrients allows the liver to maintain the homeostasis of blood glucose. Our liver also stores vitamins and minerals - such as vitamins A, D, E, K, and B12, and the minerals iron and copper - in order to provide a constant supply of these essential substances to the tissues of the body (Salem *etal.*,2011).

Production

The liver is responsible for the production of several vital protein components of blood plasma: prothrombin, fibrinogen, and albumins. Prothrombin and fibrinogen proteins are coagulation factors involved in the formation of blood clots. Albumins are proteins that maintain the isotonic environment of the blood so that cells of the

body do not gain or lose water in the presence of body fluids (Geschwind *et al.*, 2004).

Immunity

The liver functions as an organ of the immune system through the function of the Kupffer cells that line the sinusoids. Kupffer cells are a type of fixed macrophage that form part of the mononuclear phagocyte system along with macrophages in the spleen and lymph nodes. Kupffer cells play an important role by capturing and digesting bacteria, fungi, parasites, worn-out blood cells, and cellular debris. The large volume of blood passing through the hepatic portal system and the liver allows Kupffer cells to clean large volumes of blood very quickly (Geschwind *et al.*, 2004).

2.2. Liver cirrhosis

2.2.1. Definition and causes

Cirrhosis is a clinical condition in which scar tissue replaces normal, healthy liver tissue so prevents the liver from functioning properly. (Bishop *et al.*, 2010)

Also defined anatomically as diffuse fibrosis with nodular regeneration, represent the end stage of scar formation and regeneration in chronic liver injury (A. Burtis *et al.*, 2008).

Causes

Alcoholism

Chronic alcoholism particularly endangers the liver by causing alcoholic liver disease (also called alcohol-induced liver disease). Alcoholic liver disease includes fatty liver (build-up of fat cells in the liver), alcoholic hepatitis (inflammation of the liver caused by heavy drinking), and alcoholic cirrhosis. Alcoholic cirrhosis is the primary type of cirrhosis in the U.S. It develops in 10 - 20% of heavy drinkers, usually after 10 - 15 years of heavy alcohol consumption. People who drink heavily and who also have hepatitis C are at particular risk of developing cirrhosis.

In the liver, alcohol converts to toxic chemicals that trigger inflammation and tissue injury, which lead to cirrhosis(Berg *etal*,2007).

Chronic Viral Hepatitis

Chronic viral hepatitis, both hepatitis B and hepatitis C, is another primary cause of cirrhosis. Chronic hepatitis C is a more common cause of cirrhosis in developed countries, while hepatitis B is a more common cause of cirrhosis worldwide, especially in sub-Saharan Africa and parts of Asia. People with chronic hepatitis B who are co-infected with hepatitis D are especially at risk for cirrhosis. The longer a patient has had chronic hepatitis, the greater the risk for eventually developing cirrhosis.Hepatitis viruses can produce inflammation in liver cells, causing injury or destruction. If the condition is severe enough, the cell damage becomes progressive, leading to scar tissue in the liver. In advanced cases, the liver shrivels in size, a condition called postnecrotic or posthepatic cirrhosis.

Hepatitis C is a virus-caused liver inflammation which may lead to jaundice, fever, and cirrhosis. The people most at risk for contracting and spreading hepatitis C are those who share needles for injecting drugs and health care workers or emergency workers who may be exposed to contaminated blood.(Brown, 2008)

Autoimmune Hepatitis

Autoimmune hepatitis, like other autoimmune disorders, develops when a misdirected immune system attacks the body's own cells and organs. People who have autoimmune hepatitis also often have other autoimmune conditions, including systemic lupus erythematosus, rheumatoid arthritis, Sjögren syndrome, scleroderma, inflammatory bowel disease, glomerulonephritis, and hemolytic anemia. Autoimmune hepatitis typically occurs in women ages 15 - 40(Cardenas *et al.*,2011).

Bile Ducts Disorders

Disorders that block or damage the bile ducts can cause bile to back up in the liver, leading to inflammation and cirrhosis. These diseases include primary biliary cirrhosis and primary sclerosing cholangitis. Primary Biliary Cirrhosis. Up to 95% of primary biliary cirrhosis (PBC) cases occur in women, usually around age 50. In people with PBC, the immune system attacks and destroys cells in the liver's bile ducts. Like many autoimmune disorders, the causes of PBC are unknown. Primary Sclerosing Cholangitis. Primary sclerosing cholangitis (PSC) is a chronic disease that mostly affects men, usually around age 40. The cause is unknown, but immune system defects, genetics, and infections may play a role. (Chalasani *et al.*, 2012).

Nonalcoholic Fatty Liver Disease (NAFLD) and Nonalcoholic Steatohepatitis (NASH)

Nonalcoholic fatty liver disease (NAFLD) resembles alcoholic liver disease, but it occurs in people who do not drink a lot of alcohol. NAFLD is the most common liver disease in the United States. NAFLD is actually a progressive spectrum of liver diseases that include; firstly Nonalcoholic fatty liver (NAFL), or fatty liver, is the earliest stage of NAFLD. It is marked by the presence of fat in the liver (steatosis), but liver damage has not occurred. While a fatty liver is not normal, NAFL is not considered a serious condition. Secondly Nonalcoholic steatohepatitis (NASH) is the next stage of NAFLD. NASH is characterized by liver inflammation and injury, as well as a fatty liver. NASH is dangerous because it can lead to the scarring of the liver associated with cirrhosis. NASH is one of the leading causes of cirrhosis. Thirdly Cirrhosis is the final irreversible stage of NAFLD. Obesity and type 2 diabetes are the two main causes of NAFLD. Metabolic syndrome is another major factor. Metabolic syndrome is a collection of risk factors that include abdominal obesity, unhealthy blood lipid levels, high blood pressure, and insulin resistance. Nonalcoholic fatty liver disease is usually benign and very slowly

progressive. But, in certain patients, it can lead to cirrhosis and eventual liver failure. NAFLD also increases the risk for heart disease, which is the leading cause of death for these patients(Chapman *etal.*,2010).

Hereditary Disorders

Hemochromatosis is a disorder of iron metabolism. This disease interferes with the way the body normally handles iron. People with hemochromatosis absorb too much iron from the food they eat. The iron overload accumulates in organs in the body. When excess iron deposits accumulate in the liver, they can cause cirrhosis. Other Hereditary Disorders. Other inherited diseases that can cause cirrhosis include Wilson's disease (which causes an accumulation of copper in the body), alpha-1 antitrypsin deficiency (a genetic disorder caused by defective production of a particular enzyme), and glycogen storage diseases (a group of disorders that cause abnormal amounts of glycogen to be stored in the liver) (Chandok *Netal.*,2010).

Other Causes

Schistosomiasis, a disease caused by a parasite found in the Asia, Africa, and South America. And long-term or high-level exposure to certain chemicals and drugs, including arsenic, methotrexate, toxic doses of vitamin A, and certain prescription medication (Chandok *etal.*,2010).

2.2.2. Stages and Symptoms of liver cirrhosis

Cirrhosis is divided into two stages: the first stage is Compensated cirrhosis means that the body still functions fairly well despite scarring of the liver. Many people with compensated cirrhosis experience few or no symptoms. and second stage is Decompensated cirrhosis means that the severe scarring of the liver has damaged and disrupted essential body functions. Patients with decompensated cirrhosis develop many serious and life-threatening symptoms and complications. Early symptoms of compensated cirrhosis may include: Fatigue and loss of energy, Loss

of appetite and weight loss, Nausea or abdominal pain, Spider angiomas may develop on the skin. These are pinhead-sized red spots from which tiny blood vessels radiate. As cirrhosis progresses to a decompensated stage, patients may develop the following symptoms: Fluid buildup in the legs and feet (edema) and in the abdomen (ascites). (Ascites is associated with portal hypertension, which is described in the Complications section of this report), Jaundice This yellowish cast to the skin and eyes occurs because the liver cannot process bilirubin for elimination from the body, Itching (pruritus) develops from buildup of bile products, The palms of the hands may be reddish and blotchy, a condition known as palmar erythema, In men swelling of breasts or shrinkage of the testicles may occur, Easy bruising and excessive bleeding may occur (Limetal, 2009).

2.2.3. Complications

A damaged liver affects almost every bodily process, including the functions of the digestive, hormonal, and circulatory systems. Decompensated cirrhosis increases the risk of serious and potentially life-threatening complications. (Once decompensation occurs, mortality rates without liver transplantation can be as high as 85% within 5 years.) The most serious complications are those associated with portal hypertension (increased pressure in the portal vein that carries blood from the intestine to the liver), that include:

Firstly ascites is fluid buildup in the abdominal cavity. It is uncomfortable and can impair breathing and other functions. Ascites is caused by a combination of portal hypertension (high pressure in the blood vessels of the liver) and low albumin levels. Albumin is a protein produced by the liver. Although ascites itself is not fatal, it is a marker for severe progression. Hepatorenal syndrome occurs if the kidneys drastically reduce their own blood flow in response to the altered blood flow in the liver. It is a life-threatening complication of late-stage liver disease that occurs in patients with ascites. Symptoms include dark colored urine and a

reduction in volume, yellowish skin, abdominal swelling, mental changes (such as delirium and confusion), jerking or coarse muscle movement, nausea, and vomiting (Sanyaletal.,2007).

Secondly variceal Bleeding;its one of the most serious consequences of portal hypertension is the development of varices, veins that enlarge to provide an alternative pathway for blood diverted from the liver. In most patients, they form in the esophagus. They can also form in the upper stomach. Varices pose a high risk for rupture and bleeding because they are thin-walled, twisted, and subject to high pressure. Variceal intestinal bleeding is a life-threatening event. Symptoms include vomiting blood or black and tarry stools (Garciaetal., 2010) (Gonzalez *etal.*,2008).

Thirdly Spontaneous bacterial peritonitis is a life-threatening bacterial infection of the membrane that lines the abdomen. The main symptoms include confusion and altered mental status, fever, chills, and abdominal pain.(Ginès Petal,2009) Fourthly Hepatic EncephalopathyMental impairment is a common event in advanced cirrhosis. In severe cases, the disease causes encephalopathy (impaired brain function), with mental symptoms that range from confusion to coma and death. Hepatic encephalopathy is caused by a buildup in the blood of harmful intestinal toxins, particularly ammonia, which then accumulate in the brain. Encephalopathy can be triggered by many different conditions including internal bleeding, infection, constipation, and dehydration. early symptoms of hepatic encephalopathy include confusion, forgetfulness, and trouble concentrating. Sudden changes in the patient's mental state, including agitation or confusion, may indicate an emergency condition. Other symptoms include fruity-smelling bad breath and tremor. Late-stage symptoms of encephalopathy are stupor and eventually coma (Lindor, 2010).

And liver CancerPeople with cirrhosis have an increased risk for hepatocellular carcinoma, a type of liver cancer. Hepatitis B and C, alcoholism, hemo-

chromatosis, and primary biliary cirrhosis -- all causes of cirrhosis -- are some of the major risk factors for liver cancer. Cirrhosis due to hepatitis C is the leading cause of hepatocellular carcinoma in the United States(Lindor, 2010) (Gershwin *etal.*,2004).

Other Complications

Kidney Failure. Portal hypertension and spontaneous bacterial peritonitis can cause several secondary complications, including kidney failure (Gines*etal.*,2009) (Shah *etal.*,2010).

(Nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen (Advil, Motrin, generic), naproxen (Aleve, generic), and aspirin -- can also cause kidney failure in patients with cirrhosis(Gines*etal.*,2009).

Osteoporosis. Many patients with cirrhosis develop osteoporosis, a bone-thinning disease.Osteoporosis is a condition characterized by progressive loss of bone density, thinning of bone tissue, and increased vulnerability to fractures. Osteoporosis may result from disease, dietary or hormonal deficiency, or advanced age. Regular exercise and vitamin and mineral supplements may reduce and even reverse loss of bone density.

Insulin Resistance and Type 2 Diabetes. Cirrhosis often causes insulin resistance, a primary feature in type 2 diabetes. As insulin resistance progresses, it causes excess sugar (glucose) to build up in the blood, which leads to type 2 diabetes. In turn, type 2 diabetes is also a risk factor for nonalcoholic fatty liver disease, one of the causes of cirrhosis.**Heart Problems.** Cirrhosis may increase the risk for heart failure and other cardiovascular complications (Marchesini*etal.*,2008).

2.2.4. Diagnosis

A physical examination may reveal the following in a patient with cirrhosis.The cirrhotic liver is firm and often enlarged in early stages of the disease. The liver may feel rock-hard. (In advanced stages of cirrhosis, the liver may become small

and shriveled.)If the abdomen is swollen, we will check for ascites by tapping the flanks and listening for a dull thud and feeling the abdomen for a shifting wave of fluid. And also check for signs of jaundice, muscle wasting, and (in male patients) breast enlargement.A patient's medical history is another indicator of the risk for cirrhosis. Patients with a history of alcoholism, hepatitis B or C, or certain other medical conditions are at high risk.Other tests (blood tests, imaging tests, liver biopsy) may also be performed. The results of these tests along with the presence of specific complications (ascites and encephalopathy) are used for calculating the Child-Pugh Classification. This is a staging system (A to C) that helps to determine the severity of cirrhosis and predict the development of future complication(Parikh *etal.*,2007).

Blood Tests

A patient's medical history can reveal risk factors (such as alcoholism) that warrant screening for conditions such as hepatitis. Blood tests are also performed to measure liver enzymes associated with liver function. Enzymes known as aminotransferases, including aspartate transaminase (AST) and alanine transaminase (ALT), are released when the liver is damaged. Blood tests may also measure serum albumin concentration. Serum albumin measures the protein in the blood (low levels indicate poor liver function).Prothrombin time (PT) this test measures in seconds the time it takes for blood clots to form (the longer it takes the greater the risk for bleeding).Alkaline phosphatase (ALP). High ALP levels can indicate bile duct blockage.Bilirubin One of the most important factors indicative of liver damage is bilirubin, a red-yellow pigment that is normally metabolized in the liver and then excreted in the bile. In patients with hepatitis, the liver cannot process bilirubin, and blood levels of this substance rise, sometimes causing jaundice (Schuppan*etal*,2008).

Imaging Tests

Magnetic resonance imaging (MRI), computed tomography (CT), and ultrasound are all imaging techniques that are useful in detecting and defining the complications of cirrhosis, such as ascites and hepatocellular carcinoma. These imaging tests can also provide information on the extent of liver damage (Schuppan *et al.*, 2008).

Liver Biopsy

A liver biopsy is the only definite method for confirming a diagnosis of cirrhosis. It also helps determine its cause, treatment possibilities, the extent of damage, and the long-term outlook. The biopsy may be performed using various approaches, including: Firstly Percutaneous Liver Biopsy. This approach uses a needle inserted through the skin over the liver area to obtain a tissue sample from the liver. Various forms of needles are used, including those that use suction or those that cut out the tissue. This approach should not be used in patients with bleeding problems, and it must be used with caution in patients with ascites or severe obesity (Salerno *et al.*, 2007).

Secondly Transjugular Liver Biopsy This approach uses a catheter (a thin tube) that is inserted in the jugular vein in the neck and threaded through the hepatic vein (which leads to the liver). A needle is passed through the tube, and a suction device collects liver samples. This procedure is risky but may be used for patients with severe ascites (Salerno *et al.*, 2007).

Thirdly Laparoscopy. This procedure requires a small abdominal incision through which inserts a thin tube that contains small surgical instruments and a tiny camera to view the surface of the liver. This is generally reserved for staging liver cancer or for ascites of unknown cause. (Salerno *Fetal*, 2007)

2.3. Cholinesterase

2.3.1. definition and type

cholinesterase is a family of enzymes that catalyze the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid, a reaction necessary to allow a cholinergic neuron to return to its resting state after activation (Huang *et al.*, 2007).

Types of cholinesterase

There are two types of cholinesterase; firstly: Acetylcholinesterase (EC3.1.1.7) (AChE), also known as RBC cholinesterase, erythrocyte cholinesterase, or (most formally) acetylcholine acetylhydrolase, found primarily in the blood on red blood cell membranes, in neuromuscular junctions, and in neural synapses. Acetylcholinesterase exists in multiple molecular forms. In the mammalian brain the majority of AChE occurs as a tetrameric, G4 form with much smaller amounts of a monomeric G1 (4S) form. (Wang *et al.*, 2005) (Chandok *et al.*, 2010)

Secondly: Pseudocholinesterase (EC3.1.1.8) (BChE or BuChE), also known as plasma cholinesterase, butyrylcholinesterase, or (most formally) acylcholine acylhydrolase, is produced in the liver and found primarily in plasma. The half-life of pseudocholinesterase is approximately 8–16 hours. (Wang *et al.*, 2005)

The difference between the two types of cholinesterase has to do with their respective preferences for substrates: the first hydrolyses acetylcholine more quickly; but the second hydrolyses butyrylcholine more quickly. (Brash) (Miller)

2.3.2. clinical significance

Pseudocholinesterase levels may be reduced in patients with advanced liver disease. The decrease must be greater than 75% before significant prolongation of neuromuscular blockade occurs with succinylcholine (Chatterjee *et al.*, 2005). An absence or mutation of the pseudocholinesterase enzyme leads to a medical condition known as pseudocholinesterase deficiency. This is a silent condition that

manifests itself only when people that have the deficiency receive the muscle relaxants succinylcholine or mivacurium during a surgery(Chatterjea*etal.*, 2005).

Pseudocholinesterase deficiency may also affect local anesthetic selection in dental procedures. The enzyme plays an important role in the metabolism of ester-based local anesthetics, a deficiency lowers the margin of safety and increases the risk of systemic effects with this type of anesthetic. The selection of an amide-based solution is recommended in such patients (Chatterjea*etal.*, 2005).

Elevation of plasma pseudocholinesterase was observed in 90.5% cases of acute myocardial infarction(Chatterjea*etal.*, 2005).

The presence of acetylcholinesterase in the amniotic fluid may be tested in early pregnancy. A sample of amniotic fluid is removed by amniocentesis, and presence of AChE can confirm several common types of birth defect, including abdominal wall defects and neural tube defects(FBR Resource Guide, 2007).

Butyrylcholinesterase or plasma cholinesterase (most formally) acylcholineacylhydrolase can be used as a prophylactic agent against nerve gas and other organophosphate poisoning(Huang *etal.*,2007).

2.3.3. Cholinesterase inhibitor

A cholinesterase inhibitor or anticholinesterase suppresses the action of the enzyme. Because of its essential function, chemicals that interfere with the action of cholinesterase are potent neurotoxins, causing excessive salivation and eye-watering in low doses, followed by muscle spasms and ultimately death (examples are some snake venoms, and the nerve gases sarin and VX). One counteracting medication is pralidoxime. The so-called nerve gases and many substances used in insecticides have been shown to act by combining with a residue of serine in the active site of acetylcholine esterase, inhibiting the enzyme completely. The enzyme acetylcholine esterase breaks down the neurotransmitter acetylcholine, which is released at nerve and muscle junctions, in order to allow the muscle or

organ to relax. The result of acetylcholine esterase inhibition is that acetylcholine builds up and continues to act so that any nerve impulses are continually transmitted and muscle contractions do not stop. Among the most common acetylcholinesterase inhibitors are phosphorus-based compounds, which are designed to bind to the active site of the enzyme. The structural requirements are a phosphorus atom bearing two lipophilic groups, a leaving group (such as a halide or thiocyanate), and a terminal oxygen. The entry on Lawesson's reagent has some details on one sub-class of the phosphorus-based compounds. Some benzodiazepines, e.g. temazepam have an inhibitory effect on cholinesterase (Holmes *et al.*, 1978).

Also anticholinesterases are also used for reversing medication induced paralysis during anesthesia; as well as in the treatment of myasthenia gravis, glaucoma, and Alzheimer's disease. Such compounds are used for killing insects in a range of products including sheep dip, organophosphate pesticides, and carbamate pesticides. In addition to acute poisoning as described above, a semi-acute poisoning characterized by strong mental disturbances can occur. Also, prolonged exposure can cause birth defects (Holmes *et al.*, 1978).

2.4. Albumin

Albumin is synthesized in the liver from 585 amino acids at the rate of 9–12 grams per day with no reserve or storage. It is the protein present in highest concentration in the plasma. Albumin also exists in the extravascular (interstitial) space. In fact, the total extravascular albumin exceeds the total intravascular amount by 30%, but the concentration of albumin (plasma albumin concentration intravascular albumin mass/plasma volume) in the blood is much greater than its concentration in the interstitial space. Albumin leaves the circulation at a rate of 4%–5% of total intravascular albumin per hour. This rate of movement is known as

the transcapillary escape rate (TER), which measures systemic capillary efflux of albumin (Bishop *et al.*, 2010).

2.4.1. Albumin functions

responsible for nearly 80% of the colloid osmotic pressure of the intravascular fluid, buffers pH and is a negative acute-phase reactant protein, bind various substances in the blood and transports thyroid hormones; other hormones, particularly fat-soluble ones; iron; and fatty acids (Kazuyoshi *et al.*, 1998).

2.4.2. Clinical significance of albumin

Decreased concentrations of serum albumin may be caused by the following:

An inadequate source of amino acids that occurs in malnutrition and malabsorption, Liver disease, resulting in decreased synthesis by the hepatocytes (note that the increase in globulins that occurs in early cirrhosis, however, balances the loss in albumin to give a total protein concentration within acceptable limits), Protein-losing enteropathy or gastrointestinal loss as interstitial fluid leaks out in inflammation and disease of the intestinal tract as in diarrhea, Kidney loss to the urine in renal disease (Christenson *et al.*, 2008).

Albumin is normally excreted in very small amounts. This excess excretion occurs when the glomerulus no longer functions to restrict the passage of proteins from the blood as in nephrotic syndrome (Christenson *et al.*, 2008).

2.5. Prothrombin Time (PT)

The PT is functional determination of the extrinsic (tissue factor) pathway of coagulation and is extremely sensitive to the vitamin-K dependent clotting factors (factors II, VII, and X). Tissue factor (factor III) is a transmembrane protein that is widely expressed on cells of non-vascular origin, which activates factor VII during the initiation of the extrinsic coagulation pathway. A cascade mechanism results in fibrin production and clot formation.

The PT is a widely used laboratory assay for the detection of inherited or acquired coagulation defects related to the extrinsic pathway of coagulation.

2.5.1. Clinical significance of PT measurement

The PT is fundamental assay of the coagulation system. The principal clinical uses of the PT include: the detection of hereditary or acquired deficiencies or defects of the intrinsic coagulation factor (Factors XII, XI, IX, VIII, prekallikrein, high molecular weight kininogen), monitoring heparin anticoagulant therapy, the detection of coagulation inhibitors (i.e., lupus anticoagulant), and to monitor coagulation factor replacement therapy. The PT is increased with hereditary or acquired intrinsic factor deficiencies < 40%(Factor VIII:C, Factor IX, Factor XI, FactorXII, vWf), lupus anticoagulants, or specific inhibitors of the intrinsic coagulation factors (Kitchen *etal.*, 1999).

Other causes of an elevated PT include liver disease, DIC, anticoagulant therapy, a traumatic phlebotomy, and improper specimen collection (Fairweather*etal.*,1998).

3. Materials and methods

3.1 Materials:

3.1.1 Study design:

A case-control study.

3.1.2 Study groups:

This study was conducted on Sudanese cirrhotic patients (test group) and normal healthy non cirrhotic (control group).

3.1.3 Sample size:

fifty cirrhotic patients (as test group) and fifty normal healthy non cirrhotic (control group) were selected.

The test or case group classified into subgroups according to severity and the stages of disease into group A (compensated stage) contain 25 patients and group B (decompensated stage) also has 25 patients.

3.1.4 Inclusion criteria: patients with liver cirrhosis.

3.1.5 Exclusion criteria: all patients without liver cirrhosis excluded.

3.1.6 Ethical consideration:

- The aim and benefits of this study was explained to the participants.
- An informed consent was obtained from each participants.
- Health education was provided to each participants.

3.1.7 Sampling:

After informed questionnaire, and use local antiseptic for skin (70% ethanol), a venous blood sample (2.5mls) was collected from each individual include in this study, sample was collected in heparin container for choline esterase and albumin while the trisodium citrate container was used for PT.

3.1.8 Colorimeter:

Most colorimetric analytical tests are based on the beer- Lambert law which state that the correct condition the absorbance of a solution when measured at the

appropriate wave length is directly proportional to its concentration and the length of the light path through the solution.

Using a standard, this law can be applied to the measuring the concentration of the substance in an unknown (test) solution by using formula

$$\text{Concentration of test} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

3.1.9 Spectrophotometer:

one type of the colorimeter, the concentration of substance can take by using formula

$$\Delta A / \text{min} \times \frac{V_t \times 10^6}{\epsilon \times l \times V_s}$$

The molar absorbance (ϵ) of the dye, (l) the light path, (V_t) total reaction volume, (V_s) the sample volume

3.1.10 Coagulometer:

Used to measure the ability of the blood to clot

3.2 Methodology:

3.2.1 Albumin estimation method:

Bromocresol green (BCG)

Principle:

Albumin in the sample reacts with bromocresol green in acidic medium to form colored complex that measured colorimetrically at 630 nm.

Reagent composition :

Reagent A: acetate buffer 100mmol/L, bromocresol green 0.27mmol/L, detergent, pH 4.1.

Reagent S: albumin standard (bovine albumin).

Procedure:

	Blank	Standard	Sample
Albumin standard (S)	-	10 µl	-
Sample	-	-	10 µl
Reagent(A)	1.0 ml	1.0 ml	1.0 ml

3.2.2 PT estimation method:

PT with calcium

Principle:

Prothrombin time is a method of choice for monitoring oral anticoagulation therapy and fundamental screening test for acquired or inherited disorders. During oral anticoagulation therapy the activity of vitamin K-dependent clotting factors (II, VII, IX, X, protein C and protein S) is reduced and PT increased. The test is used for quantitative determination for clotting factors in the extrinsic (VII) and common pathways (II, V and X) of coagulation.

Reagent composition:

Rabbit brain thromboplastin, calcium chloride, and 0.05% sodium azide as preservative.

Procedure:

PT reagent was pre-incubated to 37°C at least 10 minutes. Maintain the suspension of reagent by magnetic stirring .

Or mixing by inversion immediately homogenize the content prior to use.

100µL was pipetted of test or control plasma into a test cuvette.

Incubate at 37°C for 1 minute.

200µL was rapidly added of the pre-incubated PT reagent , simultaneously starting timer.

Record the clotting time in second.

3.2.3 Cholinesterase(CHE) enzyme estimation method:

Butyrylthiocholine

Principle:

Cholinesterase(CHE) catalyze the hydrolysis of butyrylthiocholin to thiocholin and butyric acid. The catalytic concentration is determined from the rate of decrease of hexacyanoferrate (III), measured at 405 nm.

Reagent composition :

Reagent A:pyrophosphate 95mmol/L, hexacyanoferrate(III) 2.5mmol/L, pH7.6

Reagent B: butyrylthiocholin 60mmol/L

Procedure:

Working reagent	1.5L
Sample	25 μ L

Mix and insert a cuvette into the photometer, start a stop watch. After 90 seconds, record initial absorbance and at 30 second interval thereafter for 90 seconds Calculate the difference between consecutive absorbance, and the average absorbance per minute ($\Delta A/\text{min}$).

The activity of CHE was calculated using the following formula :

$$\Delta A/\text{min} \times \frac{V_t \times 10^6}{\epsilon \times l \times V_s}$$

3.2.4 Statistical analysis:

As appropriate descriptive and analytic procedure was followed using SPSS package (version 11.5). Independent T test was applied to compare mean of cholinesterase activity, albumin and PT level between study group who are cirrhotic individual and control who are non cirrhotic individual.

Also one sample t test was done to compare mean of cholinesterase activity between case subgroup groupA and group B

3.2.5 Quality control:

Precision and accuracy of all method used in this study were checked each time a batch was analyzed by including control sera.

Result and analysis

Table 4-1 show there is significant difference between serum cholinesterase activity among case group (2519.58 ± 1328.247) and control group (2984.00 ± 1909.877)

Cholinesterase	NO	Means (IU/L)	ST.Deviation	P.Value
Patients(case)	50	2519.58	1328.247	.000
Control	50	5984.00	1909.877	

Independent sample T test for the mean and STD of cholinesterase (IU/L) results in compared to the control ($P < 0.05$).

Table 4-2 show there is significant difference between albumin among case group (27.12 ± 8.27) and control group (42.54 ± 5.32)

Albumin	NO	Means (g/L)	ST.Deviation	P.Value
Patients	50	27.1200	8.27743	.000
Control	50	42.5400	5.32691	

Independent sample T test for the mean and STD of albumin (g/L) results in compared to the control ($P < 0.05$).

Table 4-3 show highly significant difference with mean \pm S.D at serum cholinesterase activity among case group (17.94 \pm 5.47) and control group (12.6 \pm 1.55) (P=0.000)

PT	NO	Means (sec)	ST.Deviation	P.Value
Patients	50	17.94	5.475	.000
Control	50	12.64	1.555	.000

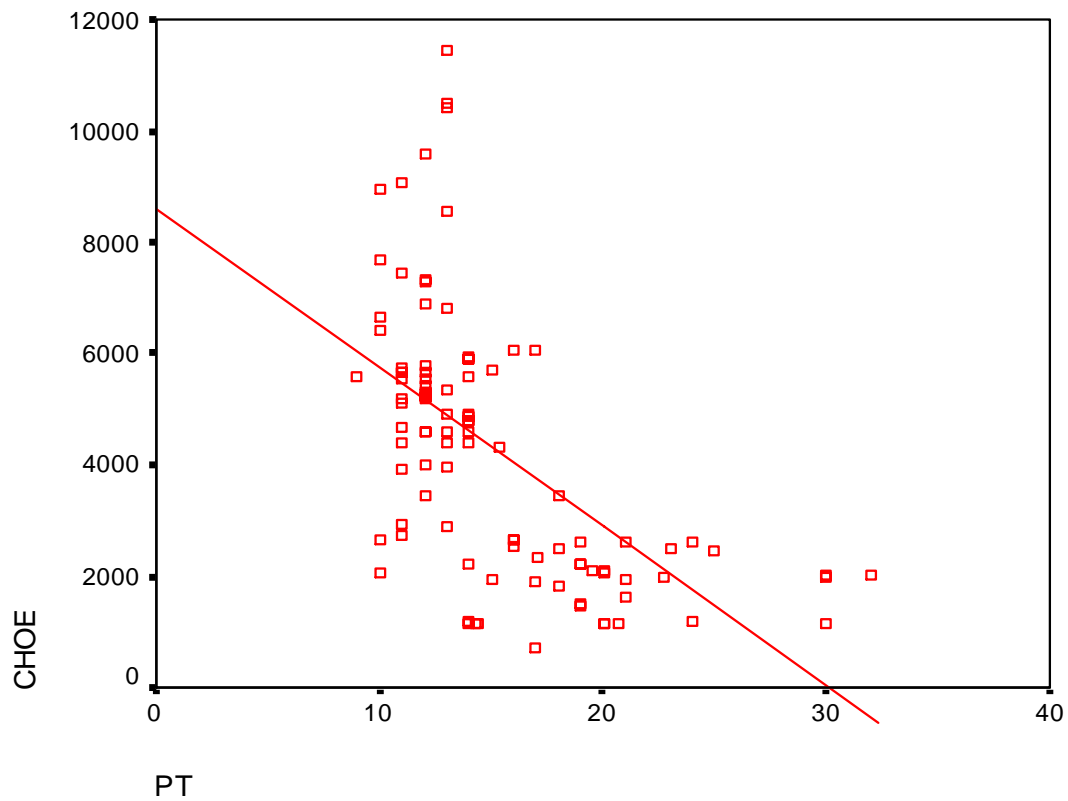
Independent sample T test for the mean and STD of PT (sec) results in compared to the control (P<0.05).

Table 4-4 show significant difference with mean \pm S.D at serum cholinesterase activity among case group A(3143.83 \pm 1583.44) and group B(1895.32 \pm 531.79)(P=0.000)

Case subgroups	No	Mean	St.deviation	P.value
Group A compensated	25	3143.84	1583.44	.000
GroupB Decompensated	25	1895.32	531.79	.000

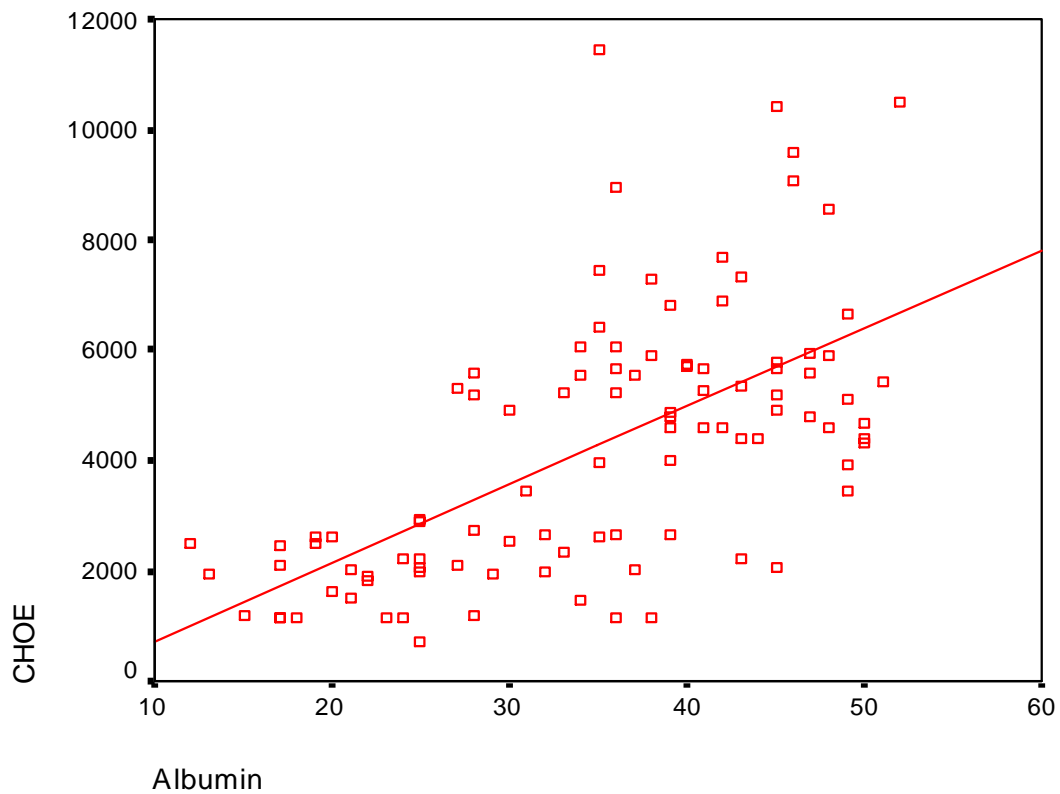
One sample T-test for serum cholinesterase activity (IU/L) among case subgroup A and subgroup B (P<0.05).

Figure4-1 show there is strong negative correlation between cholinesterase activity and PT



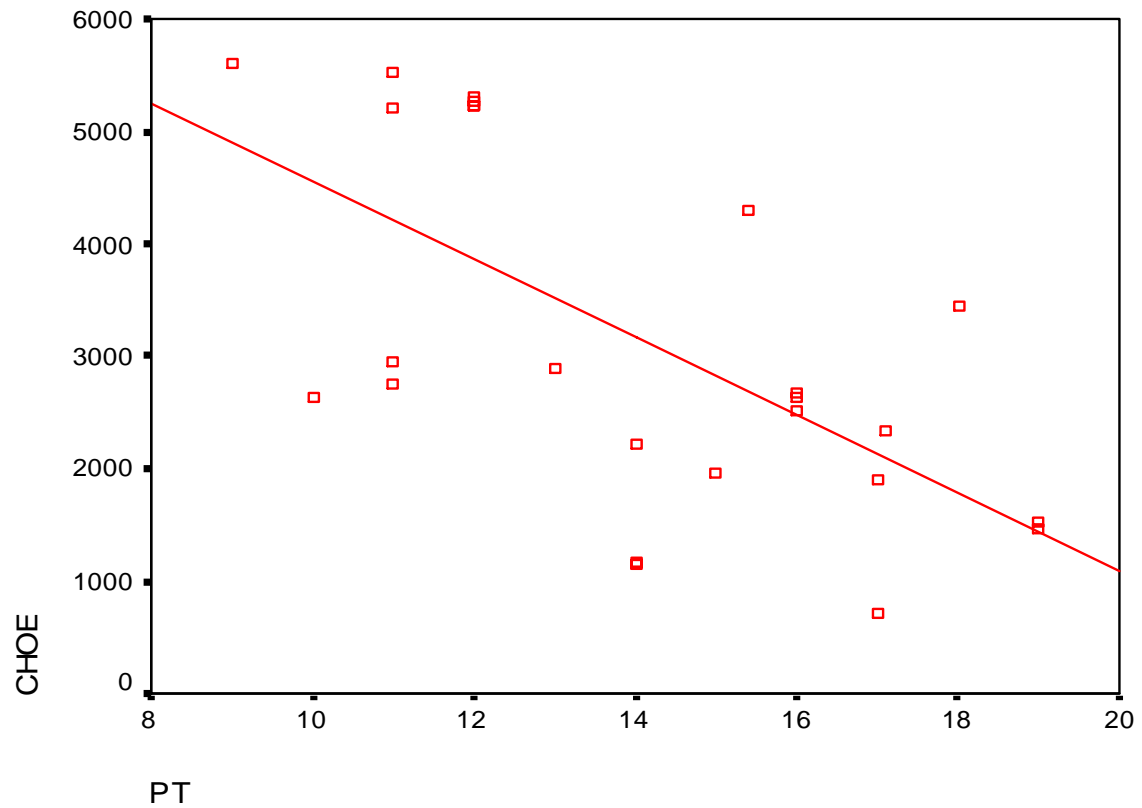
Scatter plot for CHE and PT among case group (P.value= 0.00 , r=-0.580).

Figure 4-2 show strong positive correlation between cholinesterase activity and albumin concentration among cirrhotic patients



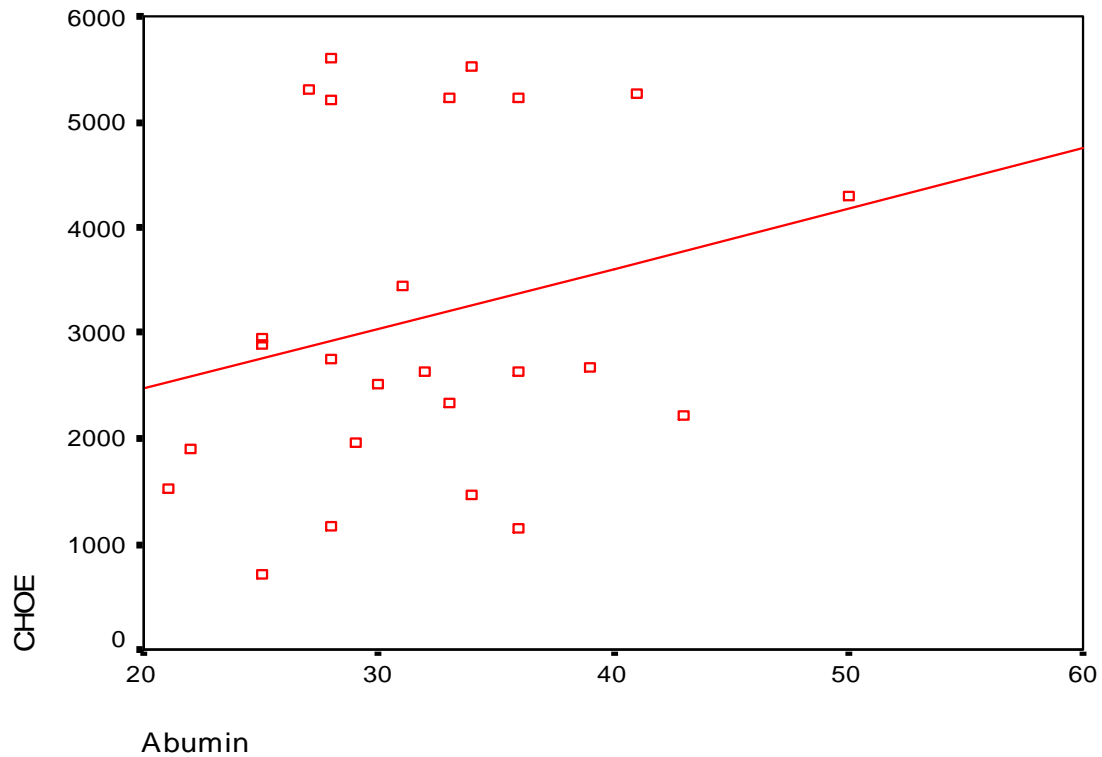
Scatter plot for CHE and albumin among case group (P.value=0.00, r=0.617).

Figure 4-3 show strong negative correlation between cholinesterase activity and PT among case subgroup A



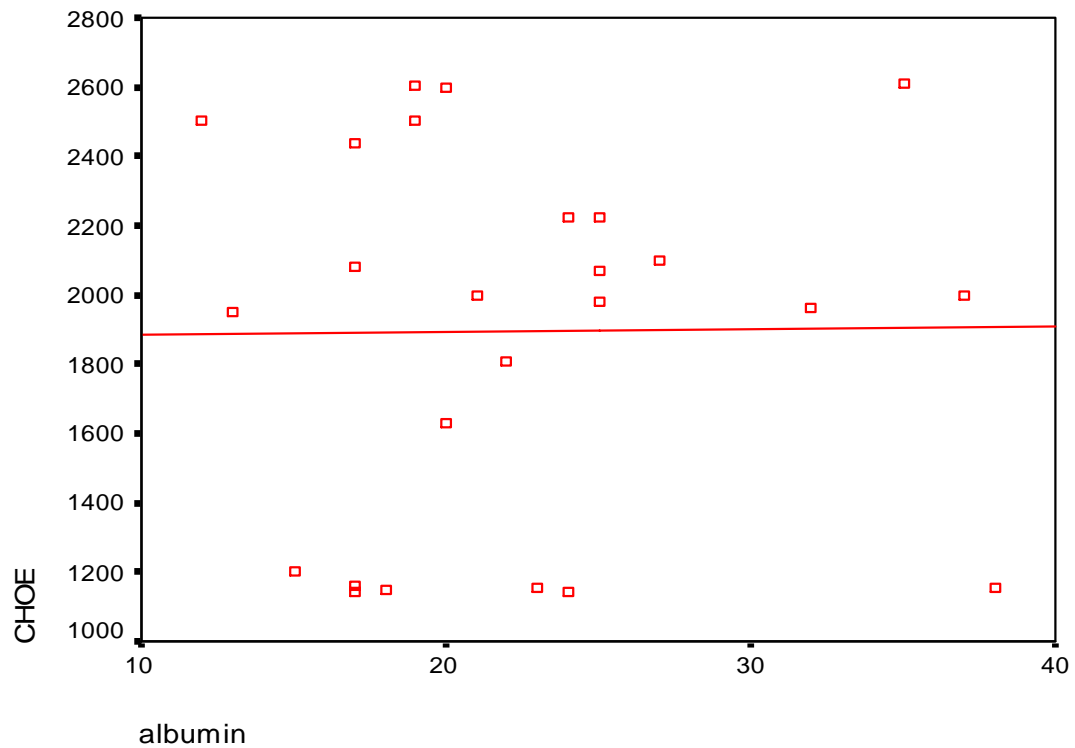
Scatter plot for CHE and PT among case group A(P.value =0.01, r=-0.632).

Figure 4-4 show there is no correlation between cholinesterase activity and albumin concentration among group A cirrhotic patients



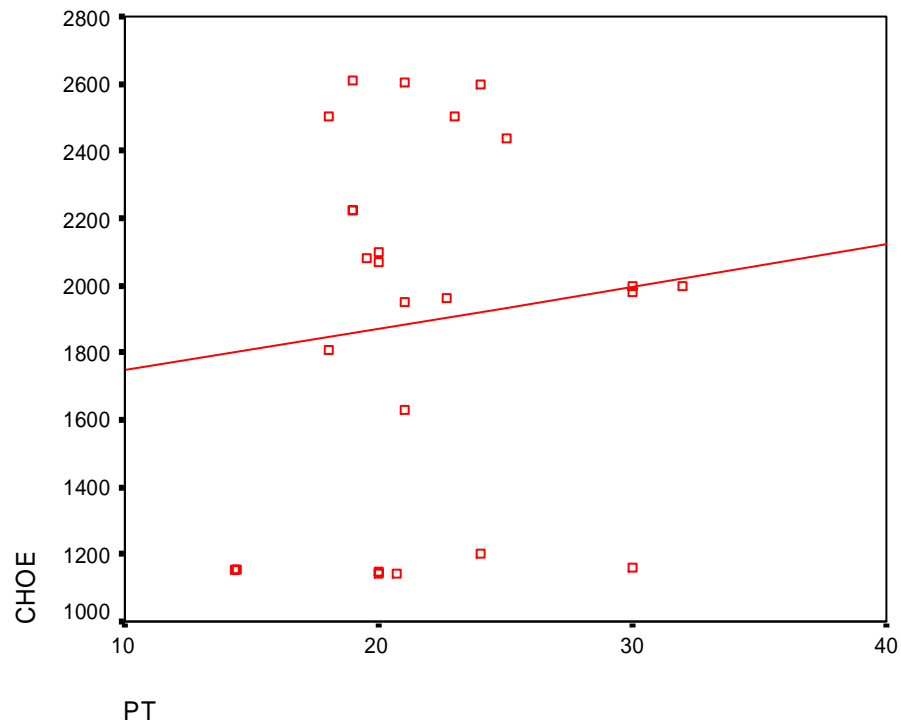
Scatter plot of CHE and albumin among case group A(P.value=0.241, r=0.244).

Figure 4-5 show there is no correlation between cholinesterase activity and albumin concentration among group B cirrhotic patients



Scatter plot of CHE and albumin among case group B (P.value=0.954, r=0.12).

Figure 4-6 show there is no correlation between cholinesterase activity and PT among group B cirrhotic patients



Scatter plot for CHE and PT among case group B ($r=0.108$, $P=0.606$)

5.Discussion, Conclusion and Recommendation

5.1Discussion

A previous study reported that cirrhosis affect the liver and prevent it from functioning properly by means impact on the power synthesis of the liver such as albumin and coagulation proteins synthesis (bishop, 2010). Pseudocholinesterase which known as plasma cholinesterase, butyrylcholinesterase, is produced in the liver and found primarily in plasma(Wang *et al*,2005) . Serum or plasma cholinesterase activity is reduced in liver dysfunction (Zhou *et al.*, 2003). From Independent T-test results presence study showed there was significant decrease in cholinesterase level among case group in compared to control group, (p.value 0.00). also albumin levels decreased significantly among case group compared to control group (p.value .000), whereas PT level tend to increase significantly among case group than control group (p.value.000). according to severity and symptoms of liver cirrhosis case group deeply classified into (group A) compensate stage and (group B) decompensate stage (Lim *et al.*,2009). the cholinesterase level tend to decrease significantly among group B more than group A (p.value .000), this strong evidence for continuously liver cells damage, thus will lead to deficiency in synthesis of cholinesterase enzyme. This result agree with mengetal whose said that the level of cholinesterase deceased significantly in cirrhotic patients (p.value .000).

After person correlation test was done this study showed there was strong positive correlation between cholinesterase and albumin (P.value 0.00) , and strong negative correlation between cholinesterase activity and PT (p.value .000), that is mean there is decrease in both albumin and cholinesterase levels, and increase in PT levels. Suitable justification for this variation in those parameters levels correlation related to the defect and deficiency of its biosynthesis by liver cells due to cirrhosis.

In case group classes group A the results revealed there is positive correlation between cholinesterase (p.value .000) and PT whereas there is no significant correlation between cholinesterase albumin level but group B show there is no significant correlation between cholinesterase, PT and albumin level, because some patients may received some albumin and blood transfusion this exactly may affect albumin and PT level that is appear clearly in correlation between case groups.

5.2 Conclusion

In conclusion the findings of present study show that plasma cholinesterase level restricted upon liver cell condition if intact the cholinesterase have normal or stable level. In contrast, if liver cells insult or damaged definitely the cholinesterase levels tend to decrease relatively with the grade of damage. Cholinesterase appear more stable and not easily affected by medications that given for cirrhotic patients thus, can be used as good parameter for diagnosis and prognosis of synthesis power of the liver with PT and albumin.

5.3Recommendations

I recommend that cholinesterase activity has effective role to assess reserve function of the liver as same as albumin and PT so we must join it within LFTs panel.

During samples collection from cirrhotic patients I observed that most causes of cirrhosis restricted upon hepatitis B,C about 80% from samples so we must increase the care with this diseases.

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