# 1. Introduction and literature review

## 1.1. Introduction:

Cigarette smoking is a major cause of preventable morbidity and mortality. Worldwide, more than 3 million people currently die each year from cigarette smoking (Aurelio, 2005). The risk of death in the smokers measured by the number of cigarettes smoked daily, the duration of smoking, the degree of inhalation and the age of initiation (Lubin .*et al*, 2007). Cigarette smoke contains over 4000 different chemicals, 400 of which are proven to be carcinogenic; it also contains various oxidants such as oxygen free radicals and volatile aldehydes which are probably the major causes of damage to biomolecules (Yeh *et al.*, 2008). Cigarette smoking yields chemical substances with high cytotoxic potentials. Cigarette smoke consists of many chemicals, including nicotine, tar with its many carcinogens, and gaseous compounds including carbon monoxide (Benowitz *et al.*, 2007).Cigarette smoke also contains large numbers of free radicals that are capable of initiating or promoting oxidative injury . Cigarette smokers are at greater risk for cardiovascular diseases, respiratory disorders, cancers, peptic ulcers and gastroesophageal reflux disease, blind-ness, bone matrix loss, and hepatotoxicity comparing with non-smokers( Spiro and Silvestri , 2005)

Cigarette smoking causes a variety of adverse effects on organs that have no direct contact with the smoke itself such as liver. The liver is an important organ that has many tasks; such as responsibility for processing drugs, alcohol and other toxins to eliminate them from the body (Sheen

*et al* .,1997) .

Some earlier studies found increase in serum ALT, ALP and AST activities conducted in Libyan population (Alsalhen, 2014). and also in other study found the smoking increased serum GGT activities (Osifio *et al.*, 2013), those studies have been reported primarily from Libya and Tanzania countries , while no study of the effects of the cigarette smoking on liver functions test for persons living in Sudan country had performed or published. Thus, this study was conducted to assess AST, ALT, GGT and ALP activities among Sudanese male smokers.

## 1.2. Literature review

### 1.2.1. Smoking

Smoking is a practice in which a substance is burned and the resulting smoke breathed in to be tasted and absorbed into the bloodstream. Most commonly the substance is the dried leaves of the tobacco plant which have been rolled into a small square of rice paper to create a small, round cylinder called a "cigarette". Smoking is primarily practiced as a route of administration for recreational drug use because the combustion of the dried plant leaves vaporizes and delivers active substances into the lungs where they are rapidly absorbed into the blood stream and reach tissue. In the case of cigarette smoking these substances are contained in a mixture of aerosol particles and gasses and include the pharmacologic- ally active alkaloid nicotine; the vaporization creates heated aerosol and gas to form that allows inhalation and deep penetration into the lungs where absorption into the bloodstream of the active substances occurs (West *et al.*, 2007).

Other smoking implements include pipes, cigars , bides , hookahs , vaporizers and bongs .it has been suggested that smoking related diseases may also be contracted by non – smokers .A 2007 report states that about 4>9million people worldwide each die as a result of smoking.(West .*et al*, 2007).

Cigars are tightly rolled bundles of dried and fermented tobacco which are ignited so that smoke may be drawn into the smoker's mouth. They are generally not inhaled because the high alkalinity of the smoke, which can quickly become irritation to the trachea and lung. The prevalence of cigar smoking varies depending on location, historical period, and

population surveyed, and prevalence estimates vary somewhat depending on survey method. The United States is the top consuming country by far, followed by Germany and United Kingdom, the US and Western Europe account for about 75% of cigar sales worldwide. (Rarick , 2008) As of 2005 it is estimated that 4.3% of men and 0.3% of women smoke cigars (Mariolis *et al.,* 2006).

Cigarettes, French for "small cigar", are a product consumed through smoking and manufactured out of cured and finely cut tobacco leaves and reconstituted tobacco, often combined with other addiditives , which are then rolled or stuffed into a paper-wrapped cylinder . Cigarettes are ignited and inhaled, usually through a cellulose acetate filter, into the mouth and lungs (Wingand .*et al* , 2006) .

### 1.2.1.1 Physiology of smoking

The active substance in tobacco, especially cigarettes, is administered by burning the leaves and inhaling the vaporized gas that results. This quickly and effectively delivers substances into the blood stream by absorption through the alveoli in the lung. The lungs contain some 300million alveoli, which amounts to a surface area of over 70 $m^2$ (about the size of tennis court). This method is inefficient as not all of the smoke will be inhaled, and some amount of the active substance will be lest in the process of combustion, pyrolysis. ( Gilman .*et al,* 2004).pipe and cigar smoke are not inhaled because of it is alkalinity , which are irritating to the trachea and lungs . However, because of it is high alkalinity (ph =8.5) compare to cigarette smoke (pH= 5.3), un-ionized nicotine is more readily absorbed through the mucous membranes in the mouth. (Turner *et al.,* 1977). Nicotine absorption from cigar and pipe,

however, is much less than that from cigarette smokes (Armitage and Turner, 1970) .

The inhaled substance triggers chemical reactions in nerve ending. The cholinergic receptors are often triggered by the naturally occurring neurotransmitter acetylcholine. Acetylcholine and nicotine express chemical similarities, which allow nicotine to trigger the receptor as well. (Wonnacott, 1997). These nicotinic acetylcholine receptors are located in the central nervous system and at the nerve muscle junction of skeletal muscle; whose activity increase heart rate, alertness and faster reaction times. Nicotine acetylcholine stimulation is not directly addictive. However, since dopamine-releasing neurons are abundant on nicotine receptors, dopamine is released.this release of dopamine, which is associated with pleasure, is reinforcing and may also increase working memory. Nicotine and cocaine activate similar patterens of neurons, which support the idea that common substrates among these drugs (Parkin *et al.,* 1998).

When tobacco is smoked, most of the nicotine is pyrolyzed. However, a dose sufficient to cause mild somatic dependency and mild to strong psychological dependency remains. There is also a formation of hormone (a MAC inhibitor) from the acetaldehyde in tobacco smoke. This seem to play an important role in nicotine addiction probably by facilitating a dopamine release in the nucleus accumbens as a receptor to nicotine stimuli .Using rate study , with after repeated exposure to nicotine results in less responsiveness nucleus accumbens cells , which produce dopamiasne responsible for reinforcement (Shoaib. *et al* , 2004).

### 1.2.1.2. Effects of smoking on general health

Smoking most commonly leads to diseases causes may affect the heart and lungs and will most commonly affect areas such as hands or feet with first signs of smoking related health issues showing up as numbness, with smoking being a major risk factor for heart attacks, Chronic Obstructive Pulmonary Disease (COPD), emphysema, and cancer, particularly lung cancer, cancers of the larynx and mouth, and pancreatic cancers

(Inflamm,2009).

About one half of long term male smokers will die of illness due to smoking .A person's increased risk of contracting disease is directly proportional to the length of time that a person continues to smoke as well as the amount smoked. However, if someone stops smoking, then these chances gradually decrease the damage to their body is repaired. A year after quitting, the risk of contracting heart disease is half that of a continuous smoking. The health risks of smoking are not uniform across all smokers. Risks vary according to amount of tobacco smoked, with those who smoke more at greater risk (Villeneuve and Mao, 1994).

### 1.2.1.3. Physical and biochemical properties of cigarettes:

Conventionally, cigarette smoke is divided into two phases: a tar phase and a gas phase. The tar or particulate phase is defined as the material that is trapped when the smoke stream is passed through the Cambridge glass-fiber filter that retains 99.9% of all particulate material with a size >0.1 μm . The gas phase is the material that passes through the filter. The particulate (tar) phase of cigarette smoke contains $>10^{17}$ free radicals/g, and the gas phase contains $>10^{15}$ free radicals/puff .The radicals associated with the tar phase are long-lived (hours to months), whereas

the radicals associated with the gas phase have a shorter life span (seconds) (Pryor .*et al*, 1993) .

Cigarette smoke that is drawn through the tobacco into an active smoker's mouth is known as mainstream smoke. Side stream cigarette smoke is the smoke emitted from the burning ends of a cigarette. Mainstream cigarette smoke comprises 8% of tar and 92% of gaseous components (Pryor .*et al*, 1993). Environmental tobacco smoke results from the combination of side stream smoke (85%) and a small fraction of exhaled mainstream smoke (15%) from smokers (Taylor .*et al*, 1992). Side stream cigarette smoke contains a relatively higher concentration of the toxic gaseous component than mainstream cigarette smoke. Of all the known constituents, nicotine, a component of the tar phase, is the addictive substance of cigarette smoke (Powell, 1998).

### 1.2.1.4. Tobacco smoke constituents

Carcinogens; Smoke, or any partially burnt organic matter, contains carcinogens (cancer-causing agents).There are over 19 known carcinogens in cigarette smoke (Jones *et al* ., 2000). The following are some of the most potent carcinogens:

**Polycyclic aromatic hydrocarbons;** are tar components produced by pyrolysis in smoldering organic matter and emitted into smoke. Several of these PAH's are already toxic in their normal form, however many of them can become more toxic in the liver. Due to the hydrophobic nature of PAH's they do not dissolve in water and are hard to expel from the body. Inorder to make the PAH more soluble in water, the liver creates an enzyme called Cytochrome P450 which adds an additional oxygen to the

PAH, turning it into a mutagenic epoxides, which is more soluble, but also more reactive(Feng *et al.*, 2006).

**Acrolein** is a pyrolysis product that is abundant in cigarette smoke. It gives smoke an acrid smell and an irritating, lachromatory effect and is a major contributor to its carcinogenity. Like PAH metabolites, acrolein is also an electrophilic alkylating agent and permanently binds to the DNA base guanine, by a conjugate addition followed by cyclization into a hemiaminal. The acrolein-guanine adduct induces mutations during DNA copying and thus causes cancers in a manner similar to PAHs (Kataoka , et al , 1997).

**Nitrosamines**; are a group of carcinogenic compounds found in cigarette smoke but not in uncured tobacco leaves. Nitrosamines form on flue-cured tobacco leaves during the curing process through a chemical reaction between nicotine and other compounds contained in the uncured leaf and various oxides of nitrogen found in all combustion gases. Switching to Indirect fire curing has been shown to reduce nitrosamine levels to less than 0.1 parts per million (Fratiglioni and Wang, 2000).

**Tar;** Tar is defined as the nicotine- free, dry, particulate mass of tobacco smoke. The nature of the chemical components in tar and their toxicity vary widely across tobacco from various sources. Tar as cilia are blocked the tars in cigarette smoke are deposited and collected on the walls of the respiratory tract and the lung, and cause them to turn block ( Talhout .*et al*, 2007).

**Gases;** In addition to the particulate fraction (tar) of tobacco smoke, many chemicals are found in the gaseous phase. The levels of these chemicals may or may not have a relationship to the yield of tar. The

most widely reported of the gaseous chemicals is carbon monoxide (CO). Carbon monoxide is emitted in high concentrations (thousands of parts per million) in cigarette smoke. The toxicity of carbon monoxide is a function of its ability to form carboxy-haemoglobin , a stable chemical complex with hemoglobin. This effectively serves to remove oxygen - carrying hemoglobin from the circulating blood and to vital tissues. Carboxyhaemoglobin concentrations in the blood of about 2% or more of hemoglobin have been associated with angina pain in people with cardiovascular disease and can result in cardiac ischaemia and diminished blood flow to the heart. Some other important chemicals in tobacco smoke, such as benzene, are also found in the gaseous phase of the smoke, but are correlated with the amount of tar (Smith .*et al,* 1997).

**Nicotine**, which is contained in cigarettes and other smoked tobacco products, is a stimulant and is one of the main factors leading to continued tobacco smoking. Nicotine is a highly addictive psychoactive chemical. When tobacco is smoked, most of the nicotine is pyrolyzed; a dose sufficient to cause mild somatic dependency and mild to strong psychological dependency remains. The amount of nicotine absorbed by the body from smoking depends on many factors, including the type of tobacco, whether the smoke is inhaled, and whether a filter is used (Talhout .*et al*, 2007).

### 1.2.2. The liver

The liver is a vital organ of vertebrates and some other animals. In the human it is located in the upper right quadrant of the abdomen, below the diaphragm. The liver has a wide range of functions, including detoxification of various metabolites, protein synthesis, and the production of bio chemicals necessary for digestion. There is currently no

way to compensate for the absence of liver function in the long term, although liver dialysis techniques can be used in the short term ( Maton *.el al*,1993).

The liver is a gland and plays a major role in metabolism with numerous functions in the human body, including regulation of glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification. It is an accessory digestive gland and produces bile, an alkaline compound which aids in digestion via the emulsification of lipids. The liver's highly specialized tissue consisting of mostly hepatocytes regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions (Maton *.et al,* 1993). Estimates regarding the organ's total number of functions vary, but textbooks generally cite it being around 500 (Zakim *.et al*, 2002).

The live 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. However, if the liver is damaged repeatedly over a long period of time, it may undergo irreversible changes that permanently interfere with it is essential function (Bishop *.et al,* 2010).

The liver gets a dual blood supply from the hepatic portal vein and hepatic arteries. Supplying approximately 75% of the liver's blood supply, the hepatic portal vein carries venous blood drained from the spleen, gastrointestinal tract, and its associated organs. The hepatic arteries supply arterial blood to the liver, accounting for the remainder of its blood flow. Oxygen is provided from both sources; approximately half

of the liver's oxygen demand is met by the hepatic portal vein, and half is met by the hepatic arteries (Schneider .*et al,* 2008).

The bile produced in the liver is collected in bile canaliculi , which merge to form bile ducts. Within the liver, these ducts are called intra-hepatic (within the liver) bile ducts, and once they exit the liver they are considered extra-hepatic (outside the liver). The intra-hepatic ducts eventually drain into the right and left hepatic ducts, which merge to form the common hepatic duct. The cystic duct from the gallbladder joins with the common hepatic duct to form the common bile duct. Bile either drains directly into the duodenum via the common bile duct, or is temporarily stored in the gallbladder via the cystic duct ( Sheporaitis and Freeny , 1998).

### 1.2.2.1. Liver Physiology

The various functions of the liver are carried out by the liver cells or hepatocytes. Currently, there is no artificial organ or device capable of emulating all the functions of the liver. Some functions can be emulated by liver dialysis, an experimental treatment for liver failure. The liver is thought to be responsible for up to 500 separate functions, usually in combination with other systems and organs (Schneider *et al .,* 2008).

The liver performs several roles in carbohydrate metabolism: *Gluconeogenesis* (the synthesis of glucose from certain amino acids, lactate or glycerol), *Glycogenolysis* (the breakdown of glycogen into glucose),*Glycogenesis* (the formation of glycogen from glucose)(muscle tissues can also do this).The liver is responsible for the mainstay of protein metabolism, synthesis as well as degradation. The liver also performs several roles in lipid metabolism: *Cholesterol synthesis*,

*Lipogenesis*, the production of triglycerides (fats), A bulk of the lipoproteins is synthesized in the liver. The liver also produces coagulation factors I (fibrinogen), II (pro-thrombin), V, VII, VIII, IX, X and XI, as well as protein C, protein S and anti-thrombin. In the first trimester fetus, the liver is the main site of red blood cell production. By the 32nd week of gestation, the bone marrow has almost completely taken over that task.

The liver produces and excretes bile (a yellowish liquid) required for emulsifying fats and help the absorption of vitamin K from the diet. Some of the bile drains directly into the duodenum, and some is stored in the gallbladder. The liver also produces insulin-like growth factor 1 (IGF-1), a polypeptide protein hormone that plays an important role in childhood growth and continues to have anabolic effects in adults. The liver also is a major site of thrombopoietin production. Thrombopoietin is a glycoprotein hormone that regulates the production of platelets by the bone marrow) (Sheporaitis and Freeny , 1998).

The breakdown of insulin and other hormones and also glucoronidates bilirubin, facilitating its excretion into bile. The liver breaks down or modifies toxic substances (e.g., methylation) and most medicinal products in a process called drug metabolism. This sometimes results in toxication, when the metabolite is more toxic than its precursor. Preferably, the toxins are conjugated to avail excretion in bile or urine. The liver converts ammonia to urea (urea cycle) (Sheporaitis and Freeny , 1998).

The liver stores a multitude of substances, including glucose (in the form of glycogen), vitamin A (1–2 years' supply), vitamin D (1–4 months' supply), vitamin B12 (1–3 years' supply), vitamin K, iron, and copper.

The liver is responsible for immunological effects the reticuloendothelial system of the liver contains many immunologically active cells, acting as a 'sieve' for antigens carried to it via the portal system. The liver produces albumin, the major osmolar component of blood serum. And also synthesizes angiotensinogen , a hormone that is responsible for raising the blood pressure when activated by renin, an enzyme that is released when the kidney senses low blood pressure (Sheporaitis and Freeny , 1998).

### 1.2.2.2. Liver disorders

There are more than a hundred kinds of liver disease. Acute and chronic Hepatitis, inflammation of the liver, is caused mainly by various viruses (viral hepatitis) but also by some liver toxins (e.g. alcoholic hepatitis), autoimmunity (autoimmune hepatitis) or hereditary conditions.

Cirrhosis is the formation of fibrous tissue (fibrosis) in the place of liver cells that have died due to a variety of causes, including viral hepatitis, alcohol overconsumption, and other forms of liver toxicity. Cirrhosis causes chronic liver failure.

Tumor of liver is tow type; the first is called primary cancer of liver (hepatocellular carcinoma or Hepatoma) and the second is Metastatic liver tumor arise from other cancerous tissue where the primary site was of lung , pancreas , gastrointestinal tract, or ovary origin .Hereditary diseases that cause damage to the liver include hemochromatosis . Hepatic jaundice ,Gilbert's syndrome, a genetic disorder of bilirubin metabolism found in about 5% of the population, can cause mild jaundice, Crigler-najjar disease :partial or complete deficiency of UDP-glycuronyltransferase , Dubin – Johnson syndrome : defective liver cell excretion of bilirubin. Intrahepatic cholestasis : may be caused by

hepatocyte injury such as cirrhosis , bile duct injury such as rotor syndrome.There are also many pediatric liver diseases including: biliary atresia, alpha-1 antitrypsin deficiency, and progressive familial intrahepatic cholestasis (Anna p.ciulla .*et al*, 2002).

### 1.2.2.3. Liver function tests

1-Markers for hepato cellular necrosis (ALT; most specific for hepatocyte injury, AST; less specific than ALT significant presence in other tissues, LD least specific and significant presence in other tissues.)

2-Marker that reflect cholestasis ( Alkaline phosphatase, Gamma-glutamyl transferase.)

3-tests to assess liver disorders (Total bilirubin , direct bilirubin (conjugated), indirect bilirubin (un conjugated) , Albumin, Ammonia, Alph fetoprotein ) (Anna.*et al*, 2002).

### 1.2.2.4. Liver enzymes

#### I. Aspartate transaminase

Enzyme code (EC 2.6.1.1) Aspartate transaminase (AST) or aspartate aminotransferase, also known as AspAT/ASAT/AAT or serum glutamic oxaloacetic transaminase (SGOT), is an enzyme belonging to the class of transferases. It's commonly referred to as a transaminase . AST catalyzes the reversible transfer of an α-amino group between aspartate and glutamate and, as such, is an important enzyme in amino acid metabolism. pyridoxal phosphate (PLP) function as coenzyme.

AST is widely distributed in human tissue the highest concentration found in cardiac tissue liver, skeletal muscle,with smaller amount in kidneys,and red blood cells (Bishop ,*et al*. 2010).

Two isoenzymes are present in a wide variety of eukaryotes. In humans: GOT1/cAST, the cytosolic isoenzyme derives mainly from red blood cells and heart and GOT2/mAST, the mitochondrial isoenzyme is present predominantly in liver.

These isoenzymes are thought to have evolved from a common ancestral AST via gene duplication, and they share a sequence homology of approximately 45 % (Hayashi. *et al*, 1990).

The clinical use of AST is limited mainly to the evaluation of hepatocellular disorder and skeletal muscle involvement. In myocardial infarction AST level begin to rise within 6 to 8 hours, peak at 24 hours, and return to normal within 5 day. AST may be elevated also in diseases affecting other organs, such as pulmonary embolism.

AST level are highest in acute hepatocellular disorders. In viral hepatitis level may reach 100 times ULN. In cirrhosis level reach 4 times URL.

Reference range 5 to 30 U/L (37C) (Bishop .*et al*, 2010).

**II. Alanine transaminase**

(EC 2.6.1.2)**,** Alanine transaminase (ALT) is a transaminase enzyme . It is also called alanine aminotransferase (ALAT) and was formerly called serum glutamate-pyruvate transaminase (SGPT) or serum glutamic-pyruvic transaminase (SGPT).ALT catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate, the products of this reversible

transamination reaction being pyruvate and L-glutamate. pyridoxal phosphate (PLP) function as coenzyme.

ALT is found in plasma and in various body tissues, but is most common in the liver. It is considered the more liver- specific enzyme of the transferase (Bishop .*et al,* 2010).

ALT is commonly measured clinically as a part of a diagnostic evaluation of hepatocellular injury, to determine liver health. Higher elevations are found in hepatocellular disorder than in extra-hepatic or intra-hepatic obstructive disorder (Bishop .*et al,* 2010).

ALT, in conjunction with an elevated AST, is used to assess liver involvement with diagnosis of AMI. ALT dose not exhibit a significant increase in muscular dystrophy, and it is not affected in case of pulmonary emboli or acute pancreatitis (Anna p.ciulla .*et al*, 2002).

Reference range of ALT 6- 37 U/L (37C) ( Bishop .*et al*, 2010).

**III. Gamma-glutamyl transferase**

(EC 2.3.2.2)Gamma-glutamyl transferase or gamma - glutamyl trans peptidase (also γ- glutamyl transferase , GGT, GGTP, gamma-GT) is an enzyme that transfers gamma -glutamyl functional groups .it is catalyzes the transfer of the gamma- glutamyl moiety of glutathione to an acceptor that may be an amino acid, a peptide or water (forming glutamate) (Bishop.*et al*, 2010).GGT plays a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione and drug and xenobiotic detoxification(Courtay .*et al*, 1992) . Other lines of evidence indicate that GGT can also exert a prooxidant role, with regulatory effects

at various levels in cellular signal transduction and cellular pathophysiology (Dominici .*et al*, 2005).

GGT activity is found primarily in tissue of kidney, brain, prostate, pancreas, and liver. Clinical applications of assay, however, are confined mainly to evaluation of liver and biliary system disorders (Bishop .*et al*, 2010).

Clinical significance OF GGT are : Increase level in all hepatobiliray disease, with levels increasing to 2-5 times the upper reference limit (example viral hepatitis, alcoholic cirrhosis) very sensitive indicator for this condition , Higher levels observed in intra – and post-hepatic biliary tract obstruction, with levels increasing to 5-3 times the URL; increases before and remains elevated longer than ALP, AST, and ALT .GGT activity induced by drugs (example Phenobarbital and phenytoin) and by alcohol consumption.GGT levels are normal in the presence of bone disease and during pregnancy in contrast to alkaline phosphatase, where levels would be elevated (Anna.*et al*, 2002).

Reference range Of GGT in Male up to 55 U/L And Female up to 38 U/L at 37C (Anna.*et al*, 2002).

**IV. Alkaline phosphatase**

**(**EC 3.1.3.1**),** Alkaline phosphatase (ALP, ALKP, ALPase , Alk Phos) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called *dephosphorylation*. As the name suggests, alkaline phosphatases are most effective in an alkaline environment. It is sometimes used synonymously as basic phosphatase (Tamas *et al.* , 2002).

In humans, alkaline phosphatase is present in all tissues throughout the entire body, but is particularly concentrated in liver, bile duct, kidney, bone, intestinal mucosa and the placenta. Humans and most other mammals contain the following alkaline phosphatase isozymes (ALPI – intestinal (molecular weight of 150 kDa),ALPL – tissue-nonspecific (liver/bone/kidney) ,ALPP – placental (Regan isozyme) (Anna p.ciulla *.et al*, 2002).

Clinical significance of ALP a, the increase serum activity seen in heptobiliary disease and bone disorders (with osteoblstic involvement) . in heptobiliary disorders, the increase levels are due to obstructive disease, and the ALP levels are increase more significantly than ALT and AST .(in biliary tract obstruction, synthesis of ALP is induce by cholestasis , which causes serum ALP levels to rise 3 to 10 times the upper reference limit. the elevation is usually greater in case of extrahepatic obstruction in contrast to intrahepatic obstruction, in hepatitis and cirrhosis , which are classified as hepatocellular conditions, ALP levels rise up to 3 times the upper reference limit ,highest elevation of ALP are seen in Paget's disease , ALP levels increase with healing bone fracture) . ALP levels are normally higher in children than adults because of bone growth. Decrease serum ALP levels are seen in Hypophosphatasia because of lack of ALP bone isoenzyme . This disorder characterized by insufficient bone calcification. ALP levels are normally higher in pregnancy because the placenta is a source of ALP.

The source of elevated ALP levels can be deduced by obtaining serum levels of gamma Glutamyl-transferase(GGT). Concomitant increases of ALP with GGT should raise the suspicion of hepatobiliray disease (Anna p.ciulla *.et al*, 2002).

## 1.3. Rationale

Every years hundreds of thousands of people around the world die from disease caused by smoking cigarettes (Aurelio, 2005)

Cigarettes smoking have many serious effect on human body and cause different disease such as cardiac disease, lung cancer, gastrointestinal, immune and metabolic system. (Spiro and Silvestri , 2005)

Nicotine is the alkaloid most t active in tobacco. Nicotine is metabolizing by various pathway. Liver is considered to be major site of nicotine biotransformation, metabolism also occurs in lung and kidney. Nicotine is responsible for high toxicity effect and also disrupt the antioxidant defense mechanism in human body which cause oxidative damage to liver ,kidney ,lung ,brain and heart (Talhout *.et al,* 2007).

Cigarette smoke propagates the lipid per-oxidation , which damage the biological cell membrane of the liver and serum aminotransferases are enzymes that act as sensitive indicators of hepatocellular damage (Rochling , 2001).The enzymes are leaked out into blood and increased the level of AST and ALT in smokers when compared non-smokers.

Some studies have demonstrated increase activity of AST, ALT, ALP and GGT in smoker compared to non smoker.

This study was conducted to assess the activity of AST,ALT, ALP and GGT in Sudanese smoker and to find if there is relationship between duration of smoking and number of cigarette smoked per day.

## 1.4. Objectives

### 1.4.1. General objective

To assess the effect of smoking on AST , ALT, ALP, and GGT activities.

### 1.5.2. Specific objective

1- To compare serum ALT, AST, ALP and GGT activities in male cigarette smokers in comparison to non smokers.

2- To correlate between serums ALT, AST, ALP and GGT activities and duration of smoking, number of cigarettes smoked per day and age of smokers.

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# 2. Materials and Methods

## 2.1. Materials

**2.1.1. Study design:** This is a case control study.

**2.1.2. Study area:** The study was conducted in Sudan in Khartoum city.

**2.1.3. Study population:** Seventy smokers were enrolled as test group and 50 non smokers as control group were enrolled in this study according to inclusion and exclusion criteria.

**Inclusion Criteria:** Cigarettes male Smokers in Khartoum state .

**Exclusion criteria:** Cigarettes male Smokers who have liver disease, alcohol abuse, bone disease, cardiac disease were excluded

**2.1.4**. **Samples:** About five ml of venous blood was collected from each patient at the fasting state. samples were collected under aseptic conditions and placed in sterile plain containers, and after clotting centrifuged for 3 minutes at 3000 RPM to obtain serum, then the obtained serum were kept at ⁻20c till the time of analysis.

**2.1.5 Ethical consideration:** Patients who voluntarily accepted to participate in the study were included.

**2.1.6. Equipments:** Mindary instrument, Centrifuge, Sterile plane containers, Disposable syringes, 70% alcohol, Tourniquets, Cotton, Micropipettes (automatic pipettes) and Graduated pipettes

**2.1.7. Data analysis:** Data was analyzed using SPSS computer program.

**2.1.8. Quality control:** at least two levels of control material should be analyzed with each batch of samples. This controls should be run with each calibration, each new reagent cartridge and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual.

## 2.2. Methods:

### 2.2.1. Estimation of serum aspartate aminotransferase :(appendix II)

**Principle of method:**

_L- aspartate + alpha- oxoglutarate $\xrightarrow{AST}$ oxaloacetate +L-glutamate

_oxaloacetate +NADH +$H^+$ $\xrightarrow{MAD}$ L-malate + $NAD^+$

In the assay reactions , the AST catalyze the reversable transamination of L-aspartate and alpha- oxoglutarate to oxaloacetate and L-glutamate . the oxaloacetate is then redused to malate in the presence of malate dehydrogenase with NADH . the rate of photometrically determined NADH decrease directly propotional to the rate of formation of oxaloacetate and thus the AST activity . **(appendix II)**

**Procedure:** (text appendix II)

**Calculation:** The analyzer calculates the activity of each sample automatically with a specified valid calibration factor from calibration process

Conversion factor of traditional unit (U/L) into SI- units (M Kat/L):

1 U/L =16.67 * $10^{-3}$ M kat/L

1 M Kat /L = 60 U/L

**Reference values:**

The refrence intervals measured at 37 C

MALE : <35 U/L , < 0.58 M kat /L .

FEMALE: <31 U/L , < 0.52 M kat/L .

**2.2.2. Estimation of serum alanin aminotransferase** : (appendix III)

**Principle of method:**

_L- alanin + alpha- oxoglutarate ALT_> pyrovate +L-glutamate

_pyrovate +NADH +$H^+$ LDH_____> L-lactat + $NAD^+$

In the assay reactions , the ALT catalyze the reversable transamination of L-alanin and alpha- oxoglutarate to pyrovat and L-glutamate . the pyrovate is then redused to lactate in the presence of lactate dehydrogenase with the concurrent oxidation of reduced B- nicotinamid adenine dinucleotide (NADH) to B- nicotinamid adenine dinucleotide(NAD) . This change in absorbance is directly propotional to the activity of ALT in the sample.

**Procedure: (text appendix III)**

**Calculation:**

The analyzer calculate the activity of each sample automatically with aspcified valid calibration factor from calibration process

Conversion factor of traditional unit (U/L) into SI- units (M Kat/L):

1 U/L =16.67 * $10^{-3}$ M kat/L

1 M Kat /L = 60 U/L

**Reference value:**

The refrence intervals measured at 37 C

MALE : <45U/L , < 0.75 *M* kat /L .

FEMALE: <34 U/L , < 0.57 *M* kat/L .

**2.2.3 Estimation of serum alkaline phosphatase : (appendix IV)**

**Principle of method:**

$$P\text{ – nitrophenyle phosphate} + H_2O \xrightarrow[Mg^{++}]{ALP} P\text{ – nitrophenol} + pi$$

By the action of ALP and Mg ions, *P* – nitrophenyle phosphate is catalysed to *P* – nitrophenol, and absorbancy increase is directly propotional to the activity of ALP .

**Procedure: (text appendix IV)**

**Calculation:**

The analyzer calculate the activity of each sample automatically with aspcified valid calibration factor from calibration process

Conversion factor of traditional unit (U/L) into SI- units (M Kat/L):

1 U/L =16.67 * $10^{-3}$ M kat/L

1 M Kat /L = 60 U/L

**Table (2-1) Reference value of Alkaline phosphatase:**

The refrence intervals measured at 37 C

| Sample type | Female | Male |
|---|---|---|
| 1_30 days | 75-316U/L | 48-406 U/L |
| 1 month – 1 years | 82-383U/L | 124-341 U/L |
| 1-3 years | 104-345U/L | 108-317 U/L |
| 4-6 years | 93-309 U/L | 96-297 U/L |
| 7-9 years | 86-315 U/L | 69-325 U/L |
| 10-12 years | 42-362 U/L | 51-332 U/L |
| 13-15 years | 74-390 U/L | 50-162 U/L |
| 16-18 years | 52-171 U/L | 47-119U/L |

**2.2.4.Estimation of serum gama-glutamyltransferase : (appendix V)**

**Principle of method:**

L-gamma- glutamyl -3- carboxy -4- nitroanilide + glycyl – glycine

GGT ⟶ ⟵ L gamma-glutamyl-glycyl-glycine + 5-amino -2-nitrobenzoate.

gama-glutamyltransferase transfere the gamma- glutamyl group of gamma- glutamyl -3- carboxy -4- nitroanilide to glycyl – glycine with the production of P- nitroaniline. The 5-amino -2-nitrobenzoate results in the elevated absorbance which is directly to the activity of GGT in the sample

**procedure: (text appendix V)**

**Calculation:**

The analyzer calculate the activity of each sample automatically with aspcified valid calibration factor from calibration process

Conversion factor of traditional unit (U/L) into SI- units (M Kat/L):

1 U/L =16.67 * $10^{-3}$ M kat/L

1 M Kat /L = 60 U/L

**Reference value:**

The refrence intervals measured at 37 C

Male : <49U/L , < 0.82 *M* kat /L .

Female : <32 U/L , < 0.53 *M* kat/L .

# 3. Results

One hundred and twenty Sudanese males (70 smokers and 50 non smokers) were enrolled in this study to study the effect of smoking on some liver enzymes.

**Table 3.1:** shows a compression between means of AST, ALT, ALP and GGT in Sudanese smokers and control group.

**Figure 3.1** a scatter plot shows a significant positive correlation (P=0.000, r =0.476) between AST activity and duration of smoking.

**Figure 3.2** a scatter plot shows a significant positive correlation (P=0.011, r =0.304) between AST activity and number of cigarette.

**Figure 3.3** a scatter plot shows a significant positive correlation (P=0.005, r = 0. 253) between AST activity and age among Sudanese smokers.

**Figure 3.4** a scatter plot shows a significant positive correlation (P=0.002, r = 0.369) between ALT activity and number of cigarette per day among Sudanese smokers.

**Figure 3.5** a scatter plot shows a significant positive correlation (P=0.000, r = 0. 325) between ALT activity and age among Sudanese smokers.

**Figure 3.6** a scatter plot shows significant positive correlation (P=0.000, r =0. 498) between ALT activity and duration of smoking among Sudanese smokers.

**Figure 3.7** a scatter plot shows a significant positive correlation (P=0.016, r =0. 287) between ALP activity and duration of smoking among Sudanese smokers.

**Figure 3.8** a scatter plot shows a significant positive correlation (P=0.05, r = 0. 231) between ALP activity and number of cigarette per day among Sudanese smokers.

**Figure 3.9** a scatter plot shows no significant correlation (P= 0.859, r =-.016) between ALP activity and age among Sudanese smokers

**Figure 3.10** a scatter plot shows a significant positive correlation (P=0.002, r = 0. 275) between GGT activity and AGE among Sudanese smokers.

**Figure 3.11** a scatter plot shows no significant correlation (P=.207, r =0.153) between GGT activity and age among Sudanese smokers

**Figure 3.12** a scatter plot shows a significant positive correlation (P=0.002, r =0. 362) between GGT activity and duration of smoking among Sudanese smokers.

**Table 3.1:** Comparison between means of AST, ALT, ALP and GGT actives in smokers and non smokers.

| Enzymes | Smokers N=(70) | Non smokers N=50 | P. value |
|---|---|---|---|
| AST (U/L) | 31.73 ± 13.47 | 21.98±2.527 | 0.000 |
| ALT(U/L) | 23.30± 18.220 | 13.74±3.498 | 0.000 |
| ALP(U/L) | 80.20±29.244 | 69.50 ±14.502 | 0.01 |
| GGT(U/L) | 32.57 ±17.967 | 23.78 ±6.640 | 0.001 |

** Independent sample T test was used for comparison, value considered significant at level ≤ 0.05.

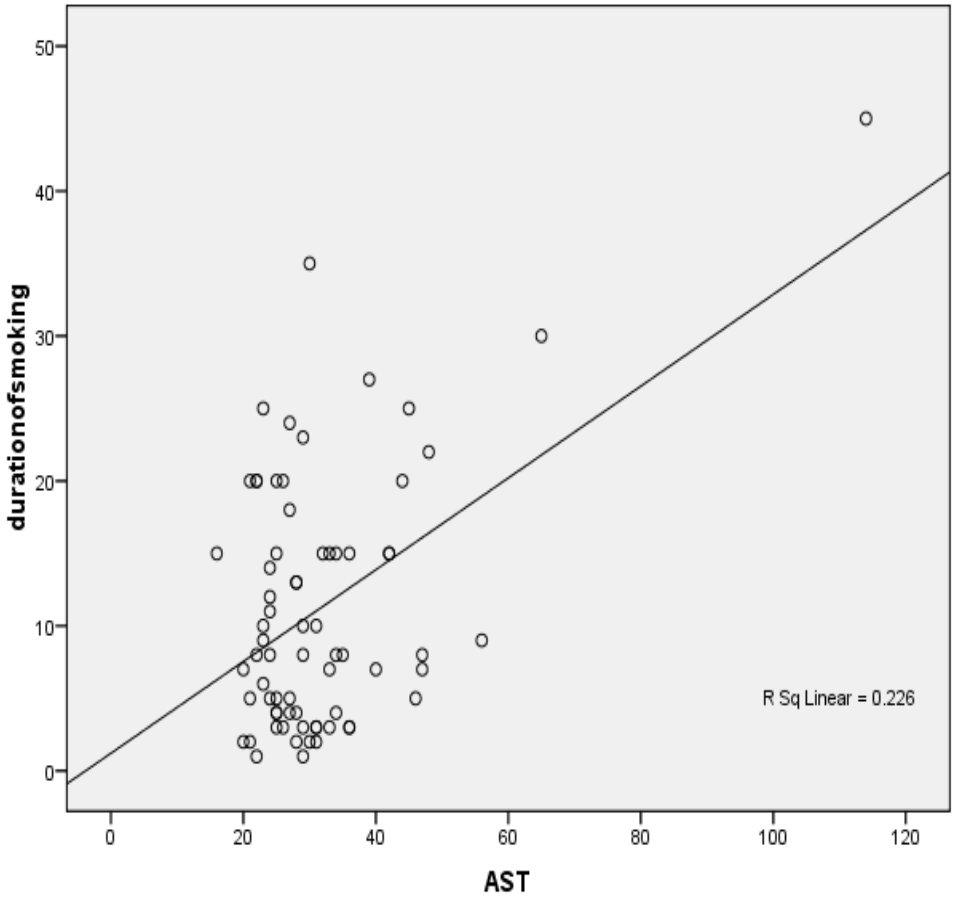


**Figure 3.1:** correlation between AST activity and duration of smoking among Sudanese smokers.
(P=0.000, r =0.476).

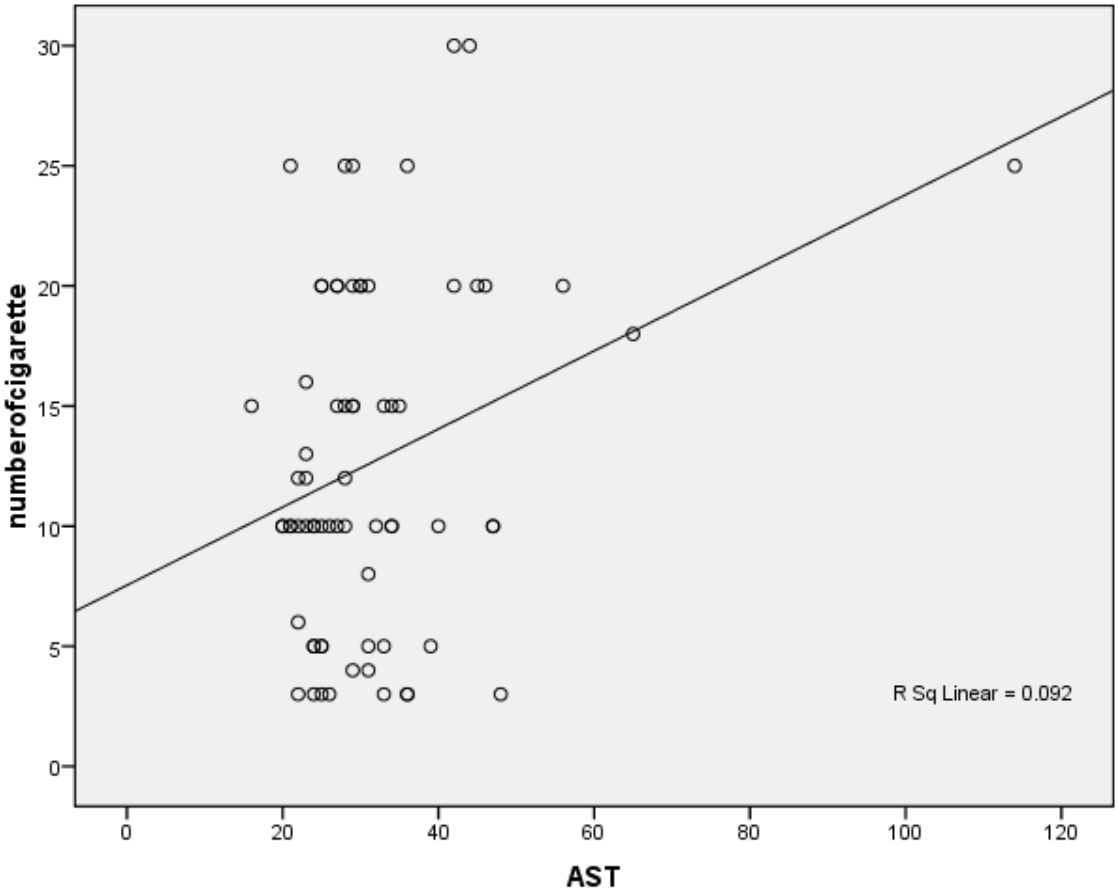


**Figure 3.2**: correlation between AST activity and number of cigarette per day among Sudanese smokers.
(P=0.011, r =0.304).

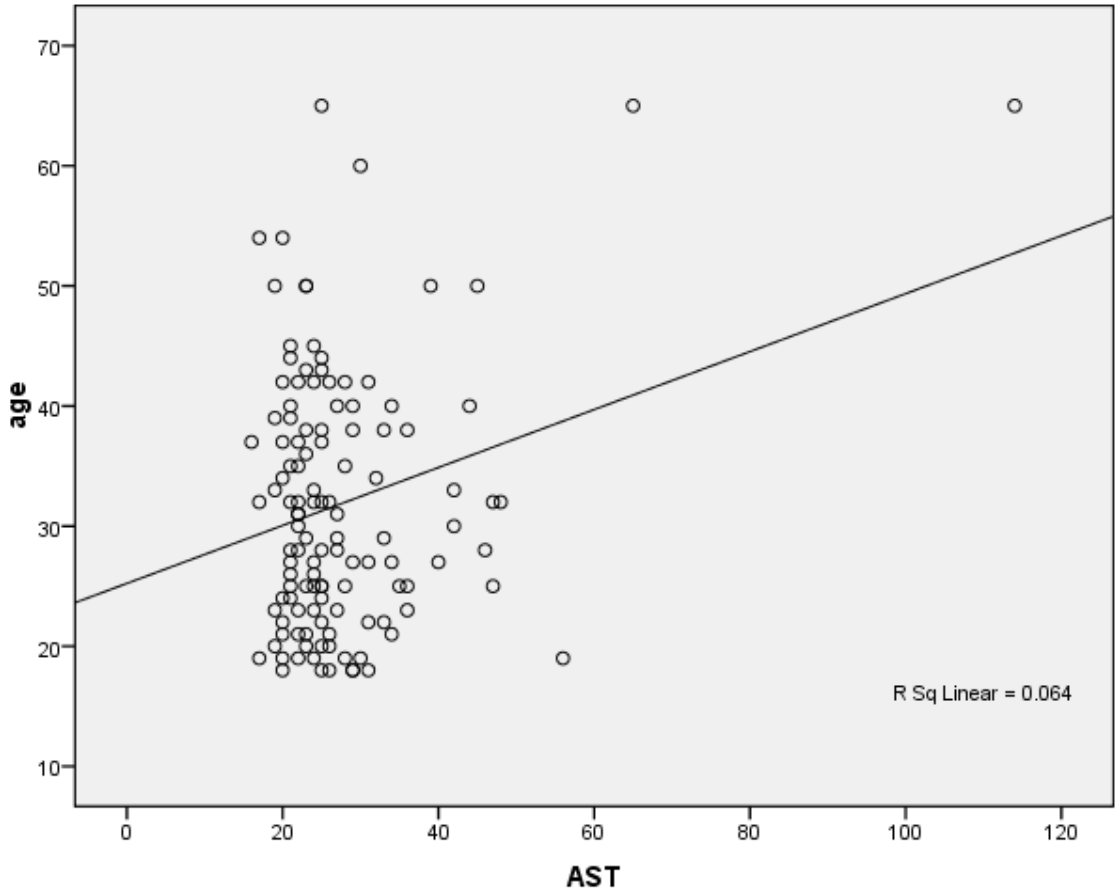


**Figure 3.3**: correlation between AST activity and age among Sudanese smokers.

(P=0.005, r = 0. 253).

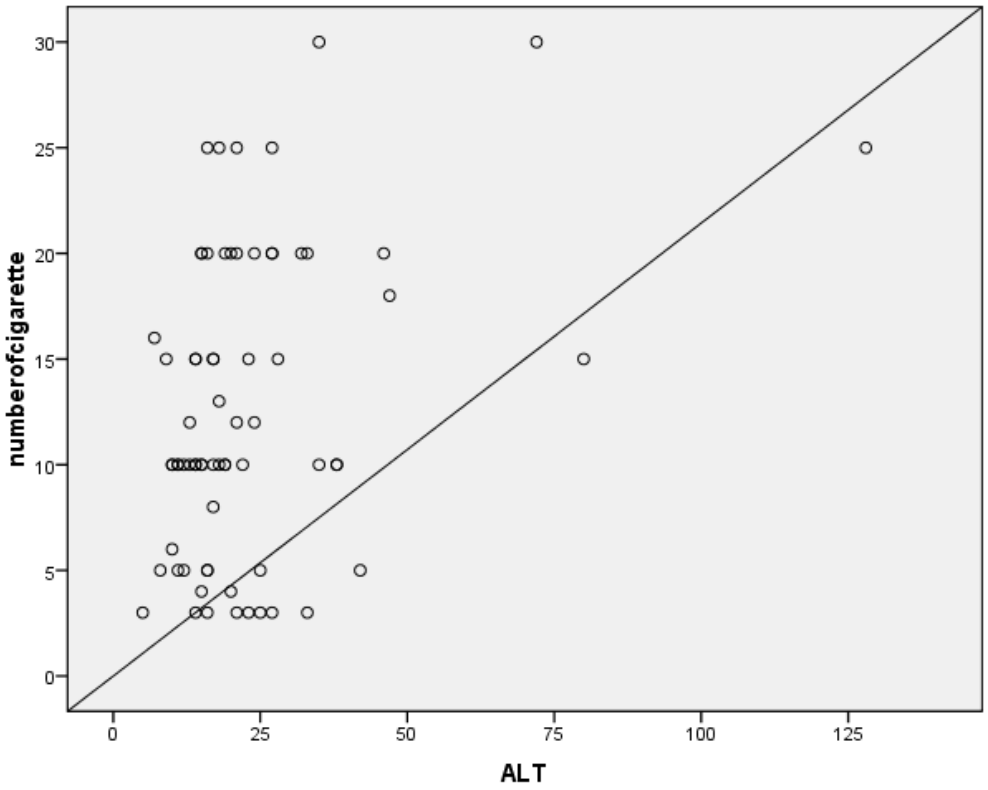


**Figure 3.4**: correlation between ALT activity and number of cigarette per day among Sudanese smokers.

(P=0.002, r = 0.369).

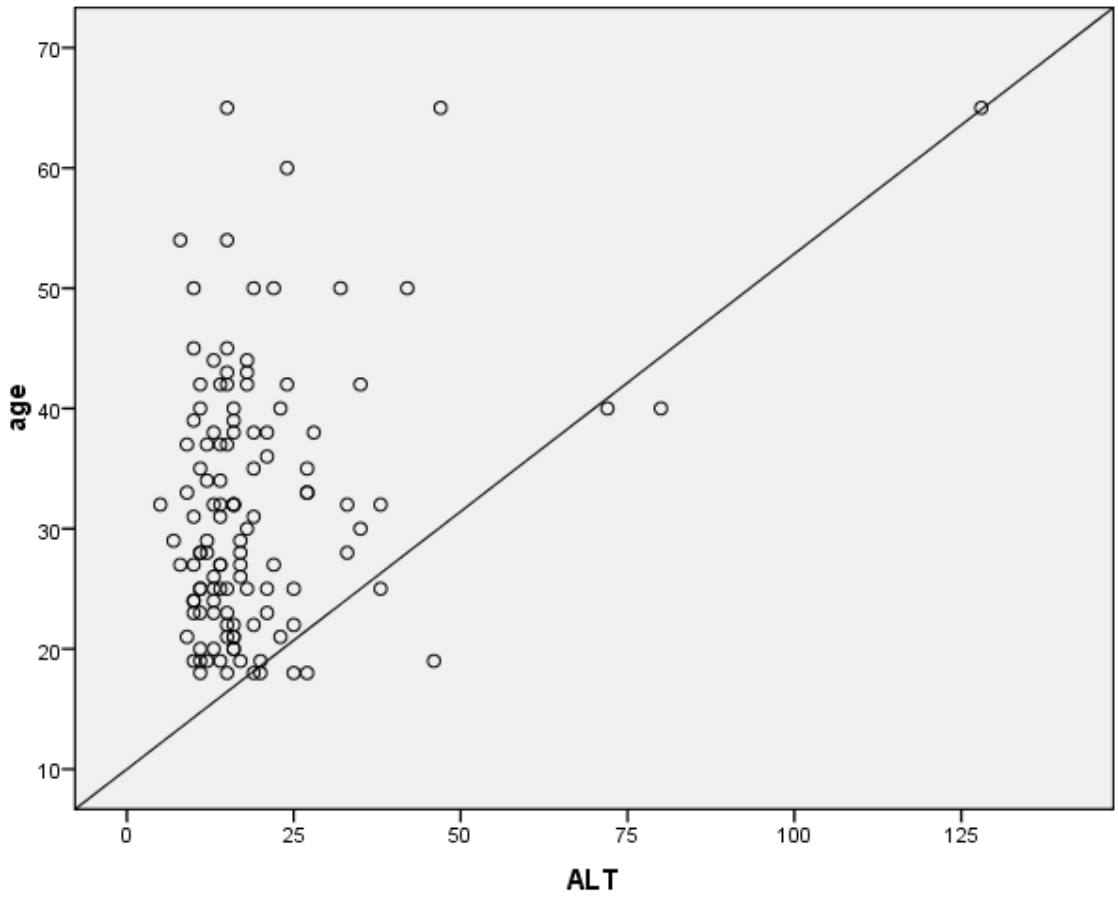


**Figure 3.5**: correlation between ALT activity and age among Sudanese smokers.
(P=0.000, r = 0. 325).

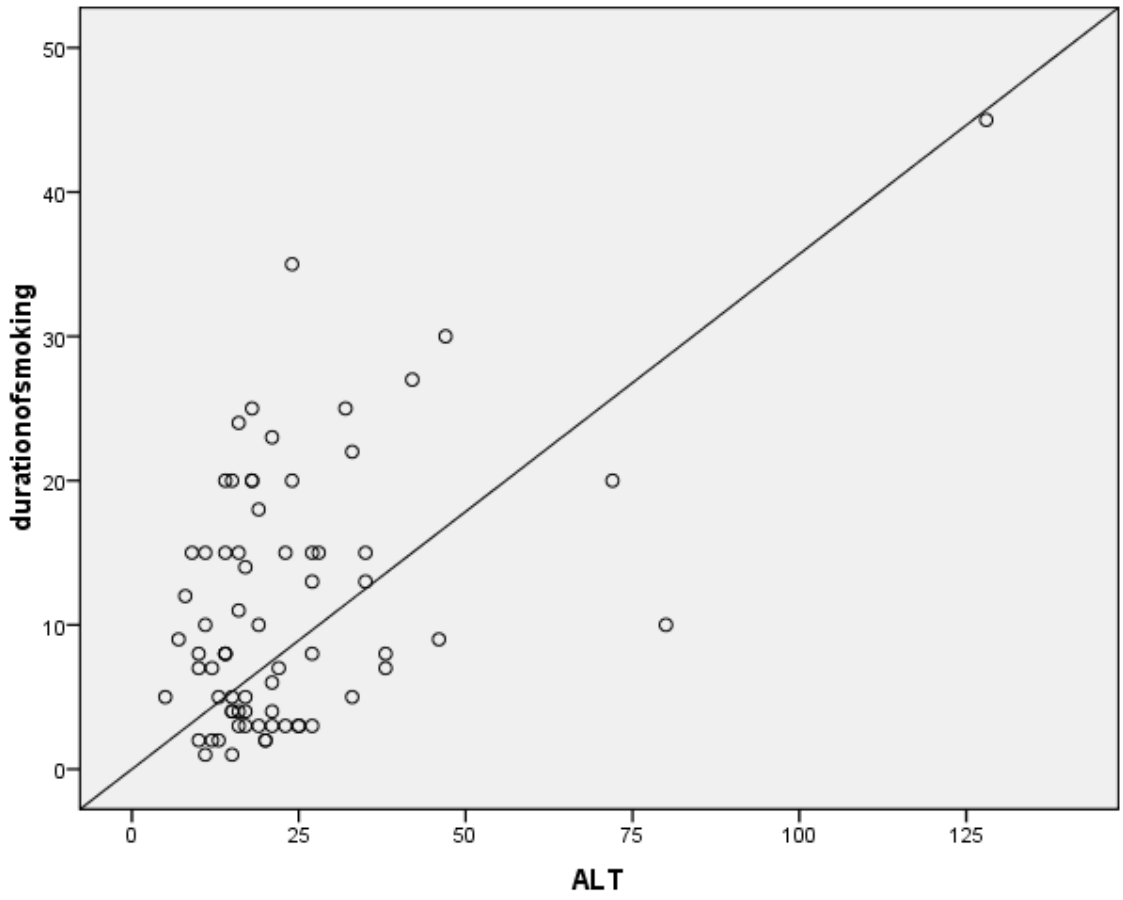


**Figure 3.6**: correlation between ALT activity and duration of smoking among Sudanese smokers.

(P=0.000, r =0. 498).

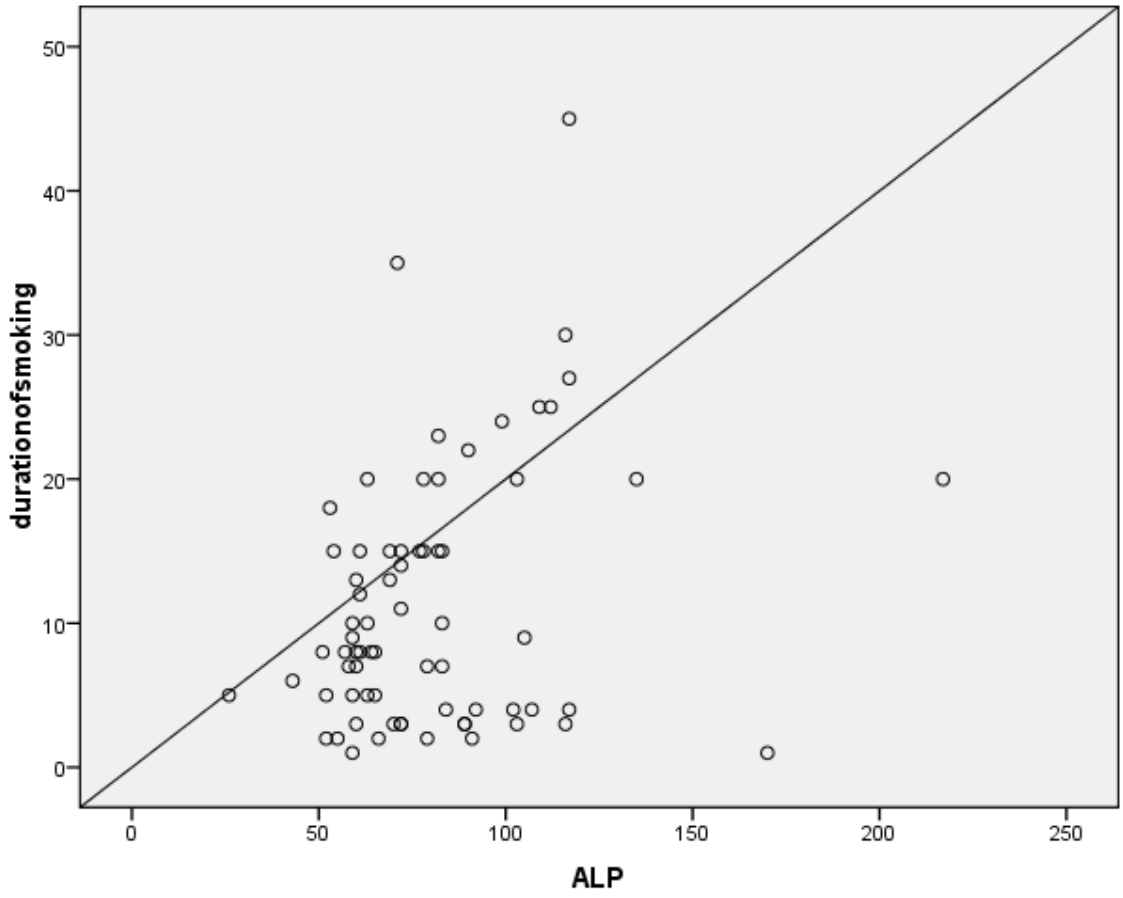


**Figure 3.7**: correlation between ALP activity and duration of smoking among Sudanese smokers.
(P=0.016, r =0. 287).

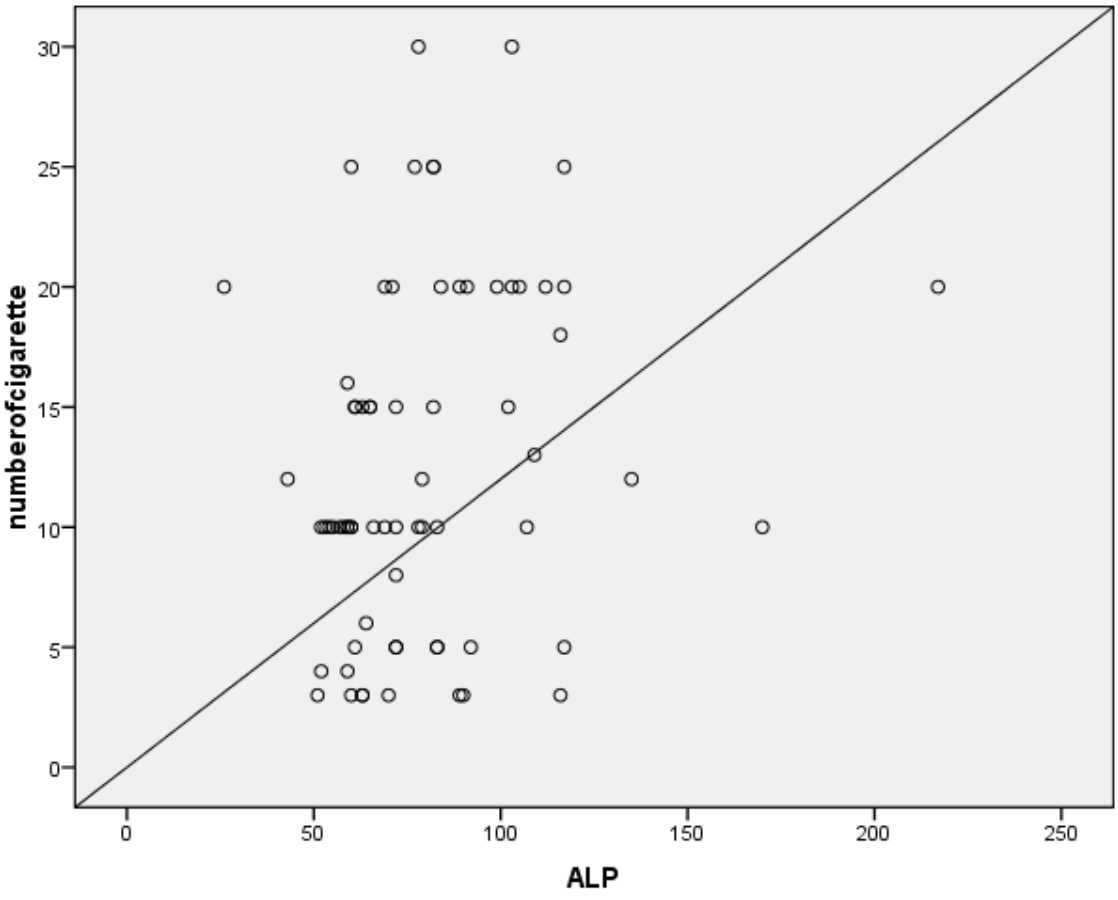


**Figure 3.8**: correlation between ALP activity and number of cigarette per day among Sudanese smokers.
(P=0.05, r = 0. 231).

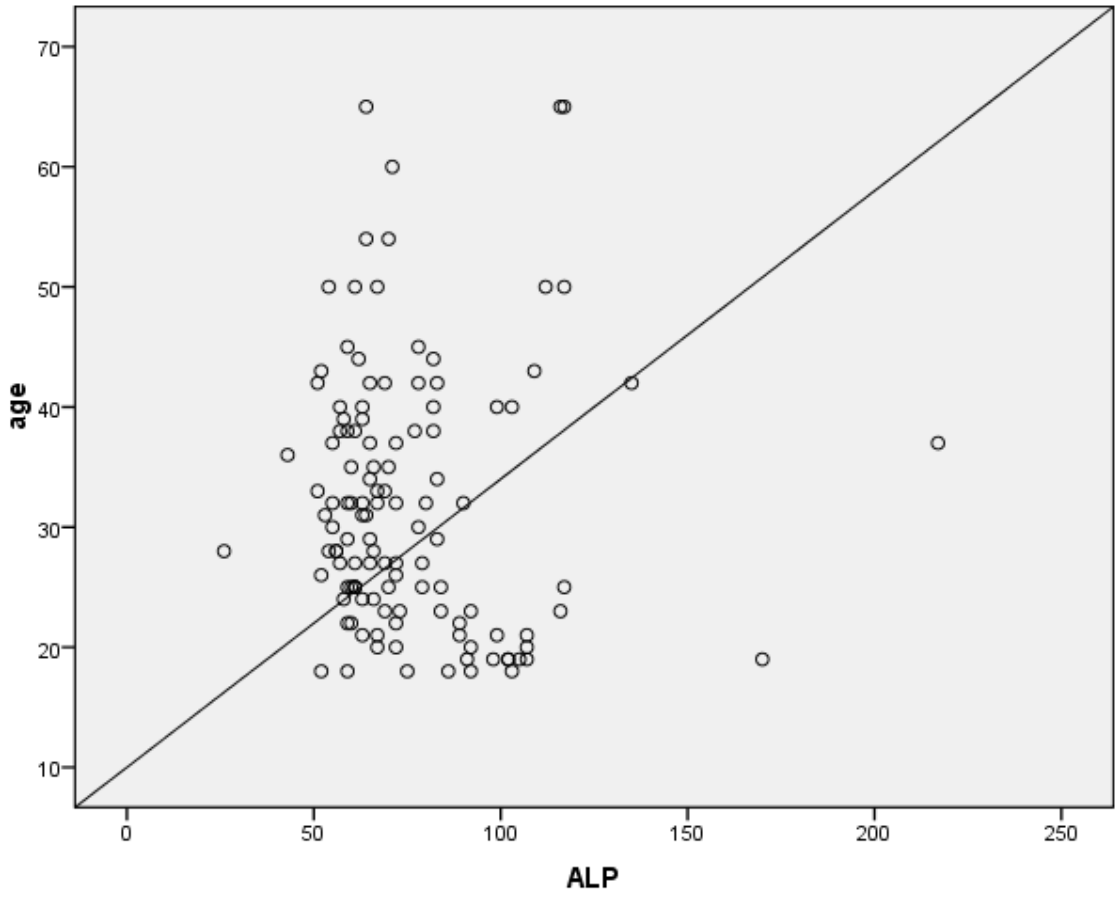


**Figure 3.9**: correlation between ALP activity and age among Sudanese smokers .
(P= 0.859, r =-.016).

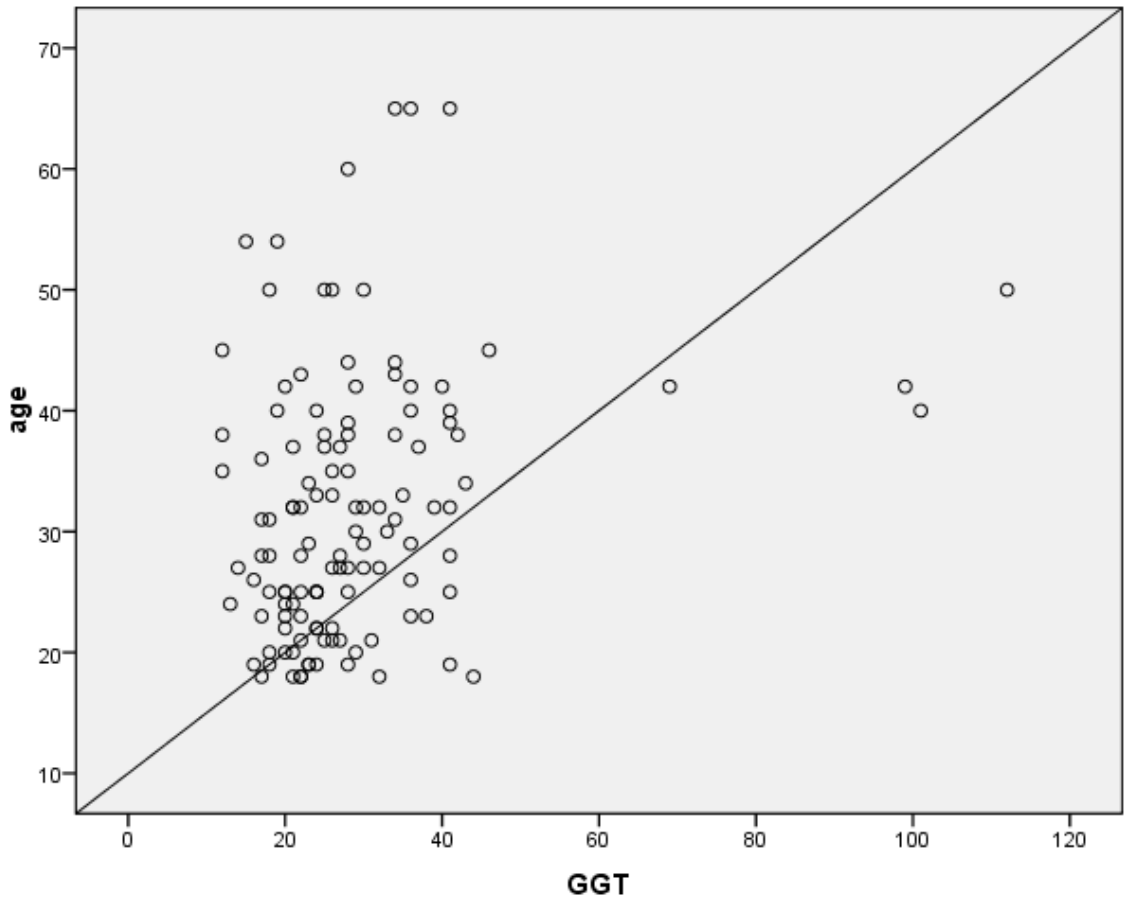


**Figure 3.10**: correlation between GGT activity and AGE among Sudanese smokers.

(P=0.002, r = 0. 275).

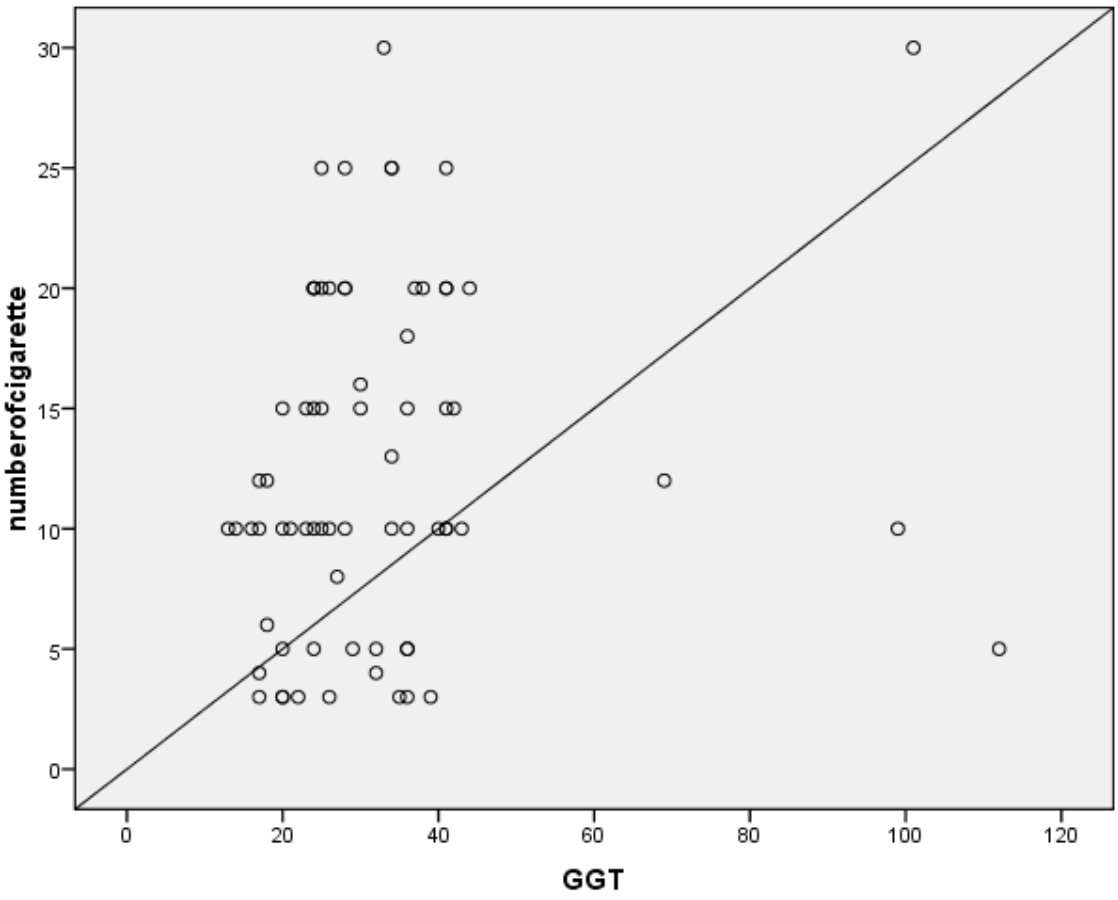


**Figure 3.11**: correlation between GGT activity and age among Sudanese smokers .

(P=.207, r =0.153).

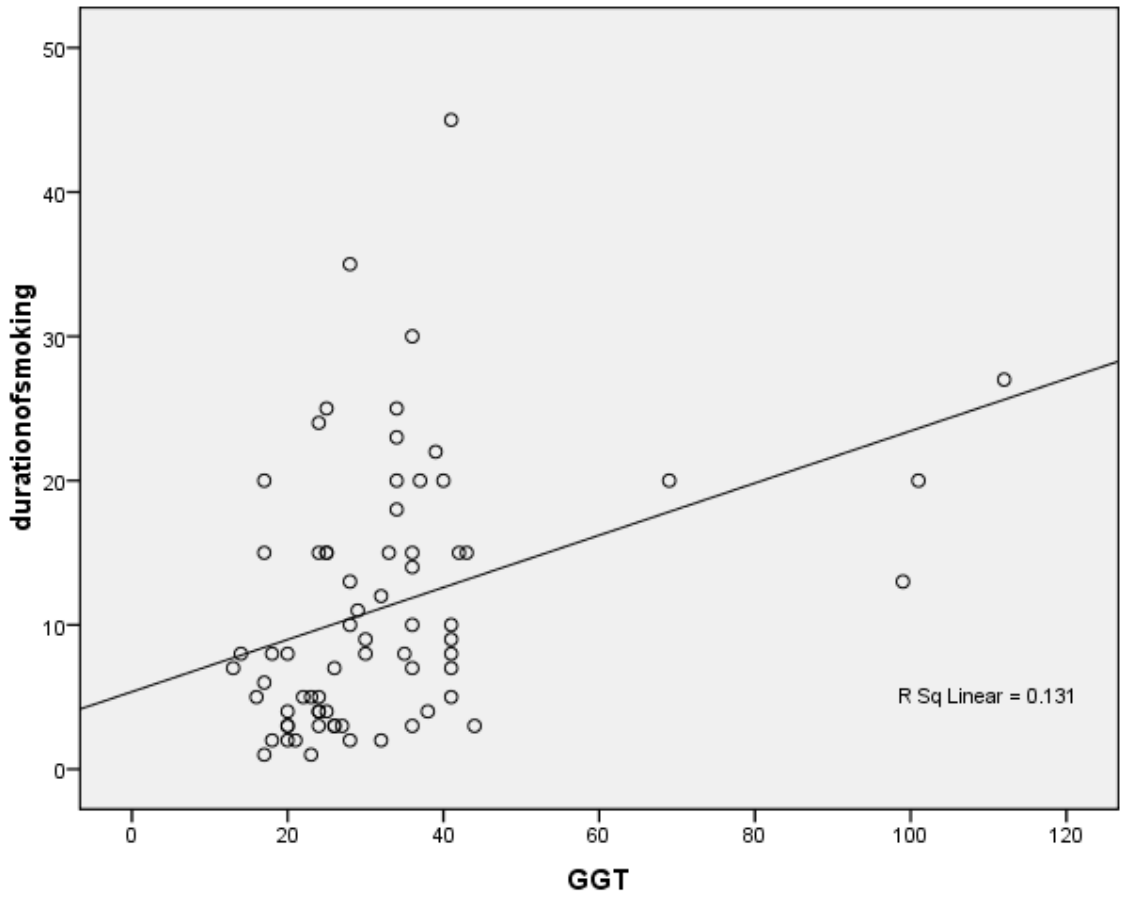


**Figure 3.12**: correlation between GGT activity and duration of smoking among Sudanese smokers .
(P=0.002, r =0. 362).

# 4. Discussion, conclusion and recommendations

## 4.1. Discussion

Nicotine is the major component of cigarette smoke plays an important role in the development of many diseases (Czernin *.et al,* 2003). It causes oxidative damage to kidney, lung, liver and heart. It is a potential oxidant, which is capable of producing free radical and reactive oxygen species (Yildiz *et al.*, 1998).

The nicotine induce free radical to react with bio membrane causing oxidative destruction of poly unsaturated fatty acid and forming cytotoxic aldhydes by lipid per-oxidation, lipid per-oxidation implicated in pathogenesis of number of disease (Mortal *et al.*, 1963).

This is a case control study aimed to study the effect of smoking on liver enzymes activities. One hundred and twenty Sudanese male (70 smokers and 50 non smokers) were enrolled in this study to study the effect of smoking on liver enzymes. After evaluation of enzymes activities by auto analyzer, the statistical analysis was done by using SPSS computer program and the results showed that all liver enzyme levels was significantly higher in smoker group when compared to non smoker group(table 3-1) , increase in activity of AST, ALT, ALP and GGT in smokers indicate tissue damage due to loss of functional integrity of cell membrane (Wetscer *.et al,* 1995).

This result agreed with results of study conducted in El-beida City, Libya done by Alsalhen to show the Effect of cigarette smoking on liver function among smokers and non-smokers male, showed that the smoker had significantly higher than the non smokers in AST , ALT and ALP activities ($P<0.05$) in smoker's plasma ,and means ± SD (22.72 ± 3.26 vs. 38.06 ± 9.79 for AST), (20.37 ± 4.33 Vs 36.24 ± 8.79 for ALT) , and

(139.0 ± 10.90 vs. 240.51 ± 63.84 for ALP) (Alsalhen ,2014) . and this is agreed with study done by Osifio and his team conducted in serum Gamma Glutamyl transferase level in cigarette smokers , show that a significant increase in serum level of GGT compared to non smoker ($p<0.05$), and mean± SD (73±4.96 U/L) VS ( 43±11.86 U/L)( Osifio .*et al*,2013).

Results of this study revealed that increases in AST,ALT ,ALP and GGT is proportional with duration of smoking per years , also The serum AST,ALT, and GGT activities are a significant positive correlation with age , and there were no correlation between ALP with age . The serum AST, ALT, ALP and GGT activities are a significant positive correlation with duration of smoking .The serum AST,ALT, ALP activities are significantly positive correlation with number of cigarettes and There were no correlation between GGT activities and number of cigarettes per day.

## 4.2. Conclusion

The study results revealed that:

1. The mean of serum AST, ALT, ALP and GGT are significantly increased in smoker when compared to non smokers group.
2. Serum AST, ALT, and GGT activities are a significant positive correlation with age, and were no correlation between ALP and age.
3. serum AST,ALT, ALP and GGT activities are a significant positive correlation with duration of smoking
4. serum AST, ALT, ALP activities showed significantly positive correlation with number of cigarettes and there were no correlation between GGT activities and number of cigarettes smoked per day.

## 4.3 Recommendations

1. health program can be designed to awareness about several side effects of smoking.

2. Smokers should estimate liver enzymes activity routinely to have an early alarm about the oxidative damage to liver.

4. Other study studies are suspected the increases in ALP activity in current smokers from bone , needed to do further studies estimate bone biomarker to reflect the effects of smoking on bone.

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# Appendix I

## Sudan University of Science and Technology

## College of Graduate Studies

## Effect of Smoking on Serum Liver Enzymes Activities among Sudanese Male Smokers

### Khartoum state 2015

### Questionnaire

Name: ..... No of sample ( ).

Age: .....

Duration of smoking/ years: .....

Number of cigarettes / day: .....

History of other diseases: .....

Results:

AST Activity : ..... U/L

ALT Activity : ..... U/L

ALP Activity:.....U/L

GGT Activity:..... U/L