**CHAPTER 1**

**1.0 Introduction**

Human Immunodeficiency Virus (HIV) is a retro virus known to be the primary aetiologic agent of Acquired Immuno Deficiency Syndrome (AIDS). People living with HIV and AIDS are prone to developing liver diseases because of the antiretroviral therapy which hamper the growth of the virus, there by, causing the suppression of viral particle multiplication and eventually leads to a decreased viral load. Liver toxicity is an important complication of HIV infection. The pathogenesis of drug-induced liver disease normally involves the participation of the parent drug or its metabolite that either affects the cell biochemistry directly or indirectly by iliciting an immune response (Neil;2004).

 All classes of antiretroviral drugs have been associated with potential risk of hepatotoxicity (sukowski,2004). There are several laboratory test used to monitor how the liver is working as well as to determine the cause of the problem. Some of them are Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) also known as transaminases. Both enzymes are present in high concentration in the liver cells (hepatocytes). Damage to the liver cell cytoplasmic membrane that may be caused by inflammation or leakage of cytoplasmic contents causes relatively greater increase in the serum AST and ALT level (Harigan; et al,2003) On the other hand if the damage occurs in both the mitochondria and cytoplasmic membrane, there is proportionally greater increase in both the serum AST and ALT levels. Hence, AST and ALT are referred to as “Hepato cellular damage” (currier, et al; 2003).

 Antiretroviral Drug – Related liver Injury (ARLT) is defined by elevations of AST and ALT in serum, with ALT characteristically greater than AST. Because AST is found in many other organs beside the liver, including the kidneys, the muscles and the heart, having a high level of AST does not always (but often does) indicate that there is a liver problem. On the other hand, because ALT is found primarily in the liver, high levels of ALT almost always indicate that there is a problem with the liver. ARLT is one of the greatest causes of treatment discontinuation in HIV infected patients (Castillo, et al; 2006). Its prevention and management is therefore very important among HIV infected patients who are to be placed on Highly Active Antiretroviral Therapy (HAART) (Pamella,et al; 2006). With the wide spread use of HAART and availability of new anti retroviral medications, ARLT has gained prominent attention owing to its negative impact of clinical outcomes. Furthermore, antiretroviral drug discontinuation hampers maintenance of HIV suppression.

 Severe HAART are hepatotoxic and the liver is one of the vital organs useful in the metabolism of the drugs as well as in detoxification. It is there fore important that the liver which is the main biochemical hub of the body be monitored and those HAART regimens that may be toxic to it identified, so that changes or modifications can be made to enhance patient care. Apart from antiretroviral drugs other factors can influence the level of transaminases, these are risk factors such as hepatitis B, hepatitis c, alcohol; pregnancy; age; physical exercise; previous intramuscular injection; gender, oral contraceptives, myocardial infarction, antibiotics like erythromycin, antihypertensives, cardiac operations, beriberi, hepatic cirrhosis, hepatic necrosis, obstructive jaundice (Anne, et al; 2006).

* 1. **Statement of the problem.**

Recent studies have suggested that HAART, may lead to rises in ALT and AST among HIV-infected patients. Highly active antiretroviral therapy (HAART) has proven remarkably effective in prolonging the life of patients with HIV (J-int; 2009). However, while most HAART agents are safe, many have the potential to cause liver toxicity.

 In different studies written by different authors, results are showing that elevated transaminase level is one of the major problems in HIV patients. Results obtained in the USA showed that 10 % of HIV patients were suffering from liver diseases (Pol,et al 2004). On the other hand, results obtained in Shisong showed that 36.4% of HIV-infected patients where suffering from liver diseases (Nadege ,2009). Furthermore, it was identified that the end –stage liver disease is the leading cause of morbidity and mortality in HIV-infected patients (Bica, et al; 2001).

More of it, it has been found that liver damage caused by medication ingestion, also known as Drug Induced Liver Injury (DILI) has become an important public health problem contributing to more than 50% of acute liver failure (Bonacini;2006)

 The results above are showing that HIV patients are prone to have elevated ALT and AST level, thus suffering from liver diseases, thus the emergence of new liver pathology in other to reduce morbidity and mortality among HIV patients. Medical personal must therefore consider the possibility of drug-induced liver injury in the management of HIV patients especially those with certain risk factor such as co-infection with hepatitis.

1-**2 Research question and hypotheses**

1-2-1 **Research question**

Are all HIV patients on antiretroviral treatment suffering from transaminitis?

1-2-2 **Hypotheses**

* Null hypothesis: All HIV patients on antiretroviral treatment are suffering from transaminitis.
* Alternative hypothesis: Not all HIV patients on antiretroviral treatment are suffering from transaminitis.

1-3 **Goals and objectives**

1-3-1 **Goals**

- To assist the therapeutic committee of the Hospital to improve upon the management of HIV patients.

- To educate HIV patients why they must live a healthy life.

1-3-2. **Objectives**

- To determine the prevalence of transaminitis in the shisong General Hospital.

- To determine other factors contributing to the elevation in transaminase levels in addition to antiretroviral medication.

 **1-4) Relevance of the study**

The outcome of this piece of work will help us.

* To raise the awareness about the importance of the liver for an HIV positive patient.
* To provide a tool for health personel taking care of HIV patients which will permit them to know much more about the liver state of their patients.
* Explain the impact of hepatic dysfunction on anti-retroviral pharmacokinetics.

1-5 **Limitations**

- School programs interfered with this work program making it difficult to have a larger sample size.

- The local dialet was a problem in answering the questionnaires

- Patients refusal to participate in the research.

**CHAPTER II**

**2.0 Introduction:**

 Management of human immunodeficiency virus (HIV) has become increasingly complex since the introduction of HAART. Hepatic complications have become common and may lead to discontinuation of treatment and significant mobidity. Up to 90% of patients with AIDS receive at least one drug that can cause hepatotoxicity(Orenstern, et al; 2002). Clinicians treating patients with HIV frequently face difficulty distinguishing abnormal liver transaminase levels and toxicities in patients receiving several drugs. Some potential causes of hepatic dysfunction are viral infections, alcohol and substance abuse. Medical personals should e aware of the potential non-antiretroviral hepatotoxic agents that are frequently administered in HIV management.

**2-1 HIV and Antiretroviral**

 **2-1-1 HIV**

Human immunodeficiency virus (HIV) is a lentivirus ( a member of the retrovirus family) that cause acquired immuno deficiency syndrome (AIDS), a condition in humans in which the immune system begins to fail, leading to life – threatening opportunistic infections. Infection with HIV occurs by the transfer of blood, semen, vagina fluids, pre-ejaculate, or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells. The main major routes of transmission are unsafe sex, contaminated needles, breast milk, and transmission from an infected mother to her baby at birth (prenatal transmission). HIV infection in humans considered pandemic by the World Health Organisation (WHO). From its discovery in 1981 to 2006, AIDS killed more than 25 million people (Greener;2002) . HIV infects primarily vital cells in the human immune system such as hyper T cells (specifically CD4 T cells) macro phages and dentritic cells (Donaghy, et al;2010). Anti retroviral treatment reduces both the mortality and morbidity of HIV infection ( palella et al 1998). Without anti retroviral therapy someone who has AIDs typically dies within a year. (Morgan, et al 2002).

**2-1-2 Antiretroviral**

 Antiretroviral drugs are medications for the treatment by retroviruses, primarily HIV. When several such drugs, typically 3 of 4 are taken in combination, the approach is known as Highly active antiretroviral therapy ( HAART). The American National Institute of health and other organizations recommend offering antiretroviral treatment to all patients with AIDS (Dybul, et al;2002).

Anti retroviral (ARV) drugs are broadly classified by the phase of the retrovirus life cycle that the drug inhibits.

* Nucleoside and nucleotide reverse transcriptase inhibitors (NRTI) inhibit revere transcription by being incorporated into the newly synthesized viral DNA strand as a faulty nucleotide.
* Non – nucleoside reverse transcriptase inhibitors ( NNRTI)

Inhibits reverse transcriptase directly by binding to the enzyme and interfering with its function.

* Protease inhibitors (PIs) target viral assembly by inhibiting the activity of protease, an enzyme used by HIV to cleave nascent proteins for final assembly of new virions.
* Integrase inhibitors inhibit the enzyme integrase which is responsible for integration of viral DNA into the DNA of the infected cell.
* Maturation inhibitors inhibit the last step in gag processing in which the viral capsid polyprotein is cleared thereby, blocking the conversion of the polyprotein into the mature capsid protein (P24)
* CCR5 recetor antagonist are the first antiretroviral drugs which do not target the virus directly instead they bind to the CCR5 receptor on the surface of the T-cell and block viral attachment to the cell.
* Entry inhibitors ( or fusion inhibitors) interfere with binding, fusion and entry of HIV-1 to the host cell by blocking one of the several targets. (Smiley, et al;2008)
	1. **Anatomy and physiology of the liver.**

**2-2-1 Anatomy of the liver**

The liver is the largest visceral organ in the body. A human liver normally weighs 1,4-1,6 kg. The liver is located in the right upper quadrant of the abdomen just below the diaphragm. A fibrous capsule of connective tissue called Glissons capsule covers the entire surface of the liver. The liver is divided into a large right lobe and a smaller left lobe. Each lobe is further divided into lobules that are approximately 2mm high and 1mm in circumference. Hepatic lobules are the functional aggregation of hepatocytes. Each of the approximately 100,000 lobules consist of mostly single cell thickness plates of hepatocytes which radiate out from a central vein. The spaces between the plates are called sinusoids. About 25% of the total cardiac output enters the sinusoid via terminal portal and arterial vessels. The blood mixes, passes through the sinusoids, bathes the hepatocytes and drains into the central vein – about 1.5 litres of blood exit the liver every minute. ( cotran, et al; 2005)

 **2-2-2 Physiology of the liver**

- Excretion of bile: The liver produces and excretes bile into the intestine.

- Carbohydrate metabolism: The main monosacharrides obtained during digestive processes are converted into glycogen and stored in the liver. When required, the glycogen is re-converted to glucose ( glycogenolysis) thus blood glucose level is maintained. But if the liver has used all the stored glycogen and blood glucose is below normal, the liver can convert protein and fats into glucose (gluconeogenesis)

- Protein production: plasma proteins mainly fibrinogen, albumin and globulin are manufactured in the liver. The liver also produces transport protein such as transferin which binds and transport irons, and haptoglobin which combines with free haemoglobin.

- Storage: In addition to glycogen, and many vitamins, the liver is the main organ of storage for iron synthesis.

- Synthesis of blood clotting factors: Many of coagulation factors, fibrinogen, prothrombin, factors V, VII, IX, X XI, and XII are synthesized in the liver.

- Detoxification: The ammonia released from amino acid deamination is detoxicated by being converted to urea for excretion by the kidney. The liver also detoxicate drugs, metabolic products, hormones and alcohol by oxidation, conjugation, methylation or reduction.

- Metabolism of lipid: The synthesis of cholesterol, phospholipid, endogenous triglycerides and liproprotein occurs mainly in the liver, including the esterification of cholesterol.

- Phagocytic: Micro - organisms are removed by the kupffer cells which form a part of mononuclear phagocytic defence system (Ochei; et al; 2008)

**2-3. Types of liver diseases**

* Hepatitis: Inflammations of the liver, usual caused by viruses like hepatitis A,B and C. hepatitis can have non infectious causes too, including heavy drinking, drugs, allergic reactions or obesity.
* Cirrhosis: long term damage to the liver from any cause can lead to permanent scaring, called cirrhosis. The liver then becomes unable to function well.
* Liver cancer: The most common type of liver cancer, hepatocellular carcinoma almost always occurs after cirrhosis is present.
* Ascites: As cirrhosis results, the liver leaks fluids ( ascites) into the belly, which becomes distended and heavy.
* Gilbert’s syndrome: A genetic disorder of bilirubin metabolism, found in about 5% of the population.
* Wilson’s disease: A hereditary disease which causes the body to retain copper.
* Haemochromatosis: A hereditary disease causing the accumulation of iron in the body eventually leading to liver damage.
* Jaundice: Also known as icterus, is a yellowish pigmentation of the skin, the conjunctiva membrane over the sclerae (whites of the eyes) and other mucous membranes caused by hyperbilirubinemia (Bathesda; 2009)

**2-4. Signs and symptoms of hypatotoxicity**.

 Symptoms partly depend on the type and the extent of liver disease. In many cases, there may be no symptoms. signs and symptoms that are common to a number of different types of liver disease include.

* Pale stools: occur when stercobilin, a brown pigment is absent from the stool. Stercobilin is derived from bilirubin merabolites produced in the liver.
* Dark Urine: When bilirubin mixes with urine.
* Swelling of the abdomen, ankles and feet occurs because the liver fails to make albumin.
* Excessive fatigue: Occurs from a generalized lost of nutrients.
* Jaundice: That is yellowing of the skin and the eyes caused by hyperbilirumia.
* Itching: Bilirubin when it deposits in skin causes an intense itch. Itching is the most common complain by people who have liver failure often, this itch cannot be relieved by drugs.
* Bruising and easy bleeding are other features of liver disease. The liver makes substances which help prevent bleeding. When liver damage occurs, these substances are no longer present and severe bleeding can occur.
* Loss of appetite: Due to incorrect metabolism of the carbohydrate, proteins and fat in the body that will eventually turn into weight loss (Admin, 2009).

**2-5 Factors affecting transaminase levels.**

* + Viral hepatitis: This is inflammation of the liver usually caused by viruses which damage the liver typically results in a leak of AST and ALT into the blood stream.
	+ Strenous exercise: Because large amount of AST are present in skeletal muscles, vigourous exercise can increase AST level.
	+ Heart failure: AST is found in cardiac muscle and when there is destruction of heart muscles, its level increases.
	+ Alcohol Ingestion: Alcoholic persons are susceptible to liver toxicity because alcohol induces liver injury and cirrhotic changes. Alcohol causes depletion of glutathione (hepatoprotective) stores that make the person more susceptible to toxicity.
	+ Age: Elderly persons are at increased risk of hepatic injury because of reduced hepatic blood flow and lower hepatic volume.
	+ Hepatotoxic drugs: Most drug metabolism occurs in the liver which converts the drug into a more water soluble compound.
	+ Herbal toxicity: Some of the herbal medicines prepared from certain roots and leaves are known to contain lead which is an environmental toxin that is capable of causing acute and chronic circulatory, hematological , gastrointestinal and sometime hepatic pathologies (Acrospace; 2008)
	+ Smoking: Cigarette smoking was reported to be a risk factor for the development for the hepatic dysfunction (sun, et al 2006). Cigarette smoke contains thousands of structurally diverse chemicals that process citotoxic, genotoxic and tumorigenic activity. A toxic air pollutant formed by smoking much as acrolein was reported to induce hepatotoxicity through direct mitochondrial damage (Benowitz, et al; 2003).
	1. **Mechanisms of drug-induced liver injury**.

 The pathophysiologic mechanisms of hepatotoxicity are still being explored and include both hepatocellular and extracellular mechanisms. The following are some of the mechanisms that have been described.

* Disruption of the hepatocyte: Covalent binding of the drug to intracellular proteins can cause a decrease in ATP level, leading to actin disruption. Disassembly of the actin fibrils at the surface of the hepatocyte causes blebs and rupture of the membrane.
* Disruption of the transport protein: drugs that affect transport proteins at the canalicular membrane can interrupt bile flow, lost of villous processes and interruption of transport pumps such as multidrug resistance associated protein 3 prevent the excretion of bilirubin, causing cholestasis.
* Cytolytic T – cell activation: Covalent binding of a drug to the P-420 enzyme acts as an immunogen activating T cells and cytokines and stimulating a multifaceted immune response.
* Apoptosis of hepatocytes: Activation of the apoptotic pathways by the tumor necrosis factor – alpha receptor of Fas may trigger the cascade of intercellular caspaces which results in programmed cell death.
* Mitochondria disruption: Certain drugs inhibit mitochondrial function by a dual effects on both beter -oxidation energy production by inhibitingthe synthesis of nicotinamine adenine dinucleotide and flaving dinucleotide , resulting in decrease ATP production.
* Bile duct injury: Tomic metabolites excreted in bile may cause injury to the bile duct epithelium (Nilesh, et al; 2010).

**2-7 Metabolism of drugs**

 The liver metabolizes virtually every drug or toxin introduced in the body. Most drugs are lipophilic (fat soluble) enabling easy absorption across cell membrane. In the body, there are rendered hydrophilic (water soluble) by biochemical processes in the hepatocyte toenable inactivation and easy excretion. Metabolism of drugs occurs in 2 phases.

 In phase 1 reaction, the drug is made polar by oxidation or hydroxylation. All drugs may not undergo this step, and some may directly undergo the phase 2 reaction. The cytochrome P-450 enzymes catalyze phase 1 reactions. These reactions may result in the formation of metabolites that are far more toxin than the parent substrate and may result in liver injury. Cytochrome P-450 enzymes are heamoprotein located in the small endoplasmic reticulum of the liver. These metabolites must quickly be shunted to phase 2 in order for them to make them safer for the body to use.

Phase 2 is the conjugation phase that increases solubility, subsequently drugs with high molecular weight may be excreted in bile while the kidneys excrete the smaller molecules. Several types of conjugation reactions are present in the body, including glucoronidation, sulfation and amino-acid conjugation (Lisa, et a; 2010)

 **2-8. Other liver tests:**

- Alkaline phosphatase: Alkaline phosphatase is present in bile-secreting cells in the liver, and also in bones. High levels often mean bile flow out of the liver is blocked.

- Bilirubin: High bilirubin levels suggest a problem with the liver.

- Albumin: As part of total protein levels, albumin helps determine how well the liver is working.

- Ammonia: Ammonia levels in the blood rise when the liver is not functioning properly.

- Hepatitis: This is an inflammation of the liver caused by viruses.

- Prothrombin time (PT): A prothrombin time or PT is commonly done to see if someone is taking the correct dose of the blood thinner warfarin. It also checks for blood clothing problems.

- Partial thromboflastin time (PTT). A PTT is done to check for blood clotting problems.

- Gramma – glutamyl transferase: hint a possible blockage of the bile ducts. AP and GGT are known as cholestatic liver enzymes. (Nyblom, et al; 2004)

**2-9 Diagnosis of drug induced liver injury.**

 Establishing with any degree of certainty as to whether the liver disease is drug induced can be very difficult. The key to causality is to assess the temporal relationship between drug initiation and development of an abnormal liver panel, the individual susceptibility to DILI, and to diligently exclude other causes of liver diseases. These include liver injury induced by alcohol, viral hepatitis, auto immune metabolic disorder, and biliary obstruction. The activities of Alanine aminotransferase ALT and Aspartrate aminotransferase (AST) can be monitored to define hepatic complications due to HAART. This can be done by the use of an automated chemistry analyzer like spectrophotometer (kaplowitz , 2011).

Antiretroviral related liver injury can then be defined by elevations in liver enzymes in serum, with ALT characteristically greater than AST.

Liver function test abnormalities require carefully interpretation because elevated aminotransferases also need to be interpreted within their clinical context. For example, increased liver enzymes in patient with chronic HBV infection do not necessarily imply drug injury but may reflect HBV-related hepatic flares which often occur during the natural course of the disease (Soriano; 2008).

**Principles of transaminases**

**- Principle of AST**

AST or glutamate oxaloacetate catalyses the reversible transfer of an aminogroup from aspartate to -ketoglutarate forming glutamate and oxaloacetate. The oxaloacetate produced is reduced to malate by malate dehydrofenase (MDH) and NADH.

Aspartate + α-xetoglulerate  gluramate + oxaloacetate

COOH COOH COOH COOH

CH2 + CH2 AST CH + CH2

 H-C-NH2 CH2 C=0 CH2

COOH C=0 COOH H-C-NH2

 COOH COOH

Oxaloacetate + NADH + H+ MDH malate+ NAD+

 The rate of decrease in concentration of NADH ,measured photometrically,is proportional to the catalytic concentration of AST present in the sample.(Young ,et al,20010).

-Principle of ALT

 ALT or glutamate pyruvate transaminase catalyses the reversible transfer of an amino group from alanine to α-ketoglutarate forming glutamate and pyruvate.the produced is reduced to lactate by lactate dehydrogenase (LDH) and NADH.

Alanine + α- ketoglurarate ALT Gluramate + pynuvate

 CH3 COOH CH3 COOH

H-C-NH2 + C=0 ALT  C=O + A-C-NH2

 COOH CH2 COOH CH2

 CH2 CH2

 COOH COOH

Pyruvate + NADH + H+ LDH lactate + NAD+

The rate of decrease in concentration of NADH, measured photometrically is proportional to the catalytic concentration of ALT present in the sample (young, et al; 2001).

**2.10). Therapeutic management of ARV drug related liver injury**

1. **Discontinuation of antiretroviral drugs.**

Once a patient develops drug -induced liver injury, (DILI) the management includes prompt discontinuation of the offending drug. However, stopping medications at the very first sign of mild injury can prevent serious consequences. For patient safety, several important principles need to be emphasized.

-Medications should be discontinued promptly if serum AST or ALT is greater than 10 times the upper limit of normal,even if the patient is asymptomatic.

* Always consider alternative causes for hepatitis including viral hepatitis, cholecystitis, opportunistic infections and alcohol abused (sulkowski; et al; 2003)
1. Spontaneous improvement in transaminases despite drug continuation.

In assessing drug toxicity, mild elevations of serum transaminases are commonly seen and often improved despite administration of the same drug ( martin, et al;1999). Some authors bave suggested that protease inhibitors containing HAART does not need immediate adjustment but simply careful monitoring (John, et al; 1998)

 **c) Liver biopsy**

Unfortunately, liver biopsy often does not contribute substantially to patient management. On occasion, histology can be helpful if eosinophils or granulomas are found, which suggest drug hypersensitivity ( puoti; et al;2003).

**2-11) Prevention of antiretroviral drug related liver injury.**

* **Patient education**

 It is often important to educate the patient about the symptoms of hepatitis, the bad effect of alcohol and cigarette related to liver injury. Patient education is particularly important when considering hypersensitivity reactions, which occur early and can be devastating if the offending drug is continued.

 **- Address modifiable risk factor.**

 Predisposing conditions such as obesity, alcohol, myocardial infarction should be addressed

**CHAPTER III**

**3.0 Methodology**

The study was a prospective study carried out on 210 specimens collect from November 2010 to march 2011,on 210 patients visiting the treatment center of the St. Elisabeth’s Catholic General Hospital and Cardiac Centre Shisong for their semestrial check up ( CDL, ALT,AST, blood sugar, hemoglobin).

 **3.1 Study area:**

The study was carried out at the treatment center of the St. Elizabeth’s Catholic General Hospital and Cardiac Center, Shisong. Shisong is situated in the Kumbo East health district of the North West Region. It was a population of about 15000 inhabitants most of who are farmers. There exist two seasons in Shisong as is the case with other towns and localities in Cameroon. It has a very hilly topography. It is a humid cold environment with temperatures ranging from 10-25 degrees centigrade with much colder mornings in the dry season.

 There are a few beer parlours, but many palm wine drinking spots in Shisong. However, there are many of such places in kumbo in general, with many of them situated in the commercial areas of squares and mbveh. In these areas, are also found a few hotels and inns.

 The Nso people who are the indigenes use “Lamnso” language to communicate amongst themselves, “Pidgin English” or English language to communicate with non- indigenes. The main occupations of the people are nursing, farming, trading, teaching and some are government public servants. The traditional dish in Shisong is corn fufu and huckle-berry known in the “Lamso” as “Kiban” and “nyosegi” while the main drinks are palm wine and “shar” or “nkang”.

 Settlement is sparse amongst trees giving this location a beautiful and green scenery, while housing is moderate, with no definite number of people per house.

**3.2 Study Population:**

 The study was carried out on all HIV/AIDS patients irrespective of age, gender, race, occupation or religion who visited the treatment centre of St. Elizabeth Catholic General Hospital and Cardiac Centre Shisong.

**3.3 Ethial Consideration:**

 Considering the fact that this study was to be carried out in St. Elizabeth General Hospital, Treatment Center Hospital laboratory, an authorization was obtained from the Catholic School of Health Sciences administration, the matron of the hospital and the head of the treatment center laboratory.

 The purpose of the study was presented and explained to each client to obtain an informed consent. There after, specimens were collected from patients who consented to the study.

 They were then assured of the confidentiality of the questionnaire and the results.

**3.4 Data Collection and Statistical Considerat**

 Data was collected by the use of closed ended structured questionnaires (see Appendix1). Since not all the respondents were literate, they were interviewed and the questionnaires filled in as they answered the questions.

 Prior to data and sample collection, respondents were educated on the purpose of the study as well as their consent for the information required.

 Data collected was then presented by the use of table bar charts, and pie charts, for analyses and hypothesis testing by the use of the Chi (X9) square independence or goodness of fittest.

**3.5 Reagent Preparation, Specimen Collection and Analysis:**

 The reagents and materials needed for the collection and analysis of sample are listed below. Above all, a well equipped laboratory was also in place for sample analysis.

**Materials**

* Syringes - Test tubes brushes
* Glass tubes - Test tube rack
* Centrifuge - Tissue paper
* Spectrophotometer - Gloves
* Tourniquet - Writing material
* Micro pipette - Typed questionnaire
* Pipette tips - Cotton wool
* Cuvettes (1cm) - Stop watch

**Reagents**

* 70% alcohol
* Distilled water
* Reagent kit for SGOT and SGPT
* La croix.

**3.2-1 Reagent Preparation**:

 The commercial kits used was produced by CHRONOLAB SYS S.L.

\* ALT (Alanine aminotransferase)

**Reagent 1:** (Buffer/Substrate)

* TRIS PH 7,8------100mmol/L
* L-alanine-----------500 mmol/L

**Reagent 2:** (Enzyme/wenzyme/∞-Ketoglutarate)

* NADH----------------------0, 18 mmol/L
* Lactate dehydrogenase (LDH0 ----1200u/L
* ∞ -ketoglutarate----------------------15mmol/L

**Working Reagent (WR)**

 The content of reagent 2 were dissolved with the corresponding volume of reagent 1, that is 10ml of reagent 1 in each container of reagent 2. Cap and mix gently to dissolve contents.

\* AST (Asparate aminotransferase)

**Reagent 1** (buffer/substrate)

* TRIS PH 7,8------------------------------800mmo/L
* Lactate dyhydrrogemase (;DJ) --------600u/L
* ∞ - ketoglutarate------------------------12mmol/L

**Working Reagent (WR)**

 The contents of reagent 2 were dissolved with the corresponding volume of reagent 1 that is 10ml reagent 1 in each container of reagent 2. (Young, et al: 2001).

**3.5.3. Specimen collection:**

 Venous blood was the appropriate specimen for the determination of transaminase levels and was collected as follows.

* Select a sterile dry syringe of the capacity required.
* Apply a soft tourniquet to the upper arm of the patients.
* Using the index finger, feel for a suitable vein
* Ask the patient to make a fist
* Cleanse the site to be punctured with 70% ethanol and allow to dry.
* With the thumb of the left hand holding down the skin below the site to be punctured, make the vein puncture with the level of the needle directed upwards in the line of the vein. Steadily withdraw the finger of the syringe at the pulse rate.
* When sufficient blood has been collected, release the tourniquet and instruct the patient to open his or her fist. Remove the needle and immediately press on the puncture site with a piece of dry cotton wall. Instruct the patient to continue pressing on the puncture site until the bleeding has stopped.
* Remove the needle from the syringe and carefully discard the needle safely.
* Check that bleeding from the vein punctured site has stopped ( cheesbrough;2005)

**3.5.4. Laboratory Analysis of Specimens.**

**3.5.4 a) Instrumentation.**

 **\* Spectrophotometer**

 A spectrophotometer consist of two instruments namely a spectrometer for producing light of any selected color (wavelength), and a photometer, for measuring the intensity of light. The instrument is arranged so that liquid in a cuvette can be placed between the spectrometer beam and the photometer. The amount of light passing through the tube is measured by the photometer. If development of colour is linked to the concentration of a substance, or solution, then that concentration can be measured by determining the extent of absorption of light at the appropriate wavelength. 9Mike:2008).

 The principle of spectrophotometer is based on Beer’s law that state that;

“the concentration of a substance is directly proportional to the amount of light absorbed and in versely proportional to the logarithm of the transmitted light”

**Components of a Spectrometer**

* **Light**: which provide energy in the form of visible or non visible light that may pass through the monochromator to be separated into discrete wavelength.
* **Entrance slit**: that minimizes stray light and prevent scattered light of entering the monochromator.
* Monochromators: these are wavelength selectors using prisms or gratings.
* **The exit slit**: that sieves stray light and undesired wavelength from entering the cuvette.
* **The cuvette**: they are transparent containers for the sample made from quartz or plastic.
* **Directors:** measures light intensity by converting the light signal into an electric signal.

**\* Centrifuge**

 A centrifuge is a piece of equipment generally driven by an electric motor ( some doler model were spun by hand) that puts an object in rotation around a fixed axis, applying a force perpendicular to the Axis. The centrifuge works using the sedimentation principle where the centripetal acceleration causes more dense substances to separate over along the radial direction. (the bottom of the tube). By the same was, lighter objects will tend to move to the top of the tube. Simple centrifuge are used in chemistry, biology and biochemistry for separating and isolating suspensions. They vary widely in speed and capacity. They usually comprise containing two, four or many more wells within which the samples containing centrifuge tips may be placed (zhang, 2006)

**b) Procedure of the test**

- About 4ml of blood sample was collected by Vein puncture into a labeled dry test tube.

- Blood sample was allowed to coagulate after which it was centrifuged at 3000 rpm for 5 minutes to obtain serum.

- The spectrophotometer was put on for 15-30minutes before the test.

- The spectrophotometer was calibrated at 340nm and adjusted to zero with air.

- 1000ul of the working reagent and 100ul of the sample were pipette into a cuvette.

- The cuvett was then placed in the spectrophotometer with the smooth face always facing the light.

- The stopwatch was started immediately since the reaction was kinetic.

- The absorbance were read at 1 minute interval thereafter for 2 minutes.

- After reading the various absorbances, the mansaminase values were calculated as indicated by the manufacturer and recorded in U/L.

**3.5.5. Reporting of Results:**

The activity was expressed in units per liter of sample (U/L). The transaminases level were determined following the kinetic method and results was given as normal or elevated following the normal value.

**Reference Value.**

* AST: Women : up to 31 U/L,

 Men up to 35 U/L

* ALT: Women : up to 40 UL:

Men: up to 45 U/L

 **3.5.6) Quality Control**

- Quality control of the spectrophotometer was done monthly

- Reagents were not use after their expiratory date

- Clean and dry cuvettes were use

- The components of the kit were protected form light and prevented from contamination during their use

- Homolysis of the sample was avoided

- The reagents were stored at the temperature 2 degrees-8degrees.

**CHAPTER V**

**DISCUSSION, CONCLUSION AND RECOMMENDATIONS**

**5.0) Introduction**

In our analysis, the elevation of transaminase was considered to be ALT or AST levels above the upper limit of normal that is up to 35 U/L for AST and up to 45 U/L for ALT. out of 210 patients, 50 patients showed with elevated transaminase, which give a prevalence of 23.8%.results showed that levels of transaminases varied according to individuals characteristics and tend to be elevated with increasing risk factors.

* 1. **Discussion**

 Level of transaminases was found to be increasing over time. Elevated levels were found to be associated with antiretroviral therapy. HAART was found to be associated with low level hepatotoxicites at initiation, regardless of drug classes and combination.

 13(71,1%) old patients out of 37, that is patients that were on antiretroviral therapy for 6 years and above, had elevated transaminases. Increases levels of transaminases showed a significant positive linear relationship with increase in the duration of treatment. This can be due to long term damage of the liver by medication.

 38(26%) females out of 146 had elevated transaminases while 12( 18,7%) males out of 64 had elevated transaminases. This can be explained by the fact that more women participated in the study and they are more infected with HIV-AIDS, than males.

 Elevated transaminases due to anti retroviral therapy were found independent on patient’s age. Our study showed significant relationship between the age of the patients and trnsaminase levels. 40(31,5%) patients out of 127 of the age group 21-40 presented with elevated transaminase while 0% of the age group 0-20 and 25% of the age group 61-80 had elevated transaminases. This can be explained by the fact the age grop 21-40, is the most active and most infected group, they are also exposed to risk factors like alcohol, cigarette, and hepatitis than the other age group.

 There was a significant variation between patients educational levels, 41(29,7%) of patients out of 138 with primary education had elevated transaminase, while 7(11,5%) out of 61 patients with secondary education and 2(18,2%) out of 11 patients presented with transaminitis. This can be explained by the fact that patients with primary education have less knowledge about HIV infection, and the risk factors associated with elevated transaminases.

 More over, there was a significant variation between patients occupations. 30(27,3%) of farmers out of 110, had elevated transaminases, while 10(21,7%) out of 46 business patients, and 8(25%) out of 32 patients exerting in the variety of work presented with elevated transaminases. Most of the patients exerting farming had elevations with respect to AST, which can be explained by the fact that their work need a lot of muscle exercise and AST is found in skeletal muscle and can be elevated due to strenuous exercise.

 Results showed that levels of transaminases varied according to individual’s characteristics and tend to be elevated with increasing number of risk factors. Further more, there was a significant variation between patients alcohol intake, cigarette smoking, herbs medication, and sport activities. However, some patients presented with elevated transaminases without any risk factor associated. This can be a justification that hepatotonicity is not only alcohol, cigarette, herbs, medication, or sport activities. But there are other factors that can lead to liver damage and were not taken into consideration, like co-infection with hepatitis B or C.

**5.2. Conclusion**

 Although every tissue in human body has the ability to metabolize drugs, the liver is the principal organ. Drug biotransformation in vivo can occur by spontaneous non – catalysed chemical reaction, but majority are catalysed by specific cellular enzymes. At sub cellular levels, these enzymes may be localized in the mitochondria, cytosol, lysosomes and plasma membrane. The activities of these drug metabolizing enzymes in drug biotransformation have been implicated in the formation of metabolites. With enhanced activity or toxicity including hepatotoxicity. This study has shown that HIV infection and intake of PRV drugs to combat the disease may expose patients, whether male or female, to potential risk of hepatotoxicity. This makes liver disease and injury important factors to consideration in making antiretroviral decisions. From the results analysis, and the X9 –Independence test, given that the calculated X9 was greater than the tabulated X2 in all the parameters, we reject the null hypothesis and accept the alternative hypothesis, which says that; Not all HIV patients on antiretroviral therapy are suffering from Hepatotoxicity.

**5.3. Recommendations**

- Health talks should be organized to HIV positive patients on the disadvantages of alcohol consumption, cigarette smoking and traditional herbs medication.

- Hepatitis B and C tests should be done with the others semestrially tests (CD4, hemoglobin, testing blood sugar, transaminases) for a better monitoring of patients.

- Further work should be done on this topic with respect to the different types of antiretroviral combinations, in other for the clinician to make a good decision on the type of drug administered to a patient, and also further work should be done with a larger sample size in other to obtain more significant results.

**CHAPTER IV**

**RESULTS, ANALYSES AND INTERPRETATIONS**

1. **Results.**

 Blood samples form 210 patients, both males and females independently of the age and individual characteristics were used to obtain their serum transaminase levels. Of the 210 who participated in the study, 50(23,8%) presented with elevated tranaminases, 25(11,9%) presented with tranaminitis with respect ot ALT, 20(9,5%) presented with AST elevations, and 5(2,4%) presented with both AST and ALT elevations. Participants were further differentiated following individual characteristics as number of years on medication, gender, age, level of education, occupation and personal characteristics.

**4-1) Variation of transaminases according to years of medication**

**Table I: Results according to number of years on medication**

|  |  |  |  |
| --- | --- | --- | --- |
| **No of years on medication** | **Number of participants** | **Elevated** | **Normal** |
| 0-2 | 65 | 7(10,8%) | 58(89,2%) |
| 3-5 | 110 | 30(27,3%) | 80(72,7%) |
| 6 and above | 35 | 13(37,1%) | 22(62,9%) |
| X 2tab(2;0,05)=5,991 ; X2 cal = 25,3 |

From x-independence test, there is a significant variation of transaminases between years of medication.

2



Patients taking medication between 3-5 years were the most involved in the study (110) and they presented with a percentage of 27, 3%. Patients on medication between 0-2 years presented with the lowest number of elevation (10,8%). Patients taking medication form 6 years and above presented with a percentage of 37,1% which is the highest number of elevation. This can be explain by long term damage of the liver caused by antiretroviral therapy.

**4-2) Variation of transaminases according to gender**

Table II: Results according to gender

|  |  |  |  |
| --- | --- | --- | --- |
| **Gender** | **Number of participants** | **Elevated** | **Normal** |
| Male | 64 | 12(18,7%) | 52(81,2%) |
| Female | 146 | 38(26%) | 108(74%) |
|  X tab (2,0,05)= 3,841 X Cal =40,2 |

From x – independence test, there is a significant variation of transaminases between gender.



More females, (146) participated in study than males (64) as shown in table II. The highest number of elevation were found in females (26) and the males were having the highest number of normal transaminases, this result can be explain by the fact that more females participated (146) which more than double of the number of males (64).

**4-3) Variation of transaminase according to age**

Table III: Results according to age groups

|  |  |  |  |
| --- | --- | --- | --- |
| **Age group** | **Number of participants** | **Elevated** | **Normal** |
| 0-20 | 11 | 0(0%) | 11(100) |
| 21-40 | 127 | 40(31,5%) | 87(68,5%) |
| 41-60 | 68 | 9(13,2%) | 59(86,8%) |
| 61-80 | 4 | 1(25%) | 3(75%) |
|  X tab (3,0,05) = 7,815 X cal = 50,4 |

From X-independent test, there is a significant variation of transaminases between the age groups.



Figure III prevalence of transaminitis according to age

 Patients of age group 21-40 years were the most involved in the study and they presented with the highest number of elevations (31,5%) followed by patients between 61-80 with percentage of 25 followed by the age group 41-60 with the percentage of 13,2%/ patients of age group 0-20 presented with the highest number of normal transaminases (100S%) with a null percentage of elevation.

**4.4) Variation of transaminases:**

Table IV) Results according to level of education

|  |  |  |  |
| --- | --- | --- | --- |
| **Level** | **Number of participants** | **Elevated** | **Normal** |
| Primary | 138 | 41(29,7%) | 97(70,3%) |
| Secondary | 61 | 7 (11,5%) | 54(88,5%) |
| Tertiary | 11 | 2(18,2%) | 9(81,8%) |
| X tab (,20,05) =5,991 X cal = 36,7 |

From X – independence test, there is a significant variation of transaminases between levels of education.



Figure IV Prevalence of transacminitis according to level of education.

Patients with primary education were the most involved in the study (138) and presented with the highest number of elevation (25,7%) as in table IV, followed by patient with tertiary level with a percentage of 18,2%.

 Patients with secondary education presented with the highest number of normal transaminases.

**4-5) Variations of transaminases according to occupation**

**Table V: Results according to occupation.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Occupation** | **Number of participants** | **Elevated** | **Normal** |
| Farming | 110 | 30(27,3%) | 80(72,7%) |
| Business | 46 | 10(21,7%) | 36(78,3%) |
| Teaching | 10 | 2(20%) | 8(80%) |
| Students | 12 | 0(0%) | 12(100%) |
| Others | 32 | 8(25%) | 24(75%) |
|  X tab (4,0,05) = 9,488 ; X cal = 26,8 |

From X – independence test, there is a significant variation of transaminases between occupations.



Figure V:

.

 Farmers were the most involved in the study (110) and presented with the highest number of education (27,3%) as I table V, followed by patients exercing in a variety of work (driver, mechanic, secretary----) with a percentage of 25% students presented with the highest number of normal transaminases and the lower number of elevated transaminases (0%).

**4-6) Variations according to individual characteristics**

 **4-6-1: Alcoholism**

Table VI: Results according to alcohol intake.

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristics** | **Elevated** | **Normal**  | **Total** |
| Alcoholics | 25(8,9) | 42(58,1) | 67 |
| Non alcoholics | 3(19,1) | 140(123,9) | 143 |
| Total | 28 | 182 | 210 |
|  X tab (2, 0, 05) = 3,841; X cal = 49,27. |

From the X-independence test, there is a insignificant variation of transaminases between alcoholics and non alcoholics.

**4-6-2: Smoking**

Table VIII: Results according to cigarette smoking.

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristic** | **Elevated**  | **Normal** | **Total** |
| Cigarette smokers | 2(0,09) | 8(9,8) | 10 |
| Non cigarette smokers | 2(3,08) | 198(196,2) | 200 |
| Total | 4 | 206 | 210 |
|  X tab (2,0,05)=3,8441; X cal = 41,23 |

From the X independence test, there is a significant variation of transaminases between cigarette smokers and non cigarette smokers.

**4-6-3) Herbs medication**

Table VIII: Results according to hers medication

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristic** | **Elevated**  | **Normal** | **Total** |
| Herbs drinker | 3(1,6) | 46(47,4) | 49 |
| Non herb drinker | 4(6,7) | 157(194,3) | 201 |
| Total | 7 | 203 | 210 |
|  X tab (2,0,05) = 3,841, X cal= 9,545 |

From X –independent test, there is a significant variation between herbs drinkers and non herbs drinkers.

**4.6.4). Sport activities**.

Table IX: Rents according to sport activities

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristic** | **Elevated**  | **Normal** | **Total** |
| Sporters | 8(3,7) | 63(67,3) | 71 |
| Non sporters | 3(7,3) | 136(131,7) | 139 |
| Total | 11 | 199 | 210 |
|  X tab (2,0,05) = 3,841; X cal =41, 23 |

From X-independence test, there is a significant variation between sporters and non sporters.

 **Definition of terms**

**Actin**: it is a globular protein found in eukaryotic cells

**Apoptosis**

 Is a process of programmed cell death that may occur in multicellular organisms.

**Acitis:** It is the accumulation of fluid in the abdominal cavity

**Bilirubin:** Is the yellow break down product of normal heam (component of heamoglobin) catabolism.

**Cholecystitis:** It is the inflammation of the gull bladder that causes severe abdominal pain.

**Glisson’s Capsule:** It is a colagenous capsule covering the external surface of the liver.

**Glucoronidation**: It is a phase II biotransformation reaction in which glucoronide act as a conjugation molecule and binds to a substrate via the analysis of glucoronosyltransferases.

**Hydroxylation:**  Is a chemical reaction that introduces a hydroxyl group (-OH) into an organic compound.

**Hepatotoxicity:**  liver damage caused by chemicals also known as hepatotoxins

**Lentivirus**: Is a genus of slow viruses of the retoviridae family, characterized by a long incubation period.

**Morbidity**: Is an abnormal condition affecting the body of an organism.

**Mortality**: Is an incidence of death in a population

**Necrosis:** is the premature death in of cells and living tissues

**Nicotinamine:** Also known as niacinamide is the amide of nicotinic acid (Vitamin B3)

**Retro Viruses**: Is an RNA virus that is replicated in a host cell via the enzyme reverse transcriptase to produce DNA from its RNA genome.

**Appendix I**

**Questionnaire**

**Topic:** Variation of transaminases in HIV infected patients visiting Saint Elizabeth General Hospital and Cardiac Centre Shisong.

**Researcher: CHIANGUEU TCHUANA LEONIE.**

**Purpose:** To evaluate the prevalence of Transaminitis in HIV Patients.

**Participation:** It will be Voluntary and free will, confidentiality of records and patients information will not be disclosed to any other person except on authorization of the patients.

Please, below are some questions for you to answer, please feel free and be sincere in answering the questions

**A) Patient’s identification and social history**

Serial number……………………. Age……… Address……………………

Profession………………………… Gender…….. Educational level…….

**B) Medical History**

|  |  |  |
| --- | --- | --- |
|  | Yes | No |
| Alcohol consumer |  |  |
| Number of years on medication |  |  |
| Sporter |  |  |
| Knowledge about hepatitis |  |  |
| History on Cardiac Problem |  |  |
| On traditional Herb Medication |  |  |
| Smoking |  |  |

**Strictly confidential**

**Appendix II**

1. X2=

 Where X2 is Chi square, 0 is observed frequency E is expected frequency.

1. 

 Where ∑R = row sum ∑C =column sum and G= Grand total

1. df= (R-1)(C-1), where R =rows and C= columns
2. Prevalence /incidence rate =

Where n+ = number of positive cases, T= total number of samples

**References**

1.Aceti A, Pasquazzi C, Zechini B, (2002): Hepatotoxicity development

 during antiretroviral therapy containing protease inhibitors in

 patients with HIV.

2. Anne Vanleeuwen M, Todd R, Lynette S, (2006): Davis’s comprehensive

 handbook of laboratory and diagnostic tests with nursing implication.

3.Barriero P, Rodriguez, Ruiz A, (2005): Influence of Liver fibrosis on HAART

 associated hepatotoxicity in patients with HIV and hepatitis C

 virus co\_ infected.

4.Bessesen M, Ives D, Shermark, (1999): Chronic active hepatitis B exacerbations

 in HIV infected patients following development of resistance to

 or with drawal of lamivudine.

5. Bonita Morrow Cauanaugh, (1999): Nurses manual of laboratory and

 diagnostic tests: 3rd Edition.

1. Bonfanti P, Landonios S, Ricci E, (2001): Risk factors for hepatotoxicity

 in patients treated with HAART.

1. Cheesbrough M, (2000): District Laboratory Practice in Tropical Countries.

 2nd Edition.

1. Clark, Creighton, Portmann, (2002): Acute liver failure associated

 with antiretroviral treatment for HIV.

1. Dybul M. Fauci As, Baitle H G, (2002): Panel on clinical practices for treatment

 of HIV. Guidelines for using antiretroviral agents among

 HIV infected adults and adolescents.

1. Evans D.M.D, (2001): Special tests and their meanings. 11th Edition.
2. Frances Fischbach, (1996): laboratory and diagnostic test. 5th Edition
3. Harris, A.D, (2002): Antiretroviral therapy in Sub Saharan Africa
4. Hewitt R, (2002): Abacavir hypersensitivity reaction
5. Joyce Lefever, (1994): Laboratory and diagnostic tests with nursing

 implications. 4th Edition.

1. Juanita Watson, Marie S, (1995): Nurse’s manual of laboratory and

 diagnostic tests. 2nd Edittion.

1. Maidal L, Babudieri S, Selva C, Solinas G, (2006): liver enzyme elevation

 in hepatis C virus: HIV co-infected patients prior and after

 initiation of HAART.

1. Maurizio Bonacini, (2006): Drug associated liver disease during HAART,

 impact of HCV co infection.

1. Maurizio, (2004): liver injury during HAART, the effect of HCV co-infection.
2. Nunez M, Martin-Carbonero, Moneroll Valencia, (2006): impact

 of antiretroviral.

1. Neil, (2004): HIV infection and hepatic enzyme abnormalities.
2. Nunez M,(2006): hepatotoxicity of antiretrovirals: Incidence, mechanisms

 and management.

1. Palella F, Baker K, Moorman, Chmeil, (2006): mortality in the highly

 active retroviral therapy era.

1. Palella FJ, Jr, Delaney, (2001): Declining Morbidity and mortality among

 patients with advanced human immunodefficiency virus infection.

1. Ritchie M, Haas D, Motsinger A, (2006): Drug transporter and

 metabolizing enzymes gene variants and NNRTI Hepatotoxicity.

1. Saves M, Vandentorren S, Daucourt V, (1999): Incidence and risk factors

 in patients treated by antiretroviral combination.

1. Soriano, puoti, Rockstroh,(2001): antiretroviral drugs and liver injury.
2. Splenger, Lichterfeld, Rockstroh, (2002): Antiretroviral drugs and liver injury
3. Sulkowski, (2004): Hepatotoxicity associated with protease inhibitor

 based antiretroviral regimens with or without ritonavir.

1. Young DS, (2001): Effects of disease on Clinical Laboratory tests. 4th Edition.
2. Zucker S, Qin X, Rousters, (2001): Mechanism of antiretroviral

 induced hyperbilirubinaemia